EQUIPMENT AND INSTRUMENTATION

Lab and Field Equipment, Analytical Instruments, and Calibration

Environmental Analysis Program
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EQUIPMENT AND INSTRUMENTATION

Equipment and Instrumentation

Lab and field equipment operation, analytical instrument procedures, and calibration

EA Program – Biogeochemistry Lab, Pomona College

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Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F100

EA Program Vehicle Use

Standard Operating Procedure

SOP Number	SOP-F100
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1. Purpose

This SOP describes the procedures for reserving, inspecting, operating, and returning EA Program vehicles. Following these procedures ensures that vehicles remain safe, reliable, and available for all program research and teaching activities.

2. Scope

- **Applies to:** All use of EA Program vehicles for field research, class field trips, and program-related transportation
- **Does not apply to:** Personal vehicles used for commuting to field sites; rental vehicles; Facilities and Maintenance fleet vehicles
- **Location:** EA Program vehicle parking (campus garage)

3. Responsibilities

Role	Responsibility
EA Coordinator	Manage reservation calendar, maintain insurance records
PI / Instructor	Approve vehicle requests, ensure drivers are authorized
Authorized Driver	Follow this SOP, complete pre-trip and post-trip checks
New Students	Submit insurance form and complete training before driving

4. Definitions

- **Authorized driver:** A person who holds a valid driver's license and has a completed, approved Pomona College driver insurance form on file with the EA Coordinator; the only persons permitted to operate EA Program vehicles.
- **Drowsy driving:** Operating a motor vehicle while significantly impaired by fatigue; a leading cause of serious crashes on long drives to remote field sites; if any crew member feels impaired by fatigue, pull over safely and rest before continuing.
- **Fuel card:** A fleet credit card stored in the center console of each EA vehicle; used to purchase regular unleaded gasoline (87 octane) at participating stations; if declined, pay out of pocket and submit the receipt to the EA Coordinator for reimbursement.
- **Pre-trip inspection:** A walk-around check of the vehicle before departure to identify safety issues (warning lights, tire damage, fluid leaks) that would make the vehicle unsafe to drive; required before every trip.
- **Post-trip return standard:** The condition in which the vehicle must be returned after every use – clean interior, at least 3/4 tank of fuel, fuel card in the center console, no warning lights, parked in the designated garage spot.
- **Pomona College driver authorization:** The college insurance form required before driving any Pomona College-owned or -leased vehicle; submitted to the EA Coordinator with a copy of a valid driver's license; must be approved before the first trip.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Motor vehicle operation on public roads and unpaved field-site access roads; loading and unloading heavy field equipment.
- **Environmental:** Driving in adverse weather, reduced visibility, or on unfamiliar roads near field sites.
- **Fatigue:** Long drives to remote field sites increase the risk of drowsy driving.

5.1. Requirements

- Valid driver's license (must be carried while driving)
- Completed and approved Pomona College driver insurance form on file with the EA Coordinator
- Familiarity with this SOP and SOP-F001 Field Safety

5.2. Emergency Procedures

In the event of an accident or breakdown:

1. Ensure all occupants are safe; move to a safe location if possible
2. Call 911 if anyone is injured
3. Contact Pomona College Campus Safety: (909) 621-8170
4. Notify the EA Coordinator and PI
5. Document the incident (photos, written description, other party information if applicable)
6. Do not admit fault at the scene; exchange insurance and contact information only
7. Complete a college incident report within 24 hours

Refer to SOP-F001 Field Safety for general emergency response procedures.

6. EA Program Fleet

Vehicle	Make / Model	Seats	Notes
EA-1	Ford Explorer	7	Garage card in center console
EA-2	Ford Explorer	7	Garage card in center console

Both vehicles are parked in the campus garage when not in use. Keys and fuel cards are stored in each vehicle's center console.

7. Procedure

7.1. Before You Drive: Insurance Authorization

Before driving any EA vehicle for the first time, you must:

1. Obtain the Pomona College driver authorization and insurance form from the EA Coordinator.
2. Complete and submit the form with a copy of your valid driver's license.
3. Wait for written approval before operating any EA vehicle.

> **!** No Exceptions
>
> You may not drive an EA vehicle until your insurance form has been approved and is on file with the EA Coordinator. This is a college requirement, not a suggestion. Driving without approval puts you and the program at serious liability risk.

7.2. Reserving a Vehicle

1. Contact the EA Coordinator to check availability.
2. Provide: date(s) and times needed, destination, number of passengers, and the name of the PI or instructor authorizing the trip.
3. Confirm the reservation before your trip; cancellations should be communicated as early as possible so others can use the vehicle.

7.3. Pre-Trip Inspection

Before departing, walk around the vehicle and verify:

- ☐ Fuel gauge reads at least 3/4 full (refuel before departing if below 3/4; see §6.5)
- ☐ Fuel card is present in the center console
- ☐ Tires appear properly inflated with no visible damage
- ☐ All lights (headlights, brake lights, turn signals) are functional
- ☐ Mirrors are adjusted for the driver
- ☐ Windshield is clean and wipers are functional
- ☐ First aid kit is present and stocked (see SOP-F001)
- ☐ No warning lights are illuminated on the dashboard
- ☐ Any pre-existing damage is noted and reported to the EA Coordinator before departing

If any safety issue is found (warning lights, tire damage, brake problems), do not drive the vehicle. Contact the EA Coordinator immediately.

7.4. During the Trip

- Obey all state and local traffic laws at all times.
- All occupants must wear seatbelts (California law).
- The driver must not use a handheld cell phone while driving (California Vehicle Code §23123).
- Do not exceed the vehicle's passenger capacity.
- Secure all field equipment so it cannot shift during transport.
- On unpaved roads, reduce speed and increase following distance.
- If conditions become unsafe (severe weather, road hazards, driver fatigue), pull over and reassess before continuing.

7.5. Refueling

1. Use the fuel card located in the center console.
2. Select regular unleaded gasoline (87 octane).
3. If the fuel card is declined or missing, pay out of pocket and submit the receipt to the EA Coordinator for reimbursement.
4. Replace the fuel card in the center console after refueling.

7.6. Returning the Vehicle

After every trip, complete the following before leaving the vehicle:

1. **Refuel:** Return with at least a 3/4 tank of gas. If you used the vehicle, you are responsible for refueling.
2. **Clean:** Remove all personal items, trash, and field equipment. Brush or vacuum out dirt and mud. Wipe down surfaces if visibly soiled.
3. **Fuel card:** Confirm the fuel card is back in the center console.
4. **Park:** Return the vehicle to its designated spot in the campus garage.
5. **Report problems:** If anything is wrong with the vehicle (warning lights, unusual noises, damage, low tire pressure), notify the EA Coordinator immediately. Do not assume someone else will report it.

> **Return It Ready**
>
> The next person to use the vehicle should find it clean, fueled to at least 3/4 tank, with the fuel card in the center console, parked in the garage, and free of warning lights.

8. Prohibited Uses

- Personal errands unrelated to EA Program activities
- Transporting hazardous materials not approved by the PI (standard field samples and preserved water samples are acceptable)
- Allowing unauthorized or uninsured individuals to drive
- Off-road driving beyond established field-site access roads
- Towing trailers or other vehicles without prior approval from the EA Coordinator

9. References

For driver authorization requirements, consult the EA Coordinator or the Pomona College Office of Risk Management directly. There is no publicly accessible bib entry for this internal policy; confirm current requirements with the EA Coordinator before each field season.

- SOP-F001 Field Safety

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required reading; emergency procedures
F002	Field Equipment Checklist	Vehicle pre-trip checklist

11. Training Record

Before driving EA Program vehicles independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Submit a completed driver insurance authorization form to the EA Coordinator and receive written approval
3. Familiarize themselves with the vehicle (controls, mirrors, fuel card location) before the first trip
4. Be signed off by the PI or EA Coordinator

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F101

Electrical Power in the Field

Standard Operating Procedure

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1. Purpose

Electrical power in the field can come from infrastructure sources (such as outdoor or indoor outlets), portable generators, or solar power. This SOP outlines the risks associated with using electricity in the field and develops strategies to mitigate those risks.

2. Scope

- **Applies to:** Use of portable generators (Honda EU2000i and Ryobi RYi2200A) and extension cords for powering field equipment
- **Does not apply to:** Permanent electrical installations or solar panel systems (to be covered in a future SOP)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, perform generator maintenance
New Students	Read and understand this SOP before operating generators; complete SOP-F001 Field Safety training

4. Definitions

- **Carbon monoxide (CO):** A colorless, odorless, tasteless toxic gas produced by incomplete combustion of gasoline and other fuels; displaces oxygen in the bloodstream and is rapidly fatal at elevated concentrations; never operate a generator in any enclosed or semi-enclosed space.
- **Carburetor:** The engine component that mixes air and fuel in the correct ratio for combustion; must be drained before storage of 30 days or more to prevent gum and varnish deposits from clogging fuel passages.
- **Choke:** A valve in the carburetor air intake that restricts airflow to enrich the fuel mixture during cold starts; must be moved to the OPEN or RUN position after the engine warms up to prevent over-fueling.
- **Eco-throttle:** An automatic throttle control (Honda EU2000i) that reduces engine speed when load demand is low, improving fuel economy and reducing noise; must be switched OFF during startup.
- **Ethanol-free gasoline:** Fuel containing no ethanol additive; recommended for small engines because ethanol absorbs water, degrades rubber fuel lines, and causes carburetor deposits during storage.
- **Fuel stabilizer:** A chemical additive mixed into gasoline before storage to prevent oxidation and gum formation; required when the generator will not be used for 30 days or more.
- **Ground fault:** An unintended electrical path between a live conductor and the ground or equipment casing; a leading cause of electrocution; mitigated by using grounded extension cords and GFCI protection.
- **Grounded extension cord:** An extension cord with a three-prong plug that includes a ground conductor; required for all generator connections to provide a safe fault-current return path.
- **Inverter generator:** A generator that produces AC power through an electronic inverter circuit, yielding cleaner, more stable voltage and frequency than a conventional generator;

both the Honda EU2000i and Ryobi RYi2200A are inverter generators, making them suitable for powering sensitive electronic field instruments.

- **Spark arrestor:** A fine wire-mesh screen fitted to the exhaust outlet that prevents hot carbon particles from escaping and igniting surrounding combustible material; required by law in many fire-prone areas; must be inspected and cleaned periodically.
- **Spark plug gap:** The distance between the center and ground electrodes of a spark plug; must be within the manufacturer-specified range (0.024–0.028 in for both units) for reliable ignition.

5. Safety and Hazards

⚠ Hazards

- **Carbon monoxide:** Engines emit carbon monoxide. Never use a generator inside an enclosed area (home, garage, crawl space). Fatal fumes can build up even with a fan or open doors and windows. Only operate generators outdoors, away from open windows, vents, or doors.
- **Fire and explosion:** Gasoline and its vapors are extremely flammable. Allow the engine to cool at least 2 minutes before refueling. Always use fresh gasoline. Never operate the generator near combustible materials.
- **Electrical shock:** Generators produce powerful voltage. Never operate under wet conditions and take precautions to protect the generator from rain and snow.
- **Electrocution / home wiring:** Never plug a generator directly into a home outlet. A qualified electrician must install a Power Transfer Switch for any home connection.
- **Heat from cables:** Ensure cables are rated for the current being carried. Never leave cables coiled, as coiled cords can generate excessive heat.

5.1. Required PPE

☐ Closed-toed shoes
☐ Work gloves (when handling hot engine components or refueling)
☐ Hearing protection (if operating near the generator for extended periods)

5.2. Emergency Procedures

Use a battery-powered carbon monoxide detector in the area where you are running a generator. If the alarm sounds, move to fresh air immediately and shut down the generator. Refer to SOP-F001 Field Safety for general field emergency procedures.

6. Equipment and Materials

Item	Notes
Honda EU2000i generator	Portable inverter generator
Ryobi RYi2200A generator	Portable inverter generator
High-capacity extension cords	Must be grounded type, rated for the application
Spare gas container	Filled with ethanol-free gasoline
Synthetic 10W-30 engine oil	Recommended oil type for both generators
Funnel	For adding oil

7. Procedure

7.1. Pre-Start Checks

1. **Check oil level.** If low, use a funnel to add synthetic 10W-30 oil. This does not need to be done every time; check every few weeks. If the generator shuts off after running for about 30 minutes, low oil is the likely cause.

2. **Check gas level.** If low, add ethanol-free gas from the spare container. On the Ryobi, fill to about an inch below the top of the tank. Both generators can be refilled while running. The Ryobi should be refilled after about 4 hours of operation; the Honda after about 6 hours.

7.2. Starting the Honda EU2000i

1. Turn the fuel tank cap vent (black cap on top of the generator) to the ON position. If this is not done, the generator will shut off after a few minutes.
2. Make sure the eco-throttle switch (on the side panel, below the fuel cap vent) is OFF.
3. For a cold start, move the choke (upper left front, near the engine switch) to the CLOSED position (slide the switch to the right).
4. Turn the engine switch to ON.
5. Pull the starter grip quickly several times until the engine starts.
6. Allow the engine a few minutes to warm up, then slowly move the choke to the OPEN position.
7. **To shut down:** Turn the engine switch to OFF. After the engine has cooled, turn the fuel tank cap vent to OFF.

7.3. Starting the Ryobi RYi2200A

1. Make sure the auto idle switch is OFF.
2. Move the choke to approximately halfway across. The marked cold-start position is slightly less than halfway, but the choke needs to go a bit further; you should feel a slight resistance at the correct position.
3. Prime the system by slowly pulling the starter grip several times.
4. Pull the grip quickly until the engine runs.
5. Allow the engine to run for 15–30 seconds, then move the choke all the way across to the RUN position.
6. **To shut down:** Switch the choke back to the OFF position.

7.4. Storage

If you do not plan to use the generator within 30 days, add fuel stabilizer to the gas and drain the carburetor.

7.5. Extension Cord Safety

- Use only grounded extension cords rated for the intended application.
- Always uncoil cords completely and lay them flat in open locations; coiled cords can generate excessive heat.

8. Maintenance

8.1. General (Both Units)

Keep generators in a clean, dry environment, not exposed to dust, dirt, moisture, or corrosive vapors. To clean, use a soft-bristle brush and/or vacuum cleaner to loosen and remove dirt and debris. Clean air vents with air pressure no more than 25 psi. Wipe exterior surfaces with a damp cloth. Perform maintenance more frequently when the generator is used in dusty areas. If you notice a power loss, the exhaust port and muffler may be blocked with carbon deposits; allow only qualified service technicians to clean these.

8.2. Ryobi RYi2200A Maintenance Schedule

Item	Frequency	Notes
Air filter	Check before each use; clean every 3 months or 50 hours; replace every year or 300 hours	Wash with soapy water, rinse, squeeze dry
Engine lubricant	Check before each use; change after first 20 hours, then every 6 months or 100 hours	Drain while warm for faster flow
Spark plug	Check every 6 months or 100 hours; replace every year or 300 hours	Gap: 0.024–0.028 in (0.60–0.70 mm)

Item	Frequency	Notes
Spark arrestor	Clean periodically	Wire brush; inspect screen for damage
Fuel tank / carburetor	Check fuel hose before each use; inspect fuel filter before each use; have tank and filter cleaned by qualified service center every 6 months or 100 hours	Drain both before storage to prevent gum deposits
Valve clearance	Check every year or 300 hours	Service by qualified technician

8.3. Honda EU2000i Maintenance Schedule

Item	Frequency	Notes
Air filter	Check before each use; clean every 3 months or 50 hours	Wash in detergent and warm water or non-flammable solvent; soak in clean engine oil and squeeze excess before re-installing
Engine lubricant	Check before each use; change after first 20 hours, then every 6 months or 100 hours	Fill to upper limit of oil filler neck

Item	Frequency	Notes
Spark plug	Check every 6 months or 100 hours; replace every year or 200 hours	Gap: 0.024–0.028 in (0.60–0.70 mm); new plug: tighten 1/2 turn after seating; used plug: tighten 1/8–1/4 turn
Spark arrestor	Clean every 6 months or 100 hours	Wire brush; inspect screen for damage
Fuel tank / combustion chamber	Clean fuel tank and filter every 6 months or 100 hours; clean combustion chamber every 300 hours; check fuel line every 2 years	Service by authorized Honda dealer
Valve clearance	Check every year or 200 hours	Service by qualified technician

9. Quality Control

TBD – establish routine checks to verify generator output voltage and frequency are within acceptable ranges for field instruments.

10. References

11. Training Record

Before operating generators independently, personnel must:

1. Read this SOP in full

2. Complete SOP-F001 Field Safety training
3. Review the manufacturer's operating instructions for the generator being used
4. Observe a trained operator start, run, and shut down the generator
5. Perform the startup and shutdown procedure under supervision
6. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F200

Eureka Manta2 Multiprobe for Water Quality Testing

Standard Operating Procedure

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1. Purpose

This SOP describes the procedures for calibrating, operating, and maintaining the Eureka Manta2 Sub2 Multiprobe for water quality measurements including temperature, pH, depth, conductivity, and dissolved oxygen (mg/L and % saturation) (APHA et al., 2012; Eureka Environmental Engineering, 2018).

2. Scope

- **Applies to:** Water quality measurements in lakes, streams, rivers, and other surface water bodies
- **Does not apply to:** Groundwater monitoring wells (requires different deployment procedures); laboratory bench-top water analysis
- **Location:** All EA Program field water sampling sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Field Lead	Verify calibration before deployment, coordinate logistics
Trained Personnel	Calibrate, operate, and clean the probe per this SOP
New Students	Complete training before independent use

3.1. Required Training

Researchers using this SOP should be trained on SOP-F001 Field Safety.

4. Definitions

- **Amphibian 2:** The ruggedized handheld field computer (portable PC) manufactured by Eureka that controls the Manta2 probe, displays real-time readings, stores data snapshots, and connects to the probe via the underwater cable (Eureka Environmental Engineering, 2018).
- **Barometric pressure:** Atmospheric pressure at the measurement location, expressed in mm Hg; required as an input for dissolved oxygen calibration because oxygen solubility decreases with decreasing pressure (increasing altitude) (APHA et al., 2012).
- **Calibration:** The process of adjusting sensor readings to match known reference standards before field use; required before every field outing to ensure data accuracy (Eureka Environmental Engineering, 2018).

- **Conductivity (specific conductance, spCond):** A measure of a water sample's ability to conduct electrical current, expressed in µS/cm or mS/cm; proportional to the concentration of dissolved ions; temperature-corrected to 25 °C for comparability across sites and seasons (APHA et al., 2012).
- **Dissolved oxygen (DO):** The concentration of oxygen dissolved in water, expressed as mg/L or percent saturation (%sat); measured by the HDO sensor using luminescent optical technology; decreases with increasing temperature, salinity, and altitude (APHA et al., 2012).
- **HDO (Handheld Dissolved Oxygen):** The luminescent optical dissolved oxygen sensor on the Manta2; measures both %sat and mg/L; calibrated in water-saturated air or with an air-saturated water standard (Eureka Environmental Engineering, 2018).
- **PDOP (Position Dilution of Precision):** A dimensionless measure of GPS accuracy based on satellite geometry; lower values indicate better positional accuracy; values below 3 are acceptable for most field applications.
- **Reference electrode:** The electrochemical half-cell that provides a stable reference potential for pH measurement; requires periodic refilling with reference electrolyte to maintain accurate pH readings (Eureka Environmental Engineering, 2018).
- **Sensor Response Factor (SRF):** A calibration diagnostic value indicating how the sensor responded to the reference standard; acceptable ranges are sensor-specific and indicate whether calibration was successful (Eureka Environmental Engineering, 2018).
- **Snapshot:** A single instantaneous record of all sensor readings captured and stored by the Amphibian at a specific date, time, and GPS location.
- **Sonde (probe):** The cylindrical instrument body of the Manta2 that houses all sensors (temperature, pH, conductivity, dissolved oxygen, and reference electrode) and is submerged during measurements (Eureka Environmental Engineering, 2018).

5. Safety and Hazards

⚠ Hazards

- **Biological/Chemical:** Water bodies may be contaminated; avoid ingestion and direct skin contact.
- **Physical:** Slippery surfaces near water; risk of submersion; electrical components near water.
- **Chemical:** pH standards (pH 4, 7, 10) and conductivity standard are mild irritants.

5.1. Required PPE

☐ Nitrile or latex gloves
☐ Water shoes or wading boots (if entering the water)
☐ Safety glasses (during calibration with standards)
☐ Sun protection (hat, sunscreen)

Refer to SOP-F001 Field Safety for general field safety, transportation, and emergency response.

6. Equipment and Materials

Item	Notes
Eureka Manta2 Sub2 Sonde	Inspect sensors before each use
Amphibian 2 field computer	Ensure fully charged (Eureka Environmental Engineering, 2018)
Underwater cable	Inspect for damage before use
pH 4, 7, and 10 standard solutions	For pH calibration; check expiration dates
Conductivity standard	Appropriate concentration for expected range
Deionized water	For rinsing sensors between calibration steps
Storage/calibration cup	Filled with tap water for storage
Field notebook	Per SOP-F003

7. Procedure

7.1. Startup

1. Connect the underwater cable to both the sonde and the Amphibian.
2. Power on the Amphibian.
3. Open the **Amp__2__2__6** app (or press **P1** shortcut).
4. Allow sensors to stabilize for 5–10 minutes before calibration.

7.2. Calibration

Calibrate before every field outing (APHA et al., 2012; Eureka Environmental Engineering, 2018). Record all calibration data (SRF values, standard concentrations, date, operator) in the field notebook.

7.2.1. pH

1. Remove the storage cup. Rinse sensors with DI water.
2. Submerge the sonde in pH 7 standard. Select **Calibrate > pH** and follow the on-screen prompts.
3. Rinse with DI water. Submerge in pH 4 standard. Accept the second calibration point.
4. (Optional) Verify with pH 10 standard. Record the SRF (acceptable range: 95–105).

7.2.2. Conductivity

1. Rinse sensors with DI water.
2. Submerge in the appropriate conductivity standard.
3. Select **Calibrate > Conductivity**. Enter the standard value and accept (Eureka Environmental Engineering, 2018).
4. Record the SRF (acceptable range: 110–120).

7.2.3. Dissolved Oxygen (HDO)

1. Remove the sonde from the calibration cup. Allow the HDO sensor to equilibrate in water-saturated air for 2 minutes (sensor face moist but not submerged).
2. Enter the current barometric pressure in mm Hg.
3. Select **Calibrate > DO** and accept the 100% saturation calibration (Eureka Environmental Engineering, 2018).
4. Record the SRF (should equal 100%).

7.3. Field Measurement

1. Lower the sonde to the desired depth. Allow 1–2 minutes for readings to stabilize before recording.
2. Press **Snapshot** on the Amphibian to save readings. Record the snapshot ID, location, and notes in the field notebook.
3. For depth profiles, lower the sonde in 0.5 or 1 m increments, pausing 30–60 s at each depth (Eureka Environmental Engineering, 2018).

7.4. Disconnecting

1. Remove the probe from the water.
2. Disconnect the underwater cable from the probe.
3. Insert the blue storage plug.
4. Cover the cable end with the plastic protector.

8. Post-Field Procedures

8.1. Cleaning the Probe

1. Clean the probe with warm soapy water (liquid dish soap or mild household cleaner).
2. Clean sensor stems with a soft brush.
3. Rinse thoroughly with tap water.
4. Store sensors with tap water inside the cup.

8.2. Downloading Data

1. Connect the Amphibian to your PC using a USB cable.
2. A green window will appear with four options; select **File Management**.
3. In the next window, navigate to the hard drive, then select **My Documents**.
4. Locate the Excel document with your data.
5. Copy the file to your project data folder.

8.3. Charging

Return the Amphibian to its charging station after each field outing.

9. Quality Control

- Calibrate all sensors before every field outing (APHA et al., 2012; Eureka Environmental Engineering, 2018).
- Verify SRF values are within acceptable ranges; do not proceed if any sensor fails calibration.
- Record a post-deployment calibration check on return to the lab; if any sensor has drifted beyond acceptable limits, flag the data.
- Compare spot readings against an independent DO meter or multiprobe where possible; readings should agree within ±0.3 mg/L for DO and ±0.1 units for pH (APHA et al., 2012).

10. Troubleshooting

Problem	Solution
No power	Check the green LED on the Manta2; if off, the voltage is insufficient. Try another cable.
pH SRF out of range	Recalibrate with fresh standards. If still out of range, refill the reference electrolyte and contact the PI.
Conductivity SRF outside 110–120	Recalibrate. If still out of range, contact the PI.
HDO %sat SRF 100	Redo the calibration with freshly aerated water.
GPS not acquiring satellites	Ensure the top cap (antenna) is uncovered; move to an open area.

11. References

- Eureka Environmental Engineering (2018)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Eureka Environmental Engineering. (2018). *Manta2 water quality multiprobe: User manual*. https://www.rshydro.co.uk/PDFs/Eureka/Manta2-manual.pdf

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F003	Field Notebook Protocols	Data recording
F209	Dissolved Oxygen Meter	Standalone DO companion
F307	Lake Profiling	Depth profile application

13. Training Record

Before using this instrument independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform calibration and field deployment
4. Perform a supervised calibration of all sensors
5. Perform a supervised field deployment with data collection
6. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F201

Pygmy and Type AA Velocity Meters

Standard Operating Procedure

SOP Number	SOP-F201
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the specifications, assembly, maintenance, and ordering of replacement parts for the Price Pygmy and Price Type AA mechanical current meters used by the EA Program. These vertical-axis cup-type meters convert water velocity into rotational counts and are the standard instruments for measuring point velocities during stream discharge measurements (Rantz et al., 1982) (see also SOP-F301 Stream Discharge Measurement).

2. Scope

- **Applies to:** Price Pygmy current meters and Price Type AA current meters owned by the EA Program, including standard cat-whisker and magnetic-head contact chamber variants
- **Does not apply to:** Electronic velocity meters (e.g., Marsh-McBirney, FlowTracker ADV), propeller-type meters (e.g., Ott), or acoustic Doppler instruments
- **Location:** Instruments stored in the EA field equipment room; maintenance performed in SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve purchases, authorize meter retirement
Field Lead	Perform pre-field spin tests, coordinate maintenance
Trained Personnel	Assemble, inspect, and clean meters per this SOP
New Students	Complete training before handling meters

4. Definitions

- **AquaCalc:** A digital flow computer that counts current meter revolutions electronically and converts them to velocity automatically using the stored rating table; eliminates manual timing and reduces transcription error.
- **Cat-whisker contact:** A spring-wire contact element in the Type AA meter's contact chamber; makes electrical contact with a solder bead on the shaft once per revolution (single-revolution contact) or once per five revolutions (penta contact); must be checked for wear and proper contact force before each field season (Rantz et al., 1982).
- **Contact chamber:** The sealed housing on the Type AA meter that contains the electrical contact mechanism (cat-whisker wire and solder bead, or magnetic head); connects to the cable leading to the headset or digital counter.

- **Penta contact:** A gear-reduction contact in the Type AA meter that closes the circuit once every five bucket-wheel revolutions; used at high velocities to reduce the counting rate to a manageable level; not available on the Pygmy meter (Rantz et al., 1982).
- **Pivot:** The tungsten carbide tip at the bottom of the current meter shaft; rides in a hardened bearing cup; the single most wear-sensitive part of a mechanical current meter; must be inspected for burrs, flat spots, and magnetization before each field season (Rantz et al., 1982; U.S. Geological Survey, Office of Surface Water, 2002).
- **Price Pygmy current meter:** A vertical-axis cup-type current meter with a 2-inch (5.1 cm) diameter bucket wheel; designed for shallow streams (depth 1.0 ft); wading rod suspension only; rated for velocities of 0.1–4.9 ft/s (Rantz et al., 1982).
- **Price Type AA current meter:** The standard USGS vertical-axis cup-type current meter with a 5-inch (12.7 cm) diameter bucket wheel; rated for velocities of 0.1–25 ft/s; can be suspended from a wading rod or cable (Rantz et al., 1982).
- **Rating table:** A lookup table, supplied with each meter by the manufacturer and approved by the USGS, that converts bucket-wheel revolutions per second to velocity in ft/s or m/s; laminated copies must be kept in each meter's carrying case (Rantz et al., 1982).
- **Raising nut:** The nut that controls the vertical position of the shaft and pivot within the yoke; must be tightened to the correct end-play setting (~0.001 in) for accurate measurements; never loosen by holding the nut and spinning the bucket wheel.
- **Spin test:** A field quality control check in which the bucket wheel is given a sharp spin by hand and the time for it to stop is measured; a proxy for bearing condition; minimum acceptable spin times are 40 s (Type AA) and 24 s (Pygmy) at room temperature (Rantz et al., 1982).
- **Wading rod (top-setting):** A graduated aluminum rod used to position the current meter at a known depth fraction (0.6 or 0.2/0.8 of total depth) below the water surface; the depth-setting mechanism is located on the handle, enabling depth changes without removing the meter from the water.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Pivot points and shaft tips are sharp; handle with care. The bucket wheel can pinch fingers if spun while adjusting the assembly.
- **Chemical:** Instrument oil is a mild skin irritant. Avoid eye contact.

5.1. Required PPE

☐ Nitrile gloves (when oiling or cleaning with solvents)
☐ Safety glasses (when using compressed air for cleaning)

6. Instrument Specifications

6.1. Price Type AA Current Meter

The Type AA is the standard USGS Price-type current meter for general streamflow measurement (Rantz et al., 1982). The bucket wheel has six conical cups (12.7 cm diameter) rotating on a vertical axis inside a brass yoke. Tungsten carbide bearings are seated in deeply recessed inverted cups that trap air and exclude water and silt. A tailpiece assembly aligns the meter with the current when suspended by cable.

Parameter	Value
Velocity range	0.1–25 ft/s (0.03–7.6 m/s)
Bucket wheel diameter	5 in (12.7 cm)
Number of cups	6
Contact types	Single revolution and penta (every 5 rev)
Suspension	Wading rod or cable with sounding weight
Construction	Brass and stainless steel, chrome plated
Rating	USGS standard rating table (two equations joined at 1 rev/s = 2.2 ft/s) (Rantz et al., 1982)

6.2. Price Pygmy Current Meter

The Pygmy meter is scaled to two-fifths the size of the Type AA, designed for shallow streams (Rantz et al., 1982). The contact chamber is integral to the yoke; single-revolution contact only (no penta gear). The meter has no tailpiece and cannot be used with cable suspension.

Parameter	Value
Velocity range	0.1–4.9 ft/s (0.03–1.5 m/s)
Bucket wheel diameter	2 in (5.1 cm)
Number of cups	6
Contact type	Single revolution only
Suspension	Wading rod only (no cable suspension)
Construction	Brass and stainless steel, chrome plated
Rating	USGS standard rating table (single equation) (Rantz et al., 1982)

6.3. Choosing Between Meters

Condition	Meter to Use
Water depth $>$ 1.0 ft (0.3 m)	Type AA
Water depth 1.0 ft (0.3 m)	Pygmy
Velocities expected $>$ 4.9 ft/s	Type AA
Shallow stream edges (supplemental data)	Pygmy

Condition	Meter to Use
Cable suspension required	Type AA

7. Equipment and Accessories

Item	Used With	Notes
Top-setting wading rod	Both	Graduated in tenths of a foot; depth-setting mechanism on handle
Round wading rod	Both	Simpler design; depth read from rod markings
Headset (earpiece)	Both	For counting clicks manually
AquaCalc or digital flow computer	Both	Automatically counts revolutions and computes velocity
Stopwatch	Both	For timed revolution counts if no digital counter
Sounding weight (Columbus type)	Type AA only	For cable-suspended measurements
Tailpiece assembly	Type AA only	Remove when using wading rod
Electrical cable	Both	Connects meter to counter or headset

7.1. Maintenance Supplies

Item	Notes
Instrument oil (light machine oil)	Supplied with meter; for pivot bearings
Lint-free cloth	For cleaning and drying
Cotton swabs (pointed)	For cleaning pivot bearing recesses
Small flat-head screwdriver	For contact chamber adjustments
Magnifying glass or loupe	For inspecting pivot points for burrs
Paper clip	For checking pivot magnetization
Bulk magnetic tape eraser	For demagnetizing pivots
Soft-bristle brush	For removing debris from cups
Compressed air (filtered)	For drying internal components

8. Procedure

8.1. Assembly

Type AA on a wading rod:

1. Remove the tailpiece assembly (used for cable suspension only).

2. Slide the meter yoke onto the wading rod and secure with the thumbscrew.
3. Connect the electrical cable from the binding posts on the contact chamber to the AquaCalc or headset.
4. For penta contact, connect to the penta binding post; for single-revolution counting, use the single-revolution binding post.

Pygmy on a wading rod:

1. Slide the pygmy yoke directly onto the wading rod and secure with the thumbscrew.
2. Connect the electrical cable to the binding post. The Pygmy has only a single-revolution contact.

8.2. Pre-Field Spin Test

Perform a spin test before every field outing (Rantz et al., 1982). Conduct with the meter level, shaft vertical, in a location with no air currents.

1. Hold the meter steady and use your finger or a pencil to give the bucket wheel a sharp, single spin.
2. Immediately start a stopwatch.
3. Stop the stopwatch when the wheel completely stops rotating.
4. Record the spin time in the field notebook.

Minimum acceptable spin times (Rantz et al., 1982):

Meter	Minimum Spin Time	Optimum Spin Time
Type AA	40 s	60–120 s
Pygmy	24 s	40–80 s

If the spin time falls below the minimum, clean and oil the pivot before proceeding (§7.3). If the spin time remains low after cleaning and oiling, replace the pivot.

8.3. Cleaning and Oiling (Pre-Season and As Needed)

1. **Inspect the pivot.** Remove the raising nut and carefully withdraw the shaft. Examine the pivot tip under a magnifying glass. A good pivot is highly polished, with no flat spots, pits, or scratches. Replace a damaged pivot immediately.
2. **Check for magnetization.** Hold the pivot near a paper clip. If the clip is attracted to the pivot, the pivot is magnetized and will cause erroneously high velocity readings. Demagnetize using a bulk magnetic tape eraser.
3. **Oil the bearings.** Apply one small drop of instrument oil to the upper shaft bearing and one drop to the pivot. Do not over-oil; excess oil attracts grit.
4. **Inspect the bucket wheel.** Spin the wheel slowly and watch for wobble. If the upper hub wobbles more than the lower, the shaft is bent and must be replaced.

5. **Inspect the contact chamber.** For cat-whisker contacts, check that the wire makes clean contact with the solder bead on each revolution. For magnetic-head contacts, verify the counter registers properly during the spin test.

> ⚠ Raising Nut Caution
>
> Never loosen the raising nut by holding the nut and spinning the bucket wheel. This will bend the shaft. Always hold the bucket wheel still and unscrew the nut (U.S. Geological Survey, Office of Surface Water, 2002).

8.4. Annual Maintenance

1. Disassemble the meter completely: remove bucket wheel, shaft, and pivot.
2. Clean all parts with a lint-free cloth dampened with instrument oil.
3. Inspect the shaft for straightness by rolling on a flat surface.
4. Replace worn or damaged parts.
5. Reassemble and adjust the pivot per the manufacturer's instructions.
6. Perform a spin test to verify performance.
7. Record maintenance date, parts replaced, and spin test results in the instrument log (U.S. Geological Survey, Office of Surface Water, 2002).

8.5. Pivot Adjustment

1. Hold the meter inverted with the pivot pointing up.
2. Release the keeper screw and unscrew the pivot-adjusting nut a few turns.
3. Advance the pivot-adjusting nut until it touches the yoke.
4. Set end play to approximately 0.001 in – the shaft should have just barely perceptible vertical play detectable by feel.
5. Tighten the keeper screw to lock the adjustment.

8.6. Storage

- Store meters in their waterproof carrying cases.
- Loosen the raising nut to relieve pressure on the pivot.
- Ensure meters are clean and lightly oiled.
- Store in a dry location away from dust and vibration.
- If a meter will not be used for more than 3 months, remove the bucket wheel assembly and store separately.

9. Rating Tables

Each meter ships with a USGS-approved standard rating table that converts bucket-wheel revolutions per second to velocity in ft/s or m/s (Rantz et al., 1982; Turnipseed & Sauer, 2010). Keep a laminated copy in each meter's carrying case and a digital copy on the lab shared drive.

- Type AA rating table: https://prph2o.com/usgs-type-aa-current-meter/
- Pygmy rating table: https://prph2o.com/usgs-pygmy-current-meter/

9.1. Meter Calibration Verification

USGS studies have found that approximately 35% of Type AA meters and 20% of Pygmy meters in routine field use fail to meet standard rating criteria, typically because of worn pivots, bent shafts, or damaged contacts (Rantz et al., 1982). The spin test (§7.2) is the primary field check.

10. Ordering Parts and Supplies

Primary supplier for USGS-type Price AA and Pygmy current meters and replacement parts:

Performance Results Plus, Inc. Website: https://prph2o.com

10.1. Replacement Parts to Stock

Part	Qty to Stock	Fits
Pivot (tungsten carbide tip)	2	Both
Shaft	1 per type	AA or Pygmy
Bucket wheel assembly	1 per type	AA or Pygmy
Bucket wheel hub nut	2	Both
Raising nut	1 per type	AA or Pygmy
Cat-whisker wire and solder bead	2 sets	AA
Instrument oil	1 bottle	Both
Pivot bearing	2	Both

11. Troubleshooting

Problem	Probable Cause	Corrective Action
Spin time below minimum	Dirty or worn pivot; bent shaft; dry bearings	Clean pivot; inspect for burrs; oil bearings; replace pivot or shaft if damaged
Counter not registering	Loose cable; worn cat-whisker; dead counter battery	Check cable; replace contact wire; replace battery
Bucket wheel wobbles	Bent shaft or frame	Replace shaft; if hub wobbles, replace bucket wheel assembly

Problem	Probable Cause	Corrective Action
Meter reads high	Magnetized pivot; worn bearing	Demagnetize pivot; replace pivot bearing
Meter reads low	Excessive friction; over-oiled	Clean and re-oil; replace pivot if burred

12. References

- Rantz et al. (1982)
- Turnipseed & Sauer (2010)
- U.S. Geological Survey, Office of Surface Water (2002)

Rantz, S. E. et al. (1982). *Measurement and computation of streamflow: Volume 1. Measurement of stage and discharge* (Water-Supply Paper 2175). U.S. Geological Survey. https://pubs.usgs.gov/wsp/wsp2175/

Turnipseed, D. P., & Sauer, V. B. (2010). *Discharge measurements at gaging stations* (Techniques and Methods 3-A8). U.S. Geological Survey. https://doi.org/10.3133/tm3A8

U.S. Geological Survey, Office of Surface Water. (2002). *Maintenance of Price type AA current meters.* U.S. Geological Survey. https://water.usgs.gov/osw/pubs/9906attachments.pdf

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site equipment cleaning
F202	SonTek Acoustic Velocity Meters	Acoustic alternative
F301	Stream Discharge Measurement	Primary application of this instrument

14. Training Record

Before handling or using current meters independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator assemble, spin-test, and clean both meter types
4. Successfully perform assembly and spin test under supervision
5. Read SOP-F301 before using meters in the field
6. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F202

SonTek Acoustic Velocity Meters

Standard Operating Procedure

SOP Number	SOP-F202
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Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the operation, calibration verification, and maintenance of SonTek acoustic Doppler velocity meters used for point velocity and discharge measurements in wadeable streams. Acoustic Doppler instruments measure water velocity by transmitting ultrasonic pulses and detecting the frequency shift of sound reflected by suspended particles in the water column (Rehmel, 2007). Unlike mechanical current meters (SOP-F201), acoustic meters have no moving parts, respond faster to turbulent fluctuations, and can measure very low velocities.

2. Scope

- **Applies to:** SonTek FlowTracker2 and FlowTracker (original model) handheld acoustic Doppler velocimeters (ADVs) used for wading discharge measurements in streams and channels
- **Does not apply to:** SonTek ADP/ADCP profiling instruments for non-wadeable rivers or boat-mounted surveys; mechanical current meters (see SOP-F201); fixed-installation acoustic flow meters in pipes or culverts
- **Location:** All EA Program wadeable stream sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument deployment; ensure firmware is current
Trained Personnel	Operate instrument per this SOP; download and archive data
New Students	Complete training before independent use

4. Definitions

- **ADV (acoustic Doppler velocimeter):** An instrument that measures water velocity at a single point by transmitting ultrasonic pulses and measuring the Doppler frequency shift of sound scattered back from suspended particles in a small sampling volume located at a fixed distance from the transducer head (Rehmel, 2007).
- **Averaging time:** The duration over which velocity samples are collected and averaged at each measurement vertical; the FlowTracker default is 40 seconds; increase to 60 seconds in low-velocity or highly turbulent flows to improve measurement stability (SonTek/Xylem, 2019).
- **Beam check:** A pre-measurement quality check in which the FlowTracker2 tests all three acoustic beams and reports signal-to- noise ratio (SNR) for each; all beams must return SNR > 4 dB before a measurement can proceed (SonTek/Xylem, 2019).
- **Boundary flag:** An automated QC flag set by the FlowTracker when the probe is too close to the streambed or water surface for the sampling volume to be fully within the water column; corrected by adjusting the depth setting.

- **Doppler effect:** The change in frequency of a wave (sound or light) observed when the source or the reflector is in motion relative to the observer; the physical basis of acoustic Doppler velocity measurement – the frequency shift of reflected ultrasound is proportional to the component of particle velocity along the acoustic beam axis (Rehmel, 2007).
- **FlowTracker2:** The current production SonTek handheld ADV for wading discharge measurements; uses three acoustic beams and integrated pressure depth sensor; stores data internally and transfers via USB; includes automated QA/QC flagging (SonTek/Xylem, 2019).
- **Mid-section method:** The standard USGS discharge calculation method in which each vertical's discharge is the product of measured velocity, depth, and the horizontal width extending halfway to the adjacent verticals on each side; computed automatically by the FlowTracker (Turnipseed & Sauer, 2010).
- **MMT file:** The proprietary binary data file format produced by the SonTek FlowTracker; stores raw velocity data, QC flags, site information, and the computed discharge summary; must be archived before exporting to CSV.
- **Sampling volume:** The small water volume approximately 10 cm from the FlowTracker transducer head from which velocity data are collected; must be free of boundary interference (streambed, water surface, or large obstacles) for valid measurements (SonTek/Xylem, 2019).
- **SNR (signal-to-noise ratio):** The ratio of acoustic signal strength returned from suspended particles to background noise; expressed in dB; the FlowTracker requires SNR > 4 dB on all three beams for a valid measurement; low SNR typically indicates insufficient suspended particles in very clear water (SonTek/Xylem, 2019).
- **Spike flag:** An automated QC flag set by the FlowTracker when a single velocity sample deviates significantly from the running mean, indicating a transient disturbance (bubble, debris, wading disturbance); the affected sample is excluded from the average.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Wading in flowing water carries drowning risk. Always work in pairs. Do not wade in flows deeper than waist height or where footing is unstable.
- **Electrical:** The FlowTracker uses internal rechargeable batteries. Do not open the sealed housing in the field.
- **Biological:** Waterborne pathogens; decontaminate equipment between sites per SOP-F007.

5.1. Required PPE

☐ Chest or hip waders with felt or studded soles
☐ Closed-toed shoes or wading boots
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

A minimum of two people must be present for all in-stream work. Refer to SOP-F001 Field Safety for general emergency procedures.

6. Instrument Specifications

6.1. FlowTracker2

Parameter	Value
Measurement principle	Acoustic Doppler velocimetry (ADV)
Velocity range	0.001–4.0 m/s (0.003–13 ft/s)
Velocity accuracy	±1% of measured velocity
Velocity resolution	0.0001 m/s
Sampling volume	Located 10 cm from the transducer
Depth sensor	Integrated pressure sensor, 0–5 m range
Data storage	Internal memory; USB download
Battery	Rechargeable lithium-ion; ~12 hr continuous
QC diagnostics	Automated QA/QC (SNR, boundary, flow angle, spike detection)

(SonTek/Xylem, 2019)

6.2. FlowTracker (Original)

Parameter	Value
Measurement principle	Acoustic Doppler velocimetry (ADV)
Velocity range	0.001–4.5 m/s
Velocity accuracy	±1% of measured velocity
Sampling volume	Located 10 cm from the transducer
Data storage	Internal memory; RS-232 download
Battery	3 C-cell alkaline batteries

7. Equipment and Materials

Item	Notes
SonTek FlowTracker2 (or original)	Charged and firmware updated (SonTek/Xylem, 2019)
Top-setting wading rod	Standard USGS-type; FlowTracker mounts on the rod

Item	Notes
Mounting bracket and thumbscrew	Specific to SonTek; included with instrument
USB cable	For data download (FlowTracker2)
RS-232 cable and adapter (original model)	For data download
Measuring tape (50 m)	For establishing the tag line
Tape stakes (2)	For anchoring the tag line
Laptop or tablet with SonTek software	FlowTracker2 uses SonTek's desktop software
Field data sheet (backup)	Printed on waterproof paper
Spare batteries	C-cells for original model

8. Procedure

8.1. Pre-Field Setup

1. Charge the FlowTracker2 battery fully before each field day (SonTek/Xylem, 2019). For the original model, install fresh batteries.
2. Power on the instrument and verify that the firmware version is current. Update if prompted.
3. Set the site information: site name, station ID, date, and operator initials.
4. Select the measurement method:
   - **Mid-section** (default, consistent with USGS practice (Turnipseed & Sauer, 2010))
   - **Mean-section** (if required by the project)
5. Set the measurement parameters:
   - Averaging time: 40 seconds (default; increase to 60 seconds for low or turbulent flows) (SonTek/Xylem, 2019)
   - Number of measurements per vertical: 1 (six-tenths method) or 2 (two-point method at 0.2 and 0.8 depth) (Turnipseed & Sauer, 2010)

8.2. Beam Check

Perform a beam check before each measurement (SonTek/Xylem, 2019).

1. Submerge the probe head at least 15 cm below the water surface in a location with minimal turbulence.
2. On the FlowTracker2, select **QC > Beam Check** from the menu.
3. The instrument tests all three acoustic beams and reports SNR for each beam.
4. All beams must report SNR > 4 dB. If any beam fails, clean the transducer faces and retest. If the beam check still fails, do not proceed with the measurement.
5. Record the beam check result in the field notebook.

8.3. Site Selection and Tag Line

Follow the site selection and tag line procedures described in SOP-F301 Stream Discharge Measurement, Sections 7.1–7.3 (Turnipseed & Sauer, 2010). The same cross-section criteria apply to acoustic and mechanical meters.

8.4. Making the Measurement

1. Mount the FlowTracker probe on the wading rod at the correct depth setting:
   - **Six-tenths method:** Set the probe at 0.6 of the total depth below the water surface (same as for mechanical meters)
   - **Two-point method:** Measure at 0.2 and 0.8 of the depth
2. At each vertical, the instrument automatically records velocity (Vx, Vy components) and runs QC checks (SNR, spike detection, boundary check, flow angle).
3. If a measurement is flagged, the instrument displays the reason. Common flags and responses:

Flag	Meaning	Response
Low SNR	Insufficient suspended particles	Increase averaging time; stir bed gently upstream
Boundary	Probe too close to bed or surface	Adjust depth setting
High flow angle	Flow not perpendicular to tag line	Reposition tag line or note angle
Spike	Velocity spike detected	Repeat measurement

(SonTek/Xylem, 2019)

4. After completing all verticals, the instrument computes total discharge automatically using the mid-section method (Turnipseed & Sauer, 2010).
5. Review the on-screen discharge summary before leaving the site.

8.5. Post-Measurement

1. Save the measurement file on the instrument.
2. Record a backup note in the field notebook: site, date, total discharge, number of verticals, and any flagged stations.
3. Rinse the probe with clean water. Do not use solvents or abrasives on the transducer faces.
4. Decontaminate per SOP-F007 before moving to the next site.

8.6. Data Download

1. Connect the FlowTracker2 to a laptop via USB.
2. Open the SonTek desktop software and download all measurement files (SonTek/Xylem, 2019).
3. Export each measurement as a `.csv` and a `.dis` (discharge summary) file.
4. Save files using the naming convention: `YYYY-MM-DD_SiteID_discharge_FT2.csv`
5. Upload to the project GitHub repository under `data/discharge/` within 48 hours.
6. Archive the raw `.mmt` file to the lab shared drive.

9. Maintenance

9.1. After Each Field Day

1. Rinse the entire probe head and cable with clean fresh water.
2. Dry with a lint-free cloth.
3. Inspect the transducer faces for scratches, algae buildup, or debris. Clean gently with a soft brush if needed.
4. Charge the battery (FlowTracker2) or remove batteries (original model) if storing for more than one week.

9.2. Periodic Maintenance

- **Monthly (active use):** Inspect the wading rod mounting bracket for wear. Verify firmware version.
- **Annually:** Contact SonTek for factory calibration verification if the instrument is used for regulatory or compliance monitoring (Rehmel, 2007).

10. Quality Control

- Beam check before every measurement (§7.2) (SonTek/Xylem, 2019).
- If two measurements are made at the same cross-section, they should agree within 5%; if they differ by more than 5%, make a third measurement and use the mean of the two closest values (Turnipseed & Sauer, 2010).
- Compare FlowTracker results with mechanical meter results at least once per year; results should agree within 5–8% (Rehmel, 2007).
- Review all automated QC flags during data processing. Do not discard flagged data without documentation.

11. References

- SonTek/Xylem (2019)
- Turnipseed & Sauer (2010)
- Rehmel (2007)

Rehmel, M. S. (2007). *Application of acoustic Doppler velocimeters for streamflow measurements* (Scientific Investigations Report 2007-5091). U.S. Geological Survey. https://doi.org/10.3133/sir20075091

SonTek/Xylem. (2019). *FlowTracker2 handheld ADV technical manual*. https://www.sontek.com/flowtracker2

Turnipseed, D. P., & Sauer, V. B. (2010). *Discharge measurements at gaging stations* (Techniques and Methods 3-A8). U.S. Geological Survey. https://doi.org/10.3133/tm3A8

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site cleaning
F201	Pygmy and Type AA Velocity Meters	Mechanical alternative
F301	Stream Discharge Measurement	Cross-section procedures

13. Training Record

Before using SonTek velocity meters independently, personnel must:

1. Read this SOP and SOP-F301 in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform a complete discharge measurement including beam check, measurement, and data download
4. Perform a supervised discharge measurement with results that agree within 5% of the trainer's independent measurement
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F203

HOBO Water Level Pressure Transducers

Standard Operating Procedure

SOP Number	SOP-F203
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment, data retrieval, and barometric compensation of Onset HOBO water level loggers for continuous monitoring of water surface elevation in streams, lakes, wetlands, and groundwater wells (Freeman et al., 2004; Onset Computer Corporation, 2020). Continuous stage records are essential for developing stage-discharge rating curves, characterizing hydrologic response to storms, and documenting seasonal water table fluctuations.

2. Scope

- **Applies to:** Onset HOBO U20, U20L, and MX2001 water level loggers deployed at EA Program surface water and groundwater monitoring sites
- **Does not apply to:** Real-time telemetered stage recorders; acoustic or radar level sensors; staff gauge readings (visual observations recorded in the field notebook)
- **Location:** All EA Program hydrologic monitoring sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve deployment locations and logging intervals
Trained Personnel	Deploy, retrieve, and download loggers per this SOP
New Students	Complete training before independent use

4. Definitions

- **Absolute pressure:** Pressure measured relative to a perfect vacuum; the HOBO U20L measures absolute pressure (water column plus atmospheric), which must be compensated to isolate water-column pressure (Freeman et al., 2004).
- **Barometric compensation:** Post-processing step in HOBOware that subtracts a concurrent barometric pressure record (from a reference logger deployed in air) from the absolute pressure record to yield true water depth (Onset Computer Corporation, 2021).
- **Datum:** A fixed reference elevation (e.g., NAVD88, local benchmark) to which stage measurements are referenced.
- **Deployment depth:** The vertical distance from the water surface to the sensor face at the time of deployment; used as the initial reference for water-level calculations.
- **HOBO U20L:** Onset Computer Corporation submersible water-level logger recording absolute pressure and temperature at user-defined intervals; rated to a maximum depth of 9 m (model U20L-01) or 4 m (model U20L-04 barometric) (Onset Computer Corporation, 2020).
- **HOBOconnect / HOBOware:** Onset software (mobile app and desktop, respectively) used to program loggers, download data, apply barometric compensation, and export time-series data (Onset Computer Corporation, 2021).
- **Stage:** Water-surface elevation at a monitoring location, expressed relative to a datum; the primary hydrologic quantity derived from pressure transducer records.

- **Stage-discharge rating curve:** An empirical relationship between stream stage and discharge (Q) at a gauging cross-section; enables conversion of continuous stage records into continuous discharge records (Freeman et al., 2004).
- **Stilling well:** A perforated pipe anchored in the stream or lake bed that protects the logger from direct current, debris, and disturbance while allowing free water exchange.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Deployment sites may involve steep banks, slippery surfaces, swift water, or confined spaces (well casings). Work in pairs at all stream and well sites.
- **Drowning:** Do not deploy loggers during high-flow events. Never wade in flows deeper than waist height.
- **Biological:** Waterborne pathogens; ticks and snakes in riparian areas.

5.1. Required PPE

☐ Wading boots or chest waders (stream deployments)
☐ Closed-toed shoes (well deployments)
☐ Nitrile gloves
☐ Sun protection (hat, sunscreen)

5.2. Required Training

- SOP-F001 Field Safety
- Work with a trained partner for all in-stream deployments

6. Instrument Specifications

6.1. HOBO U20L Water Level Logger

Parameter	Value
Measurement principle	Absolute pressure (water + atmosphere)
Pressure range	0–9 m (U20L-01) or 0–4 m barometric (U20L-04)
Accuracy	+/- 0.1% full scale (~0.5 cm water)
Logging interval	1 second to 18 hours (user-configurable)
Memory	~21,700 combined readings
Battery life	~5 years at 1-minute logging
Communication	Optical USB coupler (U20L)

6.2. HOBO MX2001 Water Level Logger

Parameter	Value
Communication	Bluetooth Low Energy; download via HOBOconnect app
Pressure range	0–9 m or 0–4 m models
Other specs	Similar to U20L

6.3. Barometric Reference Logger

Because the water level loggers measure absolute pressure, a companion barometric pressure logger must be deployed above the water surface at or near the site. An Onset HOBO U20L-04 barometric model or HOBO MX barometric logger serves this purpose.

7. Equipment and Materials

Item	Storage	Notes
HOBO U20L or MX2001 loggers	SGM 133	Check battery before deployment
Optical USB coupler (U20L)	SGM 133	For HOBOware download
HOBOconnect app (MX2001)	Phone	Requires Bluetooth pairing
Stilling well (PVC, 2 in)	SGM 132	With end cap and perforations
Stainless steel cable / line	SGM 131	For securing logger in well
Staff gauge (if not installed)	SGM 131	Painted, graduated to 0.01 m
HOBOware laptop	SGM 133	Pre-loaded with HOBOware Pro
GPS unit	SGM 133	See SOP-F006
Field data sheet	SGM 133	

8. Estimated Time

Task	Time
Logger programming (HOBOware)	10–15 min
Stilling well installation (new)	30–90 min
Logger deployment (existing site)	15–30 min
Data retrieval and export	15–30 min
Barometric compensation (HOBOware)	15–30 min

9. Procedure

9.1. Logger Programming

1. Connect the logger to HOBOware using the optical USB coupler or open HOBOconnect via Bluetooth.

2. Select **Launch** (HOBOware) or **Configure** (HOBOconnect).
3. Set the logging interval appropriate to the project (5 min is typical for hydrologic studies; 15 min for long-term deployment).
4. Enable a delayed start if synchronizing with another logger at the site.
5. Record the logger serial number, launch time, and interval in the field notebook and in the deployment spreadsheet.

9.2. Stream Deployment (Stilling Well)

1. Choose a stable bank location away from direct turbulence but representative of the cross-section stage.
2. Install the stilling well (2-inch PVC with perforations) vertically with the bottom anchored and the top above expected high-water level. Ensure the perforations face upstream and downstream.
3. Lower the logger into the stilling well and secure it with stainless steel wire or cable tie. The logger should hang freely.
4. Record the deployment elevation:
    - Distance from the top of the stilling well to the water surface and to the logger sensor
    - Staff gauge reading (if installed)
    - GPS coordinates per SOP-F006
5. Deploy the barometric reference logger nearby, above the high-water line, in a shaded and ventilated location (e.g., inside a radiation shield attached to a tree or post). Do not seal it in an airtight container.
6. Photograph the deployment.

9.3. Well Deployment

1. Remove the well cap and measure depth to water using an e-tape. Record the measurement.
2. Lower the logger into the well on stainless steel cable or braided fishing line. Position below the lowest expected water level but above the well screen bottom.
3. Secure the suspension cable to the well cap or casing.
4. Record the logger depth below the top of casing (TOC) and the TOC elevation if surveyed.
5. Replace the well cap.
6. Deploy the barometric reference logger at the wellhead or nearby.

9.4. Data Retrieval

Retrieve data at intervals appropriate for the project (typically monthly or quarterly).

U20L (optical coupler):

1. Remove the logger from the stilling well.
2. Attach the optical USB coupler and connect to the laptop.
3. In HOBOware, select **Readout**. The logger continues recording after readout.
4. Return the logger to its deployment position.

MX2001 (Bluetooth):

1. Open the HOBOconnect app on a smartphone within ~30 m line of sight.
2. Select the logger and tap **Readout**.
3. The logger does not need to be removed from the water.

9.5. Barometric Compensation

1. In HOBOware, open the water level logger file and the barometric reference logger file.
2. Select **Data Assistant > Barometric Compensation**.
3. Specify the reference water level: enter a staff gauge reading or a known water surface elevation at a specific date and time.
4. HOBOware subtracts the barometric pressure from the absolute pressure to yield the water column height.
5. Export the compensated data as `.csv`.
6. Save using the convention: `YYYY-MM-DD_SiteID_waterlevel_compensated.csv`.
7. Commit data to the project repository per SOP-L801.

10. Quality Control

- Verify that the barometric logger file overlaps the full duration of the water level record. Gaps in barometric data prevent compensation.
- Compare the compensated stage at the download visit against a simultaneous staff gauge reading. Acceptable agreement is within 1 cm.
- Flag and investigate any sudden offsets or drift in the compensated record (may indicate logger movement, sedimentation, or battery failure).

11. Data Management

1. Save all raw logger files (`.hobo`) to `data/raw/loggers/` in the project repository.
2. Save compensated `.csv` files to `data/processed/stage/`.
3. Commit all files per SOP-L801.

12. References

Freeman, L. A., Carpenter, M. C., Rosenberry, D. O., Rousseau, J. P., Unger, R., & McLean, J. S. (2004). *Use of submersible pressure transducers in water-resources investigations* (Techniques of Water-Resources Investigations Book 8, Chapter A3). U.S. Geological Survey. https://pubs.usgs.gov/twri/twri8a3/

Onset Computer Corporation. (2020). *HOBO U20L water level logger user's manual*. https://www.onsetcomp.com/files/manual_pdfs/14366-F-MAN-U20L.pdf

Onset Computer Corporation. (2021). *HOBOware user's guide*. https://www.onsetcomp.com/files/manual_pdfs/10246-K-MAN-HOBOware.pdf

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Deployment coordinates
F007	Field Decontamination	Between-site cleaning
F301	Stream Discharge Measurement	Stage-discharge rating curves
F303	Groundwater Sampling	Well deployment context
L801	RStudio and GitHub	Data version control

14. Training Record

Before deploying water level loggers independently, personnel must:

1. Read this SOP in full
2. Demonstrate logger programming (launch, interval, delayed start) using HOBOware or HOBOconnect
3. Participate in a supervised stream or well deployment
4. Demonstrate barometric compensation in HOBOware and export of compensated data
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F204

HOBO Weather Station Deployment

Standard Operating Procedure

SOP Number	SOP-F204
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment, programming, data retrieval, and maintenance of Onset HOBO pendant and MX-series data loggers used for unattended monitoring of air temperature, relative humidity, and light intensity at EA Program field sites. These compact loggers provide continuous microclimatic data for characterizing site conditions, supporting ecological studies, and supplementing manual weather observations (Onset Computer Corporation, 2023; World Meteorological Organization, 2018).

2. Scope

- **Applies to:** HOBO Pendant MX2201, MX2202 (temperature/light), HOBO MX2301 (temperature/RH), HOBO Pendant UA-002 (temperature/ light, legacy), and similar Onset loggers deployed at EA Program field sites
- **Does not apply to:** HOBO water level loggers (see SOP-F203); fixed, permanently installed weather stations managed by other departments; air quality sensors (see SOP-F502)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve deployment plan and logging interval
Trained Personnel	Deploy, retrieve, and download loggers per this SOP
New Students	Complete training before independent use

4. Definitions

- **Bluetooth logging:** A wireless communication protocol used by MX-series HOBO loggers; enables programming and data download via the HOBOconnect smartphone app without cables; requires proximity (typically within 15 m of the logger) (Onset Computer Corporation, 2023).
- **Data gap:** A period within a logger deployment during which no data were recorded; caused by battery failure, logger reset, or memory overflow; must be documented in the project data log.
- **HOBOconnect:** The Onset smartphone app (iOS and Android) used to program, launch, and download data from Bluetooth-enabled MX-series HOBO loggers; requires the logger to be within Bluetooth range (Onset Computer Corporation, 2021).
- **HOBOware:** The Onset desktop software used to program and download data from legacy UA-series HOBO loggers via optical USB coupler; also supports barometric compensation for water level loggers (Onset Computer Corporation, 2021).
- **Logging interval:** The time between successive measurements stored to the logger memory; typical intervals for EA Program deployments are 5 minutes (microclimatic studies) or 15 minutes (long-term monitoring); shorter intervals fill memory faster and require more frequent downloads (Onset Computer Corporation, 2023).
- **MX2301:** The Onset HOBO MX temperature and relative humidity logger; Bluetooth-enabled; ±0.2 °C temperature accuracy and ±2.5% RH accuracy; internal memory stores 500,000 readings (Onset Computer Corporation, 2023).
- **Radiation shield:** A ventilated, white polypropylene housing that surrounds a temperature or RH logger and prevents direct solar radiation from inflating the measured air temperature; a radiation shield is mandatory for any logger exposed to sunlight (World Meteorological Organization, 2018).
- **Relative humidity (RH):** The ratio of the partial pressure of water vapor in air to the saturation vapor pressure at the same temperature, expressed as a percentage; values above

approximately 70% promote sensor drift in some sensor types (World Meteorological Organization, 2018).

- **Sensor drift:** A gradual, systematic change in sensor readings over time not attributable to true environmental change; detected by co-deployment with a reference sensor or comparison with a nearby weather station.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Deployment may involve climbing, ladder use, or working on uneven terrain. Use a spotter when mounting loggers at height.
- **Environmental:** Heat exposure during summer deployments in Southern California. Carry adequate water and sun protection.
- **Wildlife:** Insects (bees, wasps), ticks, and snakes may be present at deployment sites.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Sun protection (hat, sunscreen)
☐ Nitrile gloves (when handling loggers at contaminated sites)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Instrument Specifications

Model	Parameters	Range	Accuracy	Memory	Communication
MX2201	Temp, light	-20 to 70 °C; 0–320,000 lux	±0.5 °C	96,000 readings	Bluetooth
MX2202	Temp, light (waterproof)	-20 to 70 °C	±0.5 °C	96,000 readings	Bluetooth
MX2301	Temp, RH	-40 to 70 °C; 0–100% RH	±0.2 °C; ±2.5% RH	500,000 readings	Bluetooth
UA-002-64	Temp, light (legacy)	-20 to 70 °C	±0.53 °C	64,000 readings	Optical USB

(Onset Computer Corporation, 2023)

7. Equipment and Materials

Item	Notes
HOBO logger(s)	Confirm model and battery status
Radiation shield (RS3 or similar)	Required for temperature loggers exposed to direct sunlight (World Meteorological Organization, 2018)
Mounting hardware (cable ties, U-bolts, brackets)	For securing shield/logger to posts, trees, or tripods
Tripod or mounting post	If no suitable tree or structure available
Smartphone with HOBOconnect app	For Bluetooth-enabled MX loggers (Onset Computer Corporation, 2021)
Laptop with HOBOware software	For legacy UA-series loggers (Onset Computer Corporation, 2021)
Optical USB coupler (for UA loggers)	For launching and downloading
Field notebook	For recording deployment metadata
GPS unit or smartphone	Per SOP-F006
Spare batteries	CR2032 (MX pendant) or 3V lithium (varies by model)

8. Procedure

8.1. Pre-Deployment Programming

MX-series (Bluetooth):

1. Open the HOBOconnect app and connect to the logger via Bluetooth (Onset Computer Corporation, 2021).
2. Configure:
   - Logging interval: 5 or 15 minutes
   - Start mode: delayed start (set to a round time, e.g., top of the next hour)
   - Logger name: use the convention `SiteID_Parameter_LoggerSN` (e.g., `BFS_TempRH_20456789`)
3. Verify the battery level. Replace if below 20%.

UA-series (legacy):

1. Connect the logger via the optical USB coupler and open HOBOware.
2. Select **Launch** and configure the logging interval and start time (Onset Computer Corporation, 2021).
3. Click **Start** to begin logging.

8.2. Deployment

1. Mount the radiation shield at 1.5 m above ground on a post, tree, or tripod in a location representative of the site. Avoid positions within 3 m of heat-absorbing surfaces (pavement, metal walls) (World Meteorological Organization, 2018).
2. Insert the logger into the shield. Ensure the sensor faces are unobstructed and the shield ventilation holes are unblocked.
3. Secure the shield to the mounting structure with cable ties.
4. Record in the field notebook:
   - Logger serial number
   - Deployment coordinates per SOP-F006
   - Date and time of deployment
   - Logging interval and start time
   - Sensor height above ground
5. Photograph the deployed logger with surrounding context visible.

8.3. Downloading Data at Regular Intervals

MX-series (Bluetooth):

1. Open HOBOconnect app and connect to the logger.
2. Select **Download** to transfer all stored data to the smartphone (Onset Computer Corporation, 2021).
3. Export the data as a CSV file.
4. Save using the naming convention: `YYYY-MM-DD_SiteID_Parameter_LoggerSN.csv`
5. Upload to the project GitHub repository under `data/microclimate/` within 48 hours.

UA-series (legacy):

Connect via optical USB coupler and use HOBOware to read out data.

8.4. Logger Retrieval (End of Deployment)

1. Download all remaining data.
2. Remove the logger and mounting hardware.
3. Clean the logger housing with a damp cloth. For waterproof models, rinse with fresh water.
4. Inspect the radiation shield for damage; replace desiccant packs if applicable.
5. Store loggers in a dry, room-temperature location.

9. Quality Control

- At each download visit, compare the logger temperature reading against a handheld thermometer. Discrepancies greater than 1 °C should be investigated (World Meteorological Organization, 2018).

- At least once per year, co-deploy two loggers of the same model at the same location for a minimum of 48 hours to check inter-unit agreement. Temperature should agree within 0.5 °C; RH within 5%.
- Inspect data for signs of sensor failure: constant readings, sudden offsets, or readings pegged at the sensor limit.
- Check battery level at every download. Replace batteries proactively if below 20%.
- For light loggers, verify that the sensor face has not shifted orientation by comparing the deployment photograph to current position.

10. References

- Onset Computer Corporation (2023)
- Onset Computer Corporation (2021)
- World Meteorological Organization (2018)

Onset Computer Corporation. (2021). *HOBOware user's guide*. https://www.onsetcomp.com/files/manual_pdfs/10246-K-MAN-HOBOware.pdf

Onset Computer Corporation. (2023). *HOBO U23 Pro v2 external temperature data logger: User manual*. https://www.onsetcomp.com/products/data-loggers/u23-004

World Meteorological Organization. (2018). *Guide to meteorological instruments and methods of observation (WMO-no. 8)* (2018th ed.). World Meteorological Organization. https://library.wmo.int/idurl/4/41650

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Deployment coordinates
F203	HOBO Water Level Loggers	Companion instrument SOP
F510	Weather Monitoring	Full meteorological context
L801	RStudio and GitHub	Data version control

12. Training Record

Before deploying HOBO loggers independently, personnel must:

1. Read this SOP in full
2. Demonstrate logger programming via HOBOconnect or HOBOware
3. Participate in a supervised deployment including shield mounting and metadata recording
4. Demonstrate data download, export, and upload to the repository
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F205

Handheld Turbidity Meter

Standard Operating Procedure

SOP Number	SOP-F205
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the calibration, operation, and maintenance of handheld nephelometric turbidity meters for field measurement of water clarity. Turbidity is a standard water quality parameter required by the Surface Water Ambient Monitoring Program (SWAMP), NPDES permits, and many ecological studies (APHA et al., 2012). Field measurements complement laboratory analysis by providing an immediate on-site result.

2. Scope

- **Applies to:** Handheld nephelometric turbidity meters (e.g., Hach 2100Q, LaMotte 2020we, Oakton T-100) used for in situ and grab-sample turbidity measurements at EA Program field sites
- **Does not apply to:** Laboratory bench-top turbidimeters; Secchi disk transparency measurements (see SOP-F207); continuous turbidity sensors on multiparameter sondes (see SOP-F200)
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument selection; review QC data
Trained Personnel	Calibrate and operate the meter per this SOP
New Students	Complete training before independent use

4. Definitions

- **Formazin:** A synthetic polymer suspension used as the primary turbidity calibration standard; traceable to NIST; typically supplied as a 4000 NTU stock that is diluted to working standards; contains hydrazine and must be handled as hazardous waste (APHA et al., 2012).
- **Nephelometric turbidity:** Turbidity measured by the intensity of light scattered at 90° to an incident beam; the standard method per APHA Method 2130B; appropriate for samples with NTU < 40; for higher values a ratio or attenuation mode may be used (APHA et al., 2012).
- **NTU (Nephelometric Turbidity Units):** The unit of turbidity measured by a 90° nephelometer; numerically equivalent to FNU (Formazin Nephelometric Units) and approximately equivalent to FAU (Formazin Attenuation Units) at low concentrations (APHA et al., 2012).
- **RPD (relative percent difference):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptance criterion for field turbidity duplicates is RPD $< 20\%$ for readings above 10 NTU.
- **Silicone oil treatment:** Application of a thin film of silicone oil to the outside of a glass sample vial before measurement to fill surface scratches and reduce spurious light scattering; standard practice for glass vial meters (Hach Company, 2022).
- **StablCal:** A sealed formazin-based calibration standard supplied by Hach; stable and ready to use without dilution; available in $<$ 0.1, 20, 100, and 800 NTU vials.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Sampling near water involves slip and fall hazards.
- **Biological:** Water samples may contain pathogens. Wear gloves.
- **Chemical:** Formazin calibration standards contain hydrazine, a suspected carcinogen. Handle with gloves; dispose of as hazardous waste. StablCal sealed standards eliminate direct hydrazine exposure.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (during calibration with bulk formazin)
☐ Closed-toed shoes or wading boots

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. For formazin contact with skin or eyes, flush with water for at least 15 minutes and consult the SDS.

6. Equipment and Materials

Item	Notes
Handheld turbidity meter	Confirm model and battery level
Calibration standards	Hach StablCal sealed vials: < 0.1, 20, 100, 800 NTU; or prepared formazin dilution series
Sample vials (matched to instrument)	Glass or optical-grade; use only with the meter they were supplied with
Silicone oil and applicator cloth	For glass vials (Hach Company, 2022)
Lint-free wipes (Kimwipes)	For cleaning vials
DI water (squeeze bottle)	For rinsing vials and blanks
Waste container (labeled hazardous)	For used formazin waste
Field notebook	Per SOP-F003
Spare batteries	Matched to meter model

7. Procedure

7.1. Calibration

Calibrate at the start of each field day or after storage of more than one week (APHA et al., 2012).

1. Turn on the meter and allow it to warm up (approximately 1 minute).
2. Clean calibration vial exteriors with a lint-free wipe. Apply a thin, even film of silicone oil for glass vials.
3. Insert the lowest standard (< 0.1 NTU) into the sample chamber with the orientation mark aligned. Close the lid.
4. Press **Cal** and follow the on-screen prompts to read the standard.
5. Repeat with each subsequent standard in ascending order (e.g., 20, 100, 800 NTU).
6. When all standards have been read, the meter confirms a successful calibration. Record the date, standard lot number, and any correction factors in the field notebook.
7. Verify calibration by re-reading the 20 NTU standard; the reading must be within ±10% of 20 NTU. If not, repeat the full calibration.

7.2. Sample Collection

1. Collect a grab sample per SOP-F300 Surface Water Sampling.
2. Avoid disturbing bottom sediments during collection.
3. Analyze as soon as possible; if delayed, keep the sample in the dark and analyze within 24 hours.

7.3. Measurement

1. Gently invert the sample container 3–4 times to resuspend particles uniformly. Do not shake vigorously (creates air bubbles).
2. Pour the sample into a clean sample vial to the fill line.
3. Cap and let sit for 1 minute to allow large air bubbles to rise.
4. Wipe the outside of the vial with a lint-free cloth. Apply silicone oil for glass vials.
5. Insert the vial with the orientation mark aligned. Close the lid.
6. Press **Read** and wait for the display to stabilize.
7. Record the result (NTU), sample ID, time, and site in the field notebook.
8. For samples above the meter range (typically > 1000 NTU), dilute with DI water, measure, and multiply by the dilution factor. Record both the diluted reading and the corrected result.
9. Rinse the vial three times with DI water between samples.

7.4. Post-Field Care

1. Rinse all sample vials with DI water; air-dry inverted.
2. Wipe silicone oil residue from vials before storage.
3. Store calibration standards in their original case, away from direct sunlight, at room temperature.
4. Turn off the meter; remove batteries if storing for more than one month.

8. Quality Control

- Calibrate at the start of each field day (§6.1) (APHA et al., 2012).

- Measure a DI water blank at the start and end of each sampling event; the blank must read < 1 NTU.
- Measure one field duplicate per 10 samples (minimum one per event); duplicate RPD must be $< 20\%$ for readings above 10 NTU.
- If the DI blank drifts above 1 NTU during the day, recalibrate and re-measure the most recent samples.
- Inspect sample vials for scratches before each use; scratches scatter light and produce false-high readings.

9. References

- APHA et al. (2012)
- Hach Company (2022)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2022). *2100Q portable turbidimeter: User manual*. https://www.hach.com/2100q-portable-turbidimeter/product?id=7640196322

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F207	Secchi Disk	Alternative transparency measure
F300	Surface Water Sampling	Sample collection procedures
L303	Hach Colorimetric Analysis	Lab-based Hach methods

11. Training Record

Before using turbidity meters independently, personnel must:

1. Read this SOP in full
2. Demonstrate a complete calibration using StablCal or formazin standards
3. Perform supervised field measurements at one site
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F207

Secchi Disk and Secchi Disk Tube

Standard Operating Procedure

SOP Number	SOP-F207
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the use of the standard Secchi disk and the Secchi disk tube (transparency tube) for measuring water transparency in lakes, reservoirs, and streams. Secchi depth is one of the oldest and simplest limnological measurements, providing a rapid estimate of the photic zone depth and an index of suspended particle concentration (Carlson, 1977; Wetzel, 2001).

2. Scope

- **Applies to:** Visual transparency measurements in lakes, ponds, and reservoirs (standard Secchi disk) and in streams, shallow wetlands, and stormwater basins (Secchi tube)
- **Does not apply to:** Quantitative turbidity measurements (see SOP-F205); laboratory transmissometry; PAR light profiles (requires an underwater quantum sensor)
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Specify measurement protocol for the project
Trained Personnel	Perform measurements per this SOP
New Students	Complete training before independent use

4. Definitions

- **Euphotic zone depth (z_{eu}):** The depth receiving at least 1% of surface irradiance; approximately 2–3 times the Secchi depth in most lakes; defines the lower boundary of net photosynthesis (Wetzel, 2001).
- **Photic zone:** The surface layer of a water body receiving sufficient light to support photosynthesis; its depth is inversely related to turbidity and Secchi depth.
- **Secchi depth (z_{SD}):** The depth at which a standard Secchi disk becomes just invisible when lowered, averaged with the depth at which it becomes visible again when raised; $z_{SD} = (D_1 + D_2)/2$; an index of water transparency inversely related to suspended particle concentration and dissolved color (Carlson, 1977).
- **Secchi tube (transparency tube):** A clear acrylic tube, typically 60 or 120 cm long, with a Secchi disk pattern printed at the bottom and a drain valve at the base; filled with sample water and drained until the pattern disappears; used in shallow or turbid waters where a standard disk cannot be deployed vertically.
- **Trophic state index (TSI):** A numerical scale relating Secchi depth, chlorophyll a, and total phosphorus to lake trophic status; Carlson's TSI for Secchi depth: $\mathrm{TSI}(SD) = 60 - 14.41 \ln(z_{SD})$ (Carlson, 1977).
- **Viewscope:** A tube, box, or bucket with a transparent bottom used to eliminate surface glare when reading Secchi depth; improves consistency between observers but must be noted and applied consistently within a dataset.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Secchi disk measurements from boats, docks, or bridges require caution to prevent falls. Wear a personal flotation device (PFD) when sampling from boats.
- **Biological:** Surface water may contain pathogens. Avoid ingestion; wear gloves if hands contact water.
- **Sun glare:** Measuring Secchi depth requires looking directly into the water; use polarized sunglasses or a viewscope to reduce glare (note use in the field notebook for consistency).

5.1. Required PPE

- ☐ Closed-toed shoes or wading boots
- ☐ Personal flotation device (boat sampling)
- ☐ Nitrile gloves (recommended for impaired waters)
- ☐ Sun protection (hat, sunglasses)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. If sampling from a boat, ensure all occupants know the location of PFDs and throwable flotation devices.

6. Equipment and Materials

6.1. Standard Secchi Disk

Item	Notes
Standard Secchi disk (20 cm diameter)	Alternating black and white quadrants; weighted to sink horizontally
Graduated line (marked every 0.1 m)	Non-stretch braided cord or chain
Viewscope (optional)	Eliminates surface glare; a capped PVC section with a clear bottom works well

6.2. Secchi Tube (Transparency Tube)

Item	Notes
Secchi tube (60 or 120 cm)	Clear acrylic tube; Secchi disk pattern at bottom; cm scale on side; drain valve at base
Sample bucket or wide-mouth bottle	For collecting water to fill the tube

6.3. General

Item	Notes
Field notebook	Per SOP-F003
Watch	For recording time of measurement
Camera	For documenting site conditions

7. Procedure

7.1. Standard Secchi Disk (Lakes, Reservoirs, Deep Pools)

Take measurements on the shaded side of the boat or dock, between 10:00 and 14:00 local time for consistency. Avoid measurements during heavy cloud cover, rain, or high wind (Wetzel, 2001).

1. Lower the Secchi disk on the shaded side until it just disappears from view. Record this depth as **D1** (disappearance depth).
2. Slowly raise the disk until it just reappears. Record this depth as **D2** (reappearance depth).
3. Calculate Secchi depth:
$$\text{Secchi depth} = \frac{D_1 + D_2}{2}$$
4. Record in the field notebook:
   - Secchi depth (m) and D1, D2 individually
   - Date and time
   - Sky conditions (clear, partly cloudy, overcast)
   - Wind and water surface conditions
   - Whether a viewscope was used
   - Observer name (readings vary between observers)
5. If the disk is visible on the bottom, record "Secchi depth > [bottom depth]" and note the bottom depth.
6. Repeat the measurement a second time; the two readings should agree within 0.5 m. Record both and report the mean.

7.2. Secchi Tube (Streams, Shallow Waters)

1. Collect a water sample from mid-depth using a bucket or wide-mouth bottle; avoid disturbing sediment.
2. Pour the sample into the Secchi tube until full.
3. Hold the tube vertically and look down through the water column at the Secchi pattern on the bottom.
4. Open the drain valve slowly. Watch the Secchi pattern.
5. When the pattern just disappears, close the valve and read the water level on the graduated scale. Record as **Secchi tube depth** (cm).
6. Refill the tube and repeat; the two readings should agree within 2 cm. Record both and report the mean.
7. Rinse the tube with DI water between sites to prevent cross-contamination.

8. Data Interpretation

Secchi depth is inversely related to turbidity and suspended particle concentration (Carlson, 1977; Wetzel, 2001). Approximate trophic-state guidelines for lakes:

Secchi Depth (m)	Trophic Class
> 5	Oligotrophic
2–5	Mesotrophic
1–2	Eutrophic
< 1	Hypereutrophic

> **Note**
>
> These categories are approximate and vary with dissolved color, algal species, and suspended sediment type. Do not use Secchi depth alone to classify trophic state; combine with nutrient and chlorophyll data (Carlson, 1977).

9. Quality Control

- Use the same observer for repeated measurements at a monitoring site whenever possible; inter-observer variability is the largest source of error (Wetzel, 2001).
- Always take duplicate measurements and report the mean.
- Record consistently whether sunglasses or a viewscope were used.
- Verify Secchi tube graduation markings against a ruler at least once per year.
- When both a Secchi disk and a turbidity meter are available, pair the measurements to develop a site-specific Secchi-turbidity regression.

10. References

- Wetzel (2001)
- Carlson (1977)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Carlson, R. E. (1977). A trophic state index for lakes. *Limnology and Oceanography*, *22*(2), 361–369. https://doi.org/10.4319/lo.1977.22.2.0361

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). Academic Press.

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F205	Handheld Turbidity Meter	Quantitative turbidity companion
F300	Surface Water Sampling	Sample collection context
L310	Chlorophyll Extraction	Trophic state companion

12. Training Record

Before performing Secchi measurements independently, personnel must:

1. Read this SOP in full
2. Perform supervised duplicate Secchi disk or Secchi tube measurements at one site
3. Demonstrate agreement within the QC criteria (0.5 m for disk, 2 cm for tube)
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F208

Portable Spectrophotometer / Colorimeter (Field Use)

Standard Operating Procedure

SOP Number	SOP-F208
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the field operation of the Hach DR900 portable colorimeter for on-site water quality analysis (Hach Company, 2024a, 2024b). The DR900 enables rapid, field-based measurement of nutrients, metals, and other parameters using Hach pre-packaged reagent kits. Field colorimetry provides same-day results when samples cannot be transported to the lab within required hold times and supports real-time decisions during stormwater events, spill response, or reconnaissance sampling (APHA et al., 2012).

This SOP focuses on field-specific procedures (transport, outdoor operation, field QC). For detailed method chemistry, reagent handling, the Method Registry, and laboratory use of the DR3900 and DR900, see SOP-L303 Hach Colorimetric Analysis.

2. Scope

- **Applies to:** Field use of the Hach DR900 portable colorimeter at EA Program field sites for grab-sample analysis of nutrients (PO_4, NO_3, NH_3), metals (Fe, Mn, Cu), and general chemistry (hardness, chloride, silica, chlorine)
- **Does not apply to:** Laboratory bench-top analysis (see SOP-L303); field turbidity (see SOP-F205); in situ sonde measurements (see SOP-F200)
- **Location:** All EA Program surface water, groundwater, and stormwater field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Select analytes and approve field methods
Trained Personnel	Operate the DR900 per this SOP; record results
New Students	Complete training on both this SOP and SOP-L303 before independent field use

4. Definitions

- **Beer-Lambert law:** The linear relationship between the absorbance of a solution and the concentration of the absorbing analyte, expressed as $A = \varepsilon c l$, where ε is the molar absorptivity, c is concentration, and l is path length; the physical basis of colorimetric analysis (APHA et al., 2012).
- **Blank (reagent blank / DI blank):** A deionized water sample carried through the complete analytical procedure; used to zero the instrument and establish the baseline absorbance of the reagent system; must read at or near zero before sample measurements begin.
- **Detection limit (MDL):** The minimum concentration reliably distinguishable from the blank; Hach method-specific MDLs are listed in the Method Registry in SOP-L303 (Hach Company, 2024b).
- **Field duplicate:** A second independent measurement of the same sample, prepared through the full analytical procedure, to assess precision; RPD must be $< 20\%$ for concentrations above 5 times the detection limit.
- **Reaction time:** The incubation period specified in the Hach method protocol after reagent addition during which the color-forming reaction proceeds to completion; reading before reaction time has elapsed gives systematically low results (Hach Company, 2024a).
- **RPD (relative percent difference):** |A - B| / ((A + B) / 2) × 100; used to assess duplicate precision.
- **Stored program:** A factory-programmed method on the DR900 that specifies wavelength, calibration curve, and units for a specific Hach reagent kit; accessed by program number on the instrument (Hach Company, 2024a).
- **Zeroing:** The process of inserting a DI water blank into the DR900 sample chamber and pressing Zero to set the baseline absorbance to zero for the selected program; required before reading samples.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Hach reagent powder pillows and liquid reagents may contain acids, oxidizers, or heavy metal indicators. Review the SDS for each kit before field use. Cadmium-containing reagents (older NitraVer 5 formulation) are hazardous waste.
- **Physical:** Glass sample vials can break during transport; pack in padded cases.
- **Biological:** Water samples may contain pathogens. Wear gloves.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (required for all reagent handling)
☐ Closed-toed shoes or wading boots

5.2. Emergency Procedures

For reagent splashes to skin or eyes, flush with clean water for at least 15 minutes. Carry a squeeze bottle of DI water for emergency rinsing. Refer to SOP-F001 Field Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

Item	Notes
Hach DR900 Portable Colorimeter	Charge before field day (Hach Company, 2024a)
Sample vials (10 mL, 25 mL, 1-inch round)	Matched to the methods being run
Hach reagent kits	Check expiration dates; select analytes per project
DI water (squeeze bottle, 500 mL)	For blanks, rinsing, and dilutions
Lint-free wipes (Kimwipes)	For cleaning vial exteriors
Silicone oil and applicator cloth	For 1-inch round vials
Timer	Phone timer or stopwatch for reaction times
Waste container (sealable)	For used reagent vials and waste solution
Padded carrying case	For protecting vials during transport
Field notebook	Per SOP-F003

7. Procedure

7.1. Pre-Field Preparation

1. Identify the analytes to be measured; pack the corresponding reagent kits and verify none are expired.

2. Pack at least 500 mL DI water and two extra clean sample vials per method.
3. Charge the DR900 battery and confirm stored programs for your methods are accessible (Hach Company, 2024a).
4. Print or photograph method procedure cards for each analyte (from the kit box or SOP-L303 Method Registry).

7.2. Field Setup

1. Place the DR900 on a flat, stable surface **out of direct sunlight**. Direct sunlight on the sample chamber causes erroneous readings.
2. If no shade is available, shade the instrument with a jacket or tarp during each reading.
3. Turn on the DR900 and allow it to complete its self-check.
4. Select the stored program for your analyte.

7.3. Blank and Calibration Check

1. Fill a clean sample vial with DI water to the fill line.
2. Wipe the vial exterior with a lint-free cloth.
3. Insert the vial, close the lid, and press **Zero**.
4. The display should read 0.00 (within the method detection limit); if not, clean the vial and re-zero.
5. If a known mid-range standard is available, read it immediately after zeroing; the result must be within ±10% of the stated value. Record the result in the field notebook (Hach Company, 2024b).

7.4. Sample Analysis

Follow the method-specific procedure from the reagent kit or SOP-L303 Method Registry:

1. Collect a grab sample per SOP-F300.
2. Fill the sample vial to the fill line with sample.
3. Add the reagent per method instructions.
4. Cap, invert, and mix as directed. Start the timer for the required reaction time.
5. When the reaction time is complete, wipe the vial exterior (apply silicone oil for round vials).
6. Insert the vial into the DR900, close the lid (shield from sunlight), and press **Read**.
7. Record the result, analyte, Hach method number, sample ID, units, date, and time in the field notebook.
8. Rinse the vial three times with DI water before the next sample.

7.5. Waste Management

1. Collect all used reagent packets, vials, and waste solution in a sealable container.
2. Do not discard reagent waste in the field.
3. Return waste to the lab and dispose of per SOP-L003.

8. Quality Control

- Zero the instrument with DI water before each batch of samples.
- Run one DI blank and one mid-range standard at the start of each field session; record results in the field notebook (Hach Company, 2024b).
- Analyze one field duplicate per 10 samples (minimum one per event); RPD must be $< 20\%$ for concentrations above $5 \times$ the detection limit.
- If the field blank reads above the MDL, investigate contamination (dirty vials, contaminated DI water) and re-run.
- Note ambient temperature; some Hach methods are temperature-sensitive. If the sample is outside the method-specified range (typically 20–25 °C), note the potential bias (Hach Company, 2024a).

9. References

- Hach Company (2024a)
- Hach Company (2024b)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2024a). *DR900 portable colorimeter: Procedures manual*. https://www.hach.com/dr900-portable-colorimeter/product?id=56440352476

Hach Company. (2024b). *Water analysis guide* (7th ed.). https://www.hach.com/wag

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F300	Surface Water Sampling	Sample collection procedures
L003	Hazardous Materials	Reagent waste disposal
L303	Hach Colorimetric Analysis	Full method registry and lab procedures

11. Training Record

Before using the DR900 in the field independently, personnel must:

1. Read this SOP and SOP-L303 in full
2. Demonstrate a complete field analysis (zero, standard check, sample analysis, waste collection) under supervision
3. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F209

Dissolved Oxygen Meter (Field)

Standard Operating Procedure

SOP Number	SOP-F209
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the calibration, field operation, and maintenance of standalone handheld dissolved oxygen (DO) meters for in situ measurement of dissolved oxygen concentration and percent saturation in surface waters. Dissolved oxygen is a fundamental indicator of water quality and

aquatic habitat condition (APHA et al., 2012). This SOP covers both optical (luminescent) and electrochemical (polarographic and galvanic) DO probes operated as standalone instruments, separate from multiparameter sondes.

2. Scope

- **Applies to:** Handheld DO meters (e.g., YSI Pro20i, YSI ProSolo, Hach HQ40d with LDO probe, Extech DO600) used for in situ DO measurements at EA Program surface water field sites
- **Does not apply to:** DO measured by the Eureka Manta2 multiprobe (see SOP-F200); Winkler titration (laboratory method); BOD_5 analysis (see SOP-L306); continuously deployed sondes
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument selection; review QC data
Trained Personnel	Calibrate and operate the meter per this SOP
New Students	Complete training before independent use

4. Definitions

- **Barometric pressure correction:** Adjustment of the 100% saturation calibration point for the ambient atmospheric pressure; oxygen solubility decreases with decreasing pressure (increasing altitude); entered in mmHg or hPa at the time of calibration (APHA et al., 2012).
- **DO percent saturation (%sat):** The measured DO concentration expressed as a percentage of the equilibrium saturation concentration at the same temperature and pressure; 100% indicates equilibrium with the atmosphere; supersaturation ($> 100\%$) indicates net photosynthesis; values well below 100% indicate high oxygen demand or poor reaeration (APHA et al., 2012).
- **Electrochemical (polarographic/galvanic) probe:** A DO sensor in which oxygen diffuses through a gas-permeable membrane and is reduced at the cathode, generating a current proportional to DO concentration; requires periodic membrane and electrolyte replacement and is flow- dependent (needs water movement past the membrane) (APHA et al., 2012).
- **KCl electrolyte:** The potassium chloride solution filling the electrode cavity of polarographic DO probes; must be replaced when the membrane is changed or when readings become erratic.
- **LDO (luminescent dissolved oxygen) probe:** An optical sensor that measures DO by the quenching of luminescence from a ruthenium-dye sensing element by oxygen molecules; requires no electrolyte or membrane, no warm-up time, and no stirring (Hach Company, 2023).

- **Membrane (DO probe):** The gas-permeable Teflon or polyethylene film covering the electrode of an electrochemical DO probe; must be free of bubbles, tears, or debris; replace if damaged or if readings are erratic.
- **Sensing cap:** The replaceable luminescent element on an optical DO probe; replace when the cap expires (typically 1–2 years) or when readings become erratic (YSI Inc., 2022).
- **Winkler titration:** The reference wet-chemical method for DO determination; used to verify DO meter accuracy and required for calibration validation in some regulatory programs (APHA et al., 2012).
- **Zero-oxygen calibration:** A secondary calibration point using an oxygen-free solution (sodium sulfite with cobalt chloride catalyst) to verify the zero response of the DO probe; recommended when expected DO values are below 2 mg/L.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Measuring DO requires placing the probe in or near water. Slip, fall, and drowning hazards apply. Work in pairs at stream sites.
- **Chemical:** Polarographic DO probes use KCl electrolyte solution, a mild irritant. Sodium sulfite (used for zero-oxygen calibration) is a mild irritant and reducing agent.
- **Biological:** Water may contain pathogens. Avoid ingestion; wash hands after field work.

5.1. Required PPE

☐ Closed-toed shoes or wading boots
☐ Nitrile gloves (especially for impaired waters)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Instrument Types

6.1. Optical (Luminescent) DO Probes

Optical probes (e.g., Hach HQ40d with LDO101 probe, YSI ProSolo ODO) measure DO by detecting luminescence quenching. They require no electrolyte, no warm-up time, no stirring, and no membrane replacement (Hach Company, 2023).

Advantage	Limitation
No membrane or electrolyte	Higher initial cost

Advantage	Limitation
No stirring required Fast stabilization Lower maintenance	Sensing cap has finite lifespan (1–2 yr)

6.2. Electrochemical (Polarographic / Galvanic) DO Probes

Electrochemical probes (e.g., YSI Pro20i) measure DO through an oxygen-permeable membrane (YSI Inc., 2022).

Advantage	Limitation
Lower initial cost	Requires membrane and electrolyte replacement
Well-established method	Needs 5–15 min warm-up
Widely available	Flow-dependent (stirring or moving water needed)

7. Equipment and Materials

Item	Notes
Handheld DO meter with probe	Fully charged; confirm calibration supplies
Water-saturated air calibration chamber	Wet sponge in probe storage cap
Barometric pressure reference	Built into some meters; otherwise Kestrel or phone app
Spare membranes and electrolyte	For electrochemical probes
Spare sensing cap	For optical probes; replace when expired or erratic
Thermometer (for verification)	Most DO probes include temperature sensor
DI water (squeeze bottle)	For rinsing the probe
Lint-free wipes	For drying the probe before calibration
Field notebook	Per SOP-F003
Spare batteries	Matched to meter model

8. Procedure

8.1. Calibration

Calibrate the DO meter before each field day using the **water-saturated air** method.

Water-saturated air calibration:

1. Ensure the calibration chamber (probe storage cap with damp sponge) is moist but not dripping; the probe tip must not touch the sponge.

2. Turn on the meter. Allow electrochemical probes to warm up for 5–15 minutes (see instrument manual) (YSI Inc., 2022).
3. Enter the current barometric pressure (mmHg or hPa). Verify the displayed value against a Kestrel or weather station.
4. Place the probe in the calibration chamber; wait for the reading to stabilize (1–3 min for optical; 5–10 min for electrochemical).
5. Press **Cal** per the instrument prompts; the meter sets 100% saturation at the current temperature and pressure.
6. Record in the field notebook: calibration date, time, barometric pressure, temperature, and whether calibration was accepted.

Zero-oxygen calibration (optional – low-DO environments):

1. Dissolve excess sodium sulfite in DI water with a pinch of cobalt chloride catalyst.
2. Immerse the probe and wait for the reading to stabilize.
3. Calibrate the zero point per meter instructions.

8.2. Field Measurement

1. Immerse the probe to mid-depth at the sampling location. For electrochemical probes, maintain a minimum flow of 0.3 m/s past the membrane (gently move the probe in still water). Optical probes do not require flow (Hach Company, 2023).
2. Wait for the reading to stabilize.
3. Record in the field notebook:
   - DO concentration (mg/L) and percent saturation (%)
   - Water temperature (°C) as reported by the probe
   - Date, time, sample ID, and measurement depth
   - Flow conditions (riffle, pool, still water with manual stirring)
4. For depth profiles, lower the probe in 0.5 or 1.0 m increments and allow stabilization at each depth.

8.3. Post-Field Care

1. Rinse the probe with DI water after each use.
2. For electrochemical probes, check the membrane for tears, bubbles, or deposits; replace membrane and electrolyte if damaged (YSI Inc., 2022).
3. For optical probes, gently wipe the sensing cap with a soft, damp cloth if biofouling is visible; do not use solvents.
4. Store the probe in the calibration chamber with a moist sponge (not submerged).
5. Turn off the meter; remove batteries if storing for more than one month.

9. Data Interpretation

DO concentrations are influenced by temperature, atmospheric pressure, salinity, photosynthesis, respiration, and reaeration (APHA et al., 2012).

DO (mg/L)	Condition
> 8	Well-oxygenated; suitable for most aquatic life
5–8	Adequate for many species; marginal for cold-water fish
2–5	Stressful for most fish; hypoxic conditions
< 2	Severe hypoxia; lethal to most aquatic organisms

10. Quality Control

- Calibrate at the start of each field day.
- At the end of the field day, check calibration by returning the probe to the water-saturated air chamber; reading must be within ±2% of 100% saturation. If drift exceeds 5%, note it and recalibrate.
- When possible, verify against a second DO meter or the Eureka Manta2; readings should agree within ±0.5 mg/L.
- For long field days (> 6 hours), perform a mid-day calibration check.
- Record all QC results in the field notebook (APHA et al., 2012).

11. References

- APHA et al. (2012)
- Hach Company (2023)
- YSI Inc. (2022)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2023). *HQ40d portable multi-parameter meter: User manual*. https://www.hach.com/hq40d-portable-multi-parameter-meter/product?id=7640196365

YSI Inc. (2022). *Pro20i portable dissolved oxygen instrument: User manual*. https://www.ysi.com/pro20i

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F200	Eureka Manta2 Multiprobe	Multiprobe includes DO sensor
F300	Surface Water Sampling	Sampling context
F304	Stream Physical Habitat	Habitat characterization

13. Training Record

Before using DO meters independently, personnel must:

1. Read this SOP in full
2. Demonstrate a complete water-saturated air calibration
3. Perform supervised DO measurements at one field site
4. Demonstrate membrane inspection and replacement (electrochemical probes) or sensing cap inspection (optical probes)
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L101

Glassware Cleaning and Preparation

Standard Operating Procedure

SOP Number	SOP-L101
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the inspection, routine cleaning, acid washing, drying, and storage of laboratory glassware used in the EA Program laboratory. Properly cleaned glassware is essential for reliable analytical results; residual detergent, organic films, or trace metals can introduce systematic bias into water quality, soil extraction, and instrumental analyses. The cleaning tier required depends on the downstream analysis, ranging from routine detergent washing for general use to trace-metal-grade acid washing for ICP-MS and other sensitive techniques.

2. Scope

- **Applies to:** All reusable borosilicate glass labware (beakers, Erlenmeyer flasks, graduated cylinders, volumetric flasks, watch glasses, funnels, and media bottles) used in the EA Program laboratory
- **Does not apply to:** Disposable glassware (e.g., serological pipettes), plastic labware, quartz cuvettes, or specialty items requiring manufacturer-specific cleaning (e.g., NMR tubes, electrochemistry cells)
- **Location:** Glassware washing station, EA Program laboratory, SGM 133

3. Definitions

- **Acid bath:** A polyethylene tub containing 10% (v/v) HCl or HNO_3 in deionized water, used to remove trace-metal contamination from glassware; kept in the fume hood at all times.
- **Acid washing:** A multi-step cleaning procedure in which glassware is soaked in dilute acid after routine detergent washing to remove adsorbed trace metals; required for ICP-MS, ICP-OES, and nutrient analyses.
- **Borosilicate glass:** A glass formulation with low thermal expansion, high chemical resistance, and suitability for laboratory use; the standard material for EA Program glassware.
- **Deionized (DI) water:** Water from which ions have been removed by ion-exchange resin; used for all rinsing steps. Type I (18.2 MOhm) water from the Milli-Q system is required for final rinses of trace-metal-grade glassware.
- **Routine cleaning tier:** Washing with Alconox detergent and tap water, followed by DI water rinses; appropriate for general-use glassware not destined for trace-metal or nutrient analysis.
- **Trace-metal grade:** A cleaning standard requiring acid washing with HNO_3 (Trace Metal grade) and Type I water rinses; used for glassware that contacts samples destined for ICP-MS or similar sensitive analyses.
- **Volumetric glassware:** Glassware calibrated to contain or deliver a precise volume (volumetric flasks, graduated cylinders, pipettes); must not be oven-dried because thermal expansion permanently alters calibration.

- **Water-break test:** A visual check for cleanliness in which DI water is rinsed through glassware; a continuous, uniform film (no beading) indicates adequate cleaning.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure supplies are stocked
Lab Manager	Maintain acid bath schedule, monitor DI water quality
Trained Personnel	Clean glassware after each use, report damaged items
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Broken or chipped glassware can cause lacerations. Hot glassware from the drying oven (105 C) causes burns on contact.
- **Chemical:** Concentrated acids (HCl, HNO_3) used in acid baths are corrosive and emit irritating fumes. Alconox detergent is a mild irritant.

5.1. Required PPE

☐ Safety glasses or splash goggles (goggles required for acid bath work)
☐ Lab coat
☐ Nitrile gloves (heavy-duty acid-resistant gloves for acid bath handling)
☐ Closed-toed shoes

5.2. Emergency Procedures

- **Acid splash to skin or eyes:** Flush immediately with water at the eyewash station or safety shower for at least 15 minutes. Refer to SOP-L003 Hazardous Materials Handling.
- **Broken glass injury:** Apply pressure with a clean cloth. If the cut is deep or contaminated with chemicals, seek medical attention.
- **Acid spill:** Neutralize small spills with sodium bicarbonate; for large spills, use the spill kit and evacuate the area. See SOP-L003.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Glassware washing sink	With hot and cold water supply
Drying oven	Set to 105 C; see SOP-L105
Drying rack (peg-style)	Wall-mounted or countertop
Acid bath (polyethylene tub with lid)	Dedicated to acid washing; kept in fume hood
Fume hood	Required for all acid bath work

6.2. Glassware Inventory

Common Fisherbrand borosilicate glassware used in the EA Program laboratory:

Item	Fisherbrand Cat. No.	Sizes in Lab
Low-Form Griffin Beakers	FB100050 – FB1002000	50 – 2000 mL
Narrow-Mouth Erlenmeyer Flasks	FB500125 – FB5001000	125 – 1000 mL
Wide-Mouth Erlenmeyer Flasks	FB501125 – FB501500	125 – 500 mL
Class A Volumetric Flasks	FB400100 – FB4002000	100 – 2000 mL
Graduated Cylinders	S63457 – S63461	50 – 1000 mL
Glass Media Bottles with Cap	FB800100 – FB8001000	100 – 1000 mL

6.3. Cleaning Reagents and Consumables

Item	Cat. No. / Source	Storage
Alconox powdered detergent	Fisher 04-320-1	Shelf above wash sink
Hydrochloric acid, ACS grade (for general acid bath)	Fisher A144-212	Acid cabinet, fume hood
Nitric acid, Trace Metal grade (for trace-metal acid bath)	Fisher A509P500	Acid cabinet, fume hood
Deionized water (Type I or II)	Milli-Q system; see *SOP-L11*	Dispenser at wash station
Glassware brushes (assorted sizes)	Fisher 03-578 series	Drawer at wash station
Lint-free wipes (Kimwipes)	Fisher 06-666 series	Benchtop dispenser

7. Procedure

7.1. Inspection Before Use

1. Inspect every piece of glassware before use. Check for chips, cracks, stars, or deep scratches, especially around rims, spouts, and the base
2. Discard any piece that is broken, chipped, starred, or badly scratched. Place it in the designated broken-glass box (rigid cardboard container labeled “BROKEN GLASS”), not in the regular trash

3. Check volumetric glassware (flasks, graduated cylinders) for legible graduation marks. Do not use glassware with faded or unreadable scales.

> **Tip**
>
> Run your fingernail gently around the rim of a beaker or flask. If you feel a nick or irregularity, the piece should be discarded. Chipped rims create stress points that can propagate into cracks during heating.

7.2. Routine Cleaning (General Laboratory Use)

Use this procedure for glassware that will be used for general chemistry, soil extractions (KCl, K_2SO_4), gravimetric analyses, and other procedures that do not require trace-metal cleanliness.

1. Rinse the glassware with tap water immediately after use to prevent residues from drying
2. Prepare a warm solution of Alconox detergent (approximately 10 g per liter of warm tap water) in the wash basin
3. Submerge the glassware and scrub all interior and exterior surfaces with an appropriately sized brush. Use brushes with wooden or plastic handles only; metal-handled brushes and worn brushes where the spine contacts the glass will scratch the surface
4. Pay special attention to the interior bottom of beakers and flasks, where residues accumulate
5. Rinse thoroughly under running tap water, inverting and filling each piece at least three times to remove all detergent
6. Perform a final rinse with three changes of deionized (DI) water
7. Invert the glassware on a peg-style drying rack or place upright in the drying oven at 105 C for 2–4 hours (see SOP-L105)

> **Critical**
>
> Do not allow acid to contact glassware before detergent is thoroughly removed. Acid reacting with residual soap creates a hydrophobic film that is difficult to remove and will interfere with subsequent analyses.

7.3. Acid Washing (Trace-Metal and Nutrient Analysis)

Use this procedure for glassware destined for trace-metal analysis (ICP-MS, ICP-OES, XRF digestions), nutrient analysis, or any procedure where the SOP specifies "acid-washed glassware." All acid bath work must be performed in the fume hood.

1. Complete the routine cleaning procedure (Section 6.2) first. Glassware must be detergent-free before entering the acid bath.
2. Prepare the acid bath in a dedicated polyethylene tub inside the fume hood:
   - **General acid bath:** 10% (v/v) HCl in DI water
   - **Trace-metal acid bath:** 10% (v/v) HNO_3 (Trace Metal grade) in DI water

3. Submerge the clean glassware completely in the acid bath, ensuring no air bubbles are trapped inside
4. Cover the tub with its lid and soak for a minimum of 4 hours; overnight soaking (12–24 hours) is preferred
5. Using acid-resistant gloves and splash goggles, remove the glassware from the acid bath
6. Rinse each piece with at least five changes of DI water. For trace-metal work, use Type I (18.2 M-ohm) water from the Milli-Q system for the final three rinses.
7. Place the rinsed glassware on a clean drying rack or in the drying oven. Do not use paper towels or Kimwipes to dry the interior of acid-washed glassware, as fibers introduce contamination.
8. Once dry, cover the opening of each piece with a clean watch glass, aluminum foil, or parafilm and label it "ACID WASHED" with the date

Note

The acid bath should be replaced when it becomes visibly discolored or after approximately 20 uses, whichever comes first. Record replacement dates in the acid bath log on the fume hood.

7.4. New Glassware

New borosilicate glassware is slightly alkaline from the manufacturing process. Before first use in quantitative analyses:

1. Soak the new glassware in a 1% HCl or 1% HNO_3 solution for several hours
2. Proceed with the routine cleaning procedure (Section 6.2)
3. For trace-metal applications, also complete the acid-washing procedure (Section 6.3)

7.5. Stubborn Residues and Special Cases

Residue Type	Cleaning Method
Dried organic matter	Soak overnight in Alconox; scrub with brush
Mineral scale or precipitates	Soak in 10% HCl for 4+ hours
Grease or stopcock lubricant	Rinse with acetone in the fume hood, then routine wash
Biological residues (agar, culture media)	Autoclave before washing; see SOP-L002
Unknown or heavily stained	Soak in 10% HNO_3 overnight; if persistent, contact the lab manager

Do Not Use Chromic Acid

Chromic acid (potassium dichromate in sulfuric acid) was historically used as a glassware cleaner but is a potent human carcinogen and is prohibited in this laboratory. Use the acid-wash procedures described above instead.

7.6. Drying and Storage

1. Air-dry inverted on a peg rack, or oven-dry at 105 C for 2–4 hours (see SOP-L105)
2. Do not oven-dry volumetric glassware (volumetric flasks, graduated cylinders). Thermal expansion can permanently alter the calibration. Air-dry volumetric glassware only.
3. Store clean, dry glassware in designated cabinets with shelf liner
4. Store glassware inverted or covered to prevent dust accumulation
5. Keep acid-washed glassware in a separate, labeled section of the cabinet or on a dedicated shelf to prevent accidental use for non-critical applications

8. Quality Control and Acceptance Criteria

8.1. Water-Break Test

The water-break test is a simple visual check for cleanliness:

1. Rinse the interior of the glassware with DI water
2. Observe the drainage pattern as water sheets off the surface
3. **Pass:** Water forms a uniform, unbroken film across the entire interior surface
4. **Fail:** Water beads up, forms droplets, or breaks into rivulets, indicating residual organic contamination or grease

If the water-break test fails, repeat the cleaning procedure. If it fails again after acid washing, discard the piece, as surface damage may be preventing adequate cleaning.

8.2. Routine Verification

- Perform the water-break test on a representative sample (1 in 10 pieces) from each cleaning batch
- For acid-washed glassware, run a DI water blank through the glassware and analyze it on the target instrument (e.g., ICP-MS) to confirm background levels are below method detection limits
- Record cleaning batch dates and water-break test results in the glassware cleaning log

9. Waste Disposal

Waste Type	Disposal Method
Spent acid bath (HCl or HNO_3)	Neutralize with sodium bicarbonate to pH 6–8, then dispose down the drain with copious water, or collect for EH&S pickup if volume is large
Broken glass	Place in the rigid "BROKEN GLASS" disposal box; do not place in regular trash or recycling
Detergent wash water	Drain; Alconox is biodegradable

Waste Type	Disposal Method
Acetone rinse waste	Collect in organic waste carboy in fume hood; EH&S pickup

10. References

- Corning Life Sciences. *Suggestions for Cleaning Glassware*. Application Note CLS-AN-112. Corning, NY.
- Sigma-Aldrich / MilliporeSigma. *Cleaning Laboratory Glassware*. Technical Document.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

11. Training Record

Before cleaning glassware independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Demonstrate proper use of brushes, detergent preparation, and the rinse sequence under supervision
4. For acid-washed glassware: demonstrate proper fume hood use, acid-resistant PPE donning, and acid bath handling under supervision
5. Perform a water-break test and have results reviewed by the lab manager
6. Be signed off by the PI or lab manager

12. Appendix A: Quick-Reference Cleaning Decision Guide

Downstream Analysis	Cleaning Tier Required
Gravimetric soil moisture (SOP-L400)	Routine (Section 6.2)
Loss on ignition (SOP-L406)	Routine (Section 6.2)
KCl extraction for NH_4^+/NO_3^- (SOP-L403)	Routine (Section 6.2)
TOC / TC analysis (SOP-L407)	Routine + combust ceramic boats at 900 C
pH and conductivity (SOP-L402	Routine (Section 6.2)
Hach colorimetric methods (SOP-L303)	Routine (Section 6.2)
ICP-MS / ICP-OES (*SOP-L31*)	Acid wash with 10% HNO_3 (Section 6.3)
XRF acid digestions (*SOP-L30*)	Acid wash with 10% HNO_3 (Section 6.3)
DNA extraction (SOP-L700)	Autoclave; routine wash
Microbial biomass carbon (SOP-L409)	Acid wash with 10% HCl (Section 6.3)

13. Appendix B: Acid Bath Preparation

13.1. 10% HCl Acid Bath

1. Place the clean polyethylene tub in the fume hood
2. Fill the tub approximately 90% full with DI water
3. Slowly add concentrated HCl (approximately 37%) to reach a final concentration of 10% (v/v). For a 20 L bath, add approximately 2 L of concentrated HCl to approximately 18 L of DI water.
4. Always add acid to water, never water to acid
5. Label the tub with: acid type, concentration, preparation date, and a hazard warning
6. Cover with the fitted lid when not in use

13.2. 10% HNO_3 Acid Bath (Trace Metal Grade)

1. Follow the same steps as above, substituting Trace Metal grade HNO_3 (Fisher A509P500) for HCl
2. Use Type I DI water from the Milli-Q system
3. This bath is reserved for trace-metal glassware only; do not cross-contaminate with the general HCl bath

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L102

Using Balances

Standard Operating Procedure

SOP Number	SOP-L102
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, zeroing, taring, and routine quality checks for the laboratory balances in the EA Program. Correct weighing technique is fundamental to virtually every quantitative laboratory procedure. For calibration and verification protocols, see SOP-L200 Analytical Balance Calibration.

2. Scope

- **Applies to:** All Mettler Toledo balances in the EA Program laboratory, including the basic balances (MS1602TS/00) and the precision balance (XPE205 DeltaRange) (Mettler Toledo, 2015a, 2015b)
- **Does not apply to:** Portable field balances or calibration procedures (see SOP-L200)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Capacity (maximum load):** The maximum mass that can be placed on the weighing pan without risking damage to the weighing cell; exceeding the rated capacity will damage the load cell and require expensive repair; the MS1602TS capacity is 1620 g and the XPE205 capacity is 220 g / 81 g (DeltaRange) (Mettler Toledo, 2015a, 2015b).
- **Draft shield:** The glass or acrylic enclosure surrounding the weighing pan on the XPE205 precision balance; reduces air current disturbances that cause reading instability; always keep closed except when placing or removing items.
- **Drift:** Gradual, directional change in the balance reading under constant load; caused by temperature changes, vibration, or electronic instability; the balance should be allowed to warm up for at least 30 minutes before critical weighing.
- **Linearity:** The degree to which balance readings are proportional to applied mass across the full weighing range; verified during calibration per SOP-L200.
- **Net weight:** The mass of sample only, after the tare weight of the container has been subtracted; what is displayed after pressing the Tare key with the container on the pan.
- **Readability (resolution):** The smallest increment the balance can display; the MS1602TS reads to 0.01 g and the XPE205 reads to 0.1 mg (0.0001 g) (Mettler Toledo, 2015a, 2015b).
- **Repeatability:** The standard deviation of repeated measurements of the same weight under identical conditions; a key short-term precision indicator; affected by vibration, draft, and sample placement position.

- **Spirit level (bubble level):** A small bubble vial mounted on the balance base; the bubble must be centered in the target circle before weighing; off-level readings can introduce systematic errors.
- **Tare:** The function that zeroes the displayed mass when a container is on the pan, so subsequent readings show only the mass of material added (Mettler Toledo, 2023).
- **Weighing cell:** The internal transducer (typically an electromagnetic force compensation cell) that converts the mechanical load on the pan to an electrical signal; the most sensitive and expensive component of the balance; protect it from impact, overloading, and corrosive chemicals.
- **Zeroing:** Setting the balance display to exactly zero with an empty pan and no load; performed at the start of each session and whenever the balance is moved; distinct from taring, which zeroes the balance with a container already on the pan.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Dropping heavy items onto the balance pan can damage the weighing cell. Handle objects carefully and never exceed the rated capacity.
- **Chemical:** Some materials weighed on the balance may be hazardous. Refer to the relevant SOP for the material being weighed. Never place chemicals directly on the weighing pan; always use a sample vessel.

5.1. Required PPE

☐ Lab coat
☐ Nitrile gloves (when handling chemical samples)
☐ Safety glasses (when weighing chemical reagents)

5.2. Emergency Procedures

If a chemical spill occurs on or near the balance, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use.

6. Equipment and Materials

6.1. EA Program Balances

Balance	Model	Capacity	Readability	Location
Precision balance (1)	XPE205	220 g / 81 g	0.1 mg	SGM 133
Basic balance (1–3)	MS1602TS	1620 g	0.01 g	SGM 133

(Mettler Toledo, 2015b, 2015a)

6.2. Weighing Supplies

Item	Notes
Weighing paper	Single use; keeps chemicals off the pan
Sample vessels	Weighing boats, crucibles, or tared containers
Forceps or tweezers	For handling calibration weights or small containers without contamination
Lint-free wipes (Kimwipes)	For cleaning the pan and draft shield
Anti-static brush	For removing powder adhering to the draft shield interior

7. Procedure

7.1. Startup and Warm-Up

Allow the balance to warm up for at least 30 minutes before making critical weighings (< 0.001 g significance) (Mettler Toledo, 2015b). This allows the internal components to reach thermal equilibrium.

7.2. Leveling the Balance

1. Check the spirit level before each use session.
2. If the bubble is not centered, adjust the leveling feet at the base of the balance.
3. For the XPE205, use the built-in leveling aid: **Balance menu > Leveling aid** (Mettler Toledo, 2015b).

7.3. Zeroing

1. Verify the balance is empty and the draft shield is closed.
2. Press **Zero** (or **On/Zero**). The display should read 0.000 g (for the MS1602TS) or 0.0000 g (for the XPE205).
3. If the display does not settle, check that the balance is level, no air currents are present, and the pan is clean and dry.

7.4. Taring

1. Zero the balance (§[sec-zeroing]).
2. Open the draft shield.
3. Place the sample vessel on the center of the weighing pan.
4. Close the draft shield.
5. Press the **Tare** key. The display reads zero and the **Net** indicator appears.

7.5. Performing a Weighing

1. Open the draft shield (if applicable).
2. Place the weighing object into the tared sample vessel.
3. Close the draft shield and wait for the stability indicator.
4. Record the displayed mass in the lab notebook.
5. If using the XPE205 electronic results feature, tap **Add result** to append the value to the results list.

7.6. Completing and Saving Results (XPE205)

1. Tap **Complete** to open the task completion dialog.
2. Select an option to save or print the results list.
3. Follow the on-screen instructions.
4. Tap **Complete** to finalize; the results list is saved or printed and then cleared.

7.7. Shutdown

1. Remove all samples and vessels from the weighing pan.
2. Clean the pan and draft shield interior with a lint-free wipe.
3. Close the draft shield.
4. To enter standby mode, hold the power/standby key. The display goes dark but the balance remains powered on and thermally stable.
5. To switch the balance off completely, disconnect from the power supply. Note that the balance will require a full warm-up before its next use.

8. Quality Control and Acceptance Criteria

8.1. Leveling the Balance

Exact horizontal positioning is essential for repeatable and accurate results (Mettler Toledo, 2015b):

1. Check the spirit level before each use session.
2. If the message “Balance is out of level” appears, tap **Level the balance** to open the leveling aid.
3. Adjust the leveling feet following the on-screen instructions until the bubble is centered.

8.2. Internal Adjustment (XPE205)

1. Navigate to **Methods > Adjustments**.
2. Verify that the adjustment strategy is set to **Internal adjustment**.
3. Select the adjustment and tap **Start**.
4. Do not touch the balance during the adjustment.
5. When complete, review the adjustment results.
6. Tap **Print** if a record is needed, then tap **Finish adjustment** (Mettler Toledo, 2015b).

For external calibration verification using traceable reference weights, see SOP-L200.

9. Waste Disposal

No waste is generated by this procedure. Cleaning materials (Kimwipes) can be disposed of in standard trash unless they contacted hazardous chemicals, in which case follow SOP-L003.

10. References

Mettler Toledo. (2015a). *MS1602TS basic balance: Operating instructions*. https://www.mt.com

Mettler Toledo. (2015b). *XPE205 DeltaRange precision balance: Operating instructions*. https://www.mt.com

Mettler Toledo. (2023). *Good weighing practice: The SOP for balances and scales*. https://www.mt.com/us/en/home/library/guides/laboratory-weighing/good-weighing-practice.html

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Chemical spills on/near balance
L200	Balance Calibration	Calibration and external verification
L400	Soil Moisture	Weighing application
L406	Loss on Ignition	Weighing application

12. Training Record

Before using the laboratory balances independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper leveling, zeroing, taring, and weighing technique under supervision
4. For the XPE205 precision balance, also demonstrate use of the draft shield and internal adjustment procedure
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L103

Using Micropipettes

Standard Operating Procedure

SOP Number	SOP-L103
Revision	0.3
Author(s)	Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, volume setting, tip loading, sample aspiration and dispensing, and care of Gilson Pipetman micropipettes. Micropipettes are precision instruments used to transfer small volumes of liquid (less than 1 mL) with high accuracy. Correct technique is essential for reproducible results in chemical, biological, and environmental analyses.

2. Scope

- **Applies to:** All Gilson Pipetman micropipettes (P10, P20, P200, P1000) in the EA Program laboratory
- **Does not apply to:** Serological pipettes, Pasteur pipettes, bottle- top dispensers, or Repipet-type dispensers
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure annual calibration
Lab Manager	Schedule calibration, maintain tip inventory
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Aerosol-resistant (filter) tip:** A pipette tip containing a hydrophobic filter that prevents aerosols and liquid from entering the pipette barrel; required when pipetting volatile, radioactive, infectious, or PCR-sensitive samples.
- **Air-displacement pipette:** The standard laboratory micropipette in which a piston displaces air in the tip, which in turn displaces liquid; the most common type; accuracy depends on tip seal quality and pipetting technique.
- **Dead volume:** The small volume of liquid remaining in the tip after dispensing; varies by tip type and affects accuracy at low volumes.
- **Forward pipetting:** The standard technique in which the plunger is depressed to the first stop before immersing the tip, then liquid is aspirated by releasing the plunger; used for aqueous solutions.
- **Reverse pipetting:** A technique in which the plunger is depressed past the second stop before aspirating; liquid is dispensed only to the first stop, leaving excess in the tip; used for viscous or volatile liquids to improve accuracy.

- **Systematic error:** A consistent, directional bias in pipette delivery; detected by gravimetric calibration; caused by worn seals, incorrect technique, or using tips not matched to the pipette model.
- **Volume range:** The minimum and maximum volume a given pipette model is designed to deliver accurately; pipetting below 10% of the maximum volume significantly increases relative error.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Liquids being pipetted may be hazardous. Consult the relevant SOP and Safety Data Sheet for the material being transferred.
- **Physical:** Repetitive pipetting can cause repetitive strain injuries. Take breaks during extended pipetting sessions.
- **Biological:** When pipetting biological samples, use filter tips to prevent aerosol contamination of the pipette barrel.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

If hazardous liquid enters the pipette barrel, stop immediately. Decontaminate the pipette per the manufacturer's instructions and refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Volume Range	Notes
Pipetman P10	1.0 – 10.0 μL	Yellow or white tip
Pipetman P20	2.0 – 20.0 μL	Yellow tip
Pipetman P200	20 – 200 μL	Yellow tip
Pipetman P1000	200 – 1000 μL	Blue tip

6.2. Consumables

Item	Notes
Disposable pipette tips	Match tip size to pipette; see Section 6.3
Filter pipette tips	Required for biological or volatile samples
Tip waste container	Benchtop receptacle for used tips

7. Procedure

7.1. Selecting the Correct Pipette

Always select the smallest pipette that covers the target volume. using an unnecessarily large pipette for a small volume reduces accuracy. The volume ranges are:

Pipette	Range	Smallest Graduation
P10	1.0 – 10.0 μL	0.02 μL
P20	2.0 – 20.0 μL	0.02 μL
P200	20 – 200 μL	0.2 μL
P1000	200 – 1000 μL	2 μL

! Important

Never set the pipette below the minimum or above the maximum of its range. Exceeding these limits will damage the piston seal and put the pipette out of calibration.

7.2. Setting the Volume

1. Identify the volume display window on the front face of the pipette. The three-digit display reads differently for each pipette size:
   - **P10:** digits represent ones, tenths, and hundredths of uL
   - **P20:** digits represent tens, ones, and tenths of uL
   - **P200:** digits represent hundreds, tens, and ones of uL
   - **P1000:** digits represent thousands, hundreds, and tens of uL
2. Turn the volume adjustment ring clockwise to increase volume or counterclockwise to decrease volume
3. If you overshoot the target while dialing down, dial below the desired setting and then dial back up to the correct value. This avoids mechanical backlash in the piston mechanism.

7.3. Loading a Tip

1. Select the correct tip size for the pipette being used. Tip boxes are labeled with the compatible pipette sizes. When in doubt, confirm with the lab manager.
2. Press the pipette shaft firmly into a tip in the tip box. A slight twist while pressing helps ensure a secure, airtight seal.

3. Do not touch the tip with your hands.
4. Always use a fresh tip for each different reagent or sample to prevent cross-contamination.

7.4. Aspirating (Loading) a Sample

1. Depress the plunger slowly to the **first stop** (the point of initial resistance). This is the calibrated load volume.
2. While holding the plunger at the first stop, immerse the tip into the liquid just enough to cover the opening (3–4 mm). Do not plunge the tip to the bottom of the vessel.
3. Slowly and steadily release the plunger to draw liquid into the tip. Do not let the plunger snap back.
4. Wait one to two seconds before removing the tip from the liquid, especially with viscous solutions. This allows the full volume to enter the tip and prevents air bubbles.
5. Visually inspect the tip: there should be no air gap or bubble at the distal end. If there is, dispense the liquid back and repeat.

> **Tip**
>
> For viscous liquids (glycerol, concentrated DNA solutions), use the "reverse pipetting" technique: depress the plunger to the **second stop** before immersing the tip, draw up the liquid by releasing to the rest position, and then dispense by pressing only to the **first stop**. The small amount of liquid remaining in the tip is discarded.

7.5. Dispensing a Sample

1. Touch the tip to the inner wall of the receiving vessel at a slight angle
2. Depress the plunger slowly to the **first stop** to dispense most of the liquid
3. Continue pressing to the **second stop** to expel the final drop
4. Hold the plunger at the second stop while withdrawing the tip from the vessel
5. Release the plunger only after the tip is clear of the liquid

7.6. Ejecting the Tip

1. Hold the pipette over the tip waste container
2. Press the tip ejector button on the back of the pipette
3. Never reuse a tip between different solutions

7.7. Important Precautions

- Never point a pipette upward with liquid in the tip. Liquid can flow back into the barrel and damage the piston and seals.
- Never lay a pipette down with a loaded tip.
- When not in use, set the pipette to its maximum volume and hang it vertically on the pipette stand. This relaxes the piston spring and extends the instrument's life.
- Do not use a pipette as a stirring rod.

- If the plunger action feels rough, sticky, or inconsistent, stop using the pipette and report the issue to the lab manager.

8. Quality Control and Acceptance Criteria

8.1. Gravimetric Accuracy Check

Verify pipette accuracy by weighing dispensed volumes of distilled water on the analytical balance (see SOP-L200):

1. Tare a clean microcentrifuge tube on the analytical balance
2. Pipette the test volume of distilled water into the tube
3. Record the mass. At 20 C, 1 μL of water weighs approximately 1.00 mg.
4. Repeat for a total of 10 replicates
5. Calculate the mean and coefficient of variation (CV)

Pipette	Test Volume	Acceptable CV
P10	10 μL	less than 3%
P20	20 μL	less than 2%
P200	200 μL	less than 1%
P1000	1000 μL	less than 0.5%

If the CV exceeds the acceptable threshold, the pipette should be removed from service and sent for professional calibration.

8.2. Calibration Schedule

All micropipettes must be professionally calibrated at least once per year. Calibration stickers on each pipette show the date of last calibration and the next due date. Do not use a pipette with an expired calibration.

9. Waste Disposal

Waste Type	Disposal Method
used pipette tips (non-hazardous)	Standard tip waste box; dispose in regular trash when full
used tips contaminated with hazardous chemicals	Chemical waste per SOP-L003
used tips contaminated with biological material	Biohazard bag; autoclave before disposal

10. References

- Gilson. *Pipetman Classic: user's Guide*. Gilson, Inc., Middleton, WI.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

11. Training Record

Before using micropipettes independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate correct volume setting, tip loading, aspiration, and dispensing technique under supervision
4. Perform a gravimetric accuracy check (Section 7.1) with acceptable results for at least two pipette sizes
5. Be signed off by the PI or lab manager

12. Appendix A: Volume Display Reading Guide

The three-digit volume display reads differently for each Pipetman size. The table below shows the maximum display setting and the place value of each digit:

Pipette	Max Display	Digit 1 (top)	Digit 2 (middle)	Digit 3 (bottom)
P10	1 0 . 0 0	10 μL	1 μL	0.1 μL
P20	2 0 . 0	10 μL	1 μL	0.1 μL
P200	2 0 0	100 μL	10 μL	1 μL
P1000	1 0 0 0	1000 μL	100 μL	10 μL

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Liquid drips from tip after aspiration	Tip not seated properly; damaged tip	Reseat or replace tip
Air bubble in tip after aspiration	Plunger released too quickly; tip not immersed deep enough	Re-aspirate slowly with tip 3–4 mm below surface
Dispensed volume too low	Pipette out of calibration; wrong tip size	Perform gravimetric check; verify tip compatibility
Plunger feels sticky or rough	Liquid entered barrel; worn piston seal	Clean barrel; send for service

Symptom	Probable Cause	Corrective Action
Volume display will not turn	Mechanical jam	Do not force; contact lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L104

Using a Vortex Mixer

Standard Operating Procedure

SOP Number	SOP-L104
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation and care of vortex mixers used in the EA Program laboratory. Vortex mixers produce a rapid orbital or oscillating motion that creates a vortex inside a tube or small container, providing fast and thorough mixing of liquids, suspensions, and reagents. They are used routinely in DNA extraction (SOP-L700), Qubit sample preparation (SOP-L213), chemical extractions, and general reagent mixing.

2. Scope

- **Applies to:** All vortex mixers in the EA Program laboratory, including the Fisher Scientific vortex mixers (SGM 134, qty 3) and the Vortex-Genie 2 (SGM 133)
- **Does not apply to:** Orbital shakers (for extended, gentle agitation of multiple samples); magnetic stir plates; sonicators (see SOP-L115)
- **Location:** SGM 133 and SGM 134

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain equipment, replace cup attachments
Trained Personnel	Operate the vortex mixer per this SOP
New Students	Complete training before independent use

4. Definitions

- **Continuous mode:** A vortex mixer operating mode in which the platform spins continuously when the power switch is on; used when mixing many tubes in sequence without holding each one.
- **Touch mode:** A vortex mixer operating mode in which the platform spins only when downward pressure is applied to the platform; allows the user to control mixing duration for each tube individually.
- **Vortex:** A spiral or whirling motion of liquid produced when a tube's closed end is pressed off-center against a spinning platform; efficient for resuspending pellets, dissolving solids, and homogenizing small volumes.
- **Vortex mixer (Vortex-Genie):** A benchtop mixer with an eccentric rotating cup or platform that creates a vortex in tubes pressed against it; operated at variable speeds (typically 0–3,200 rpm).

5. Safety and Hazards

⚠ Hazards

- **Aerosol generation:** Vortexing uncapped or loosely capped tubes can spray liquid and generate aerosols. Always cap tubes securely before vortexing. If the sample contains hazardous biological or chemical material, vortex inside a fume hood or biological safety cabinet.
- **Tube ejection:** At high speed, improperly held tubes can slip from the cup head and be thrown from the mixer. Hold tubes firmly against the cup or use the hands-free cup attachment.

- **Breakage:** Thin-walled glass tubes may shatter under vigorous vortexing. Use polypropylene or thick-walled borosilicate tubes whenever possible.
- **Entanglement:** Keep hands, hair, jewelry, and loose clothing away from the spinning cup head.
- **Electrical:** Do not operate the mixer with a damaged power cord or loose components. Inspect the cord before use; report damage to the lab manager.

5.1. Required PPE

☐ Safety glasses
☐ Nitrile gloves (standard lab PPE)
☐ Lab coat

6. Equipment

Item	Location	Notes
Fisher Scientific vortex mixer	SGM 134	Qty 3; touch or continuous mode
Vortex-Genie 2 (Scientific Industries)	SGM 133	Variable speed; touch or continuous mode
Cup head (standard)	Attached	For single tubes (1.5 mL to 50 mL)
Flat head or platform head (optional)	SGM 134	For microplates or multiple small tubes held in a rack

7. Procedure

7.1. Pre-Use Inspection

1. Verify the vortex mixer is clean and free from visible damage
2. Ensure the power cord is intact and properly connected
3. Check that the rubber cup head or attachment is securely installed and not cracked or deformed

7.2. Setup

1. Verify that the vortex mixer is on a stable, flat bench surface and that the rubber feet are intact (prevents the unit from walking during operation)
2. Confirm that the appropriate head is installed:
    - **Cup head:** for individual tubes (microcentrifuge tubes, 15 mL and 50 mL centrifuge tubes, glass test tubes)
    - **Platform head:** for microplates or tube racks (if available)

3. Set the speed dial to the desired setting. For most routine mixing, a medium speed (setting 5–7 on the Vortex-Genie 2 dial) is sufficient. Use lower speeds for gentle mixing and higher speeds for vigorous vortexing (e.g., bead disruption steps in DNA extraction).

7.3. Operation – Touch Mode

Touch mode is the default for most applications. The mixer runs only while the tube is pressed against the cup head.

1. Cap the tube securely
2. Hold the tube at a slight angle (approximately 15–30 degrees from vertical) and press the bottom of the tube firmly into the center of the cup head
3. The motor engages automatically. A visible vortex should form in the liquid.
4. Vortex for the duration specified in the analytical SOP (typically 2–5 seconds for brief mixing, 10–30 seconds for thorough resuspension)
5. Release the tube from the cup to stop

7.4. Operation – Continuous Mode

Use continuous mode for hands-free operation or when extended vortexing is required.

1. Switch the mode selector to **Continuous** (or **On**)
2. Adjust the speed dial to the desired setting
3. Place the capped tube in the cup head or in a tube holder secured to the platform head
4. The motor runs continuously at the set speed
5. After the prescribed duration, switch back to **Touch** mode or turn off the unit
6. Remove the tube

7.5. Vortexing Multiple Tubes

For rapid sequential vortexing of many tubes (e.g., a rack of Qubit assay tubes):

1. Set the mixer to touch mode at the desired speed
2. Work through the rack systematically, pressing each tube against the cup for 2–3 seconds
3. Return each tube to the rack before picking up the next

For simultaneous vortexing of a plate or rack, attach the platform head and secure the plate or rack with the provided clamp or tape. Run in continuous mode for the prescribed duration.

7.6. Special Applications

Application	Speed Setting	Duration	Notes
Brief reagent mixing	Medium (5–7)	2–5 s	Touch mode; just to mix

Application	Speed Setting	Duration	Notes
Qubit assay tube mixing (L213)	Medium (5–7)	2–3 s	Brief vortex after adding sample to working solution
DNA extraction bead disruption (L700)	Maximum (10)	Per kit protocol (often 10 min on adapter)	Use continuous mode with horizontal adapter if available
Resuspending pellets after centrifugation	High (8–10)	10–30 s	Until pellet is fully resuspended
Mixing soil extracts (L403, L409)	Medium (5–7)	5–10 s	Ensure homogeneous suspension before filtering

8. Maintenance

Task	Frequency
Wipe the cup head and body with 70% ethanol	After each use session
For eDNA/PCR work: wipe with 10% bleach, then ethanol	After each use session
Check rubber feet for wear	Monthly
Inspect cup head for cracks or deformation	Monthly
Replace cup head	When cracked or deformed

> **Note**
>
> If liquid spills into the cup head or body of the mixer, unplug the unit immediately, wipe up the spill, and allow to dry completely before re-use. Liquid inside the motor housing can cause electrical failure.

9. Waste Disposal

No waste is generated by the vortex mixer itself. Dispose of sample waste per the relevant analytical SOP and SOP-L006 Waste Disposal.

10. References

- Scientific Industries. *Vortex-Genie 2 Instruction Manual.* Scientific Industries, Bohemia, NY.

11. Related SOPs

SOP	Title	Relationship
L115	Sonicator	Alternative mixing/disruption
L213	Qubit Fluorometer	Sample preparation requires vortexing
L403	KCl Extraction	Extract mixing
L700	DNA Extraction	Bead disruption step

##Training Record

Before using the vortex mixer independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper tube holding technique in touch mode (cap secured, slight angle, firm contact with cup)
3. Demonstrate switching between touch and continuous mode
4. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Mixer does not start	Power disconnected or cord damaged	Check power supply; inspect cord; try another outlet
Excessive vibration or walking	Unit on uneven surface; rubber feet worn	Reposition on a flat surface; replace rubber feet
Poor or incomplete mixing	Speed too low; tube not centered on cup	Increase speed; press tube firmly into center of cup
Sample leakage during vortexing	Tube cap not secured; tube overfilled	Cap tightly; reduce fill volume to leave headspace
Abnormal noise or grinding	Cup head loose or damaged; internal bearing wear	Tighten or replace cup head; report to lab manager for service
Liquid entered motor housing	Spill from uncapped tube	Unplug immediately; wipe and dry thoroughly; do not use until fully dry

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L105

Using Drying Ovens

Standard Operating Procedure

SOP Number	SOP-L105
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe operation of gravity convection and forced-air drying ovens used in the EA Program laboratory. Drying ovens are used to determine gravimetric moisture content of soil, sediment, and plant tissue samples (see SOP-L400 Soil Moisture), to pre-dry samples before loss on ignition (see SOP-L406 Loss on Ignition), to dry glassware, and to regenerate desiccants. Consistent oven operation ensures accurate and reproducible mass-loss measurements across the laboratory (Gardner, 1986; Robertson et al., 1999).

2. Scope

- **Applies to:** All gravity convection and forced-air drying ovens in the EA Program laboratory, operated at temperatures up to 250 C
- **Does not apply to:** Muffle furnaces (550+ C; see SOP-L50), vacuum ovens, microwave drying, or autoclaves
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Desiccator:** An airtight container holding a hygroscopic drying agent (typically silica gel or Drierite) used to cool crucibles or dried samples to room temperature without re-absorbing atmospheric moisture.
- **Forced-air oven (mechanical convection):** A drying oven with a fan that circulates hot air to produce uniform temperature and faster drying; preferred when rapid or even drying is required.
- **Gravity convection oven:** A drying oven without a fan; relies on natural convection. Slightly less temperature-uniform than forced-air but adequate for most soil and sediment drying.
- **Gravimetric moisture content:** The mass of water lost on drying expressed as a proportion of the dry mass: $\theta_g = (M_{wet} - M_{dry})/M_{dry}$; expressed as g g^{-1} or as a percentage.
- **Oven-dry basis:** The convention of expressing soil or sediment concentrations relative to the mass of material dried at 105 °C to constant mass; the standard reference state for soil analysis.
- **Set point:** The target temperature programmed into the oven controller; the actual chamber temperature should be verified with a calibrated thermometer and may differ from the set point by a few degrees.
- **Tare mass:** The mass of an empty, pre-dried container (crucible, tin, or pan) recorded before adding sample; subtracted from the gross mass to obtain sample mass.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain ovens, schedule annual calibration checks
Trained Personnel	Operate ovens following this SOP, report malfunctions
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Oven surfaces and contents reach temperatures up to 250 C. Severe burns can result from touching hot surfaces, shelves, or sample containers without proper PPE. Steam may escape when opening the door if drying wet samples.
- **Chemical:** Do not dry samples containing volatile organic solvents, flammable materials, or corrosive chemicals in these ovens. Drying ovens are not explosion-proof. Volatile fumes can accumulate inside the oven chamber and pose fire or explosion risks.
- **Electrical:** Do not operate ovens with damaged power cords or plugs. Ensure the oven is properly grounded.

5.1. Required PPE

☐ Heat-resistant gloves or oven mitts (required when loading/unloading)
☐ Safety glasses
☐ Lab coat
☐ Closed-toed shoes

5.2. Emergency Procedures

For burns, cool the affected area under cool running water for at least 10 minutes. Seek medical attention for burns with blistering. If smoke or unusual odors are detected, turn off the oven, unplug it, and ventilate the area. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Drying oven	Lab bench, SGM 133	*TBD – confirm manufacturer and model number*
Calibrated thermometer or thermocouple	Inside oven	For independent temperature verification

Item	Location	Notes
Desiccator with indicating desiccant	Lab bench	For cooling samples after removal from oven
Metal tongs or oven mitts	Near oven	For handling hot containers

6.2. Consumables

Item	Notes
Aluminum weighing tins	Pre-labeled or numbered
Ceramic crucibles	For higher-temperature applications
Indicating desiccant (silica gel or Drierite)	Replace or regenerate when color changes

7. Procedure

7.1. Pre-Use Checks

1. Verify the oven is clean and free of residue from previous use
2. Check that the oven vent is unobstructed (gravity convection ovens rely on passive airflow; blocked vents cause uneven heating)
3. Confirm the temperature setpoint using the oven's built-in display or controller
4. Verify the temperature against an independent thermometer or thermocouple placed inside the oven. The reading should agree within +/- 2 C of the setpoint. If it does not, contact the lab manager.

7.2. Setting the Temperature

Common drying temperatures used in the EA Program laboratory:

Application	Temperature	Duration	Reference SOP
Gravimetric soil moisture	105 C	24+ hours	SOP-L41
Pre-drying soil for LOI	105 C	24+ hours	SOP-L50
Drying glassware	105 C	2–4 hours	–
Drying plant tissue	65–70 C	48–72 hours	–
Regenerating Drierite desiccant	210 C	1–2 hours	–
Acid-treated soil (IC removal)	105 C	Until dry	SOP-L51

1. Set the temperature using the oven controller dial or digital display
2. Allow the oven to reach the target temperature and stabilize before loading samples (typically 30–60 minutes)
3. Record the setpoint and actual temperature in your lab notebook

7.3. Loading Samples

1. Arrange samples on oven shelves with adequate spacing to allow air circulation. Do not stack containers or block vents.
2. Place containers with the lids removed or loosely resting to allow moisture to escape
3. For large batches, distribute samples evenly across shelves to minimize temperature gradients
4. Close the oven door promptly after loading

> **Tip**
>
> Place a small label or sign on the oven door indicating: your name, the setpoint temperature, the date and time samples were loaded, and the expected removal time. This prevents others from accidentally changing the temperature or disturbing your samples.

7.4. Monitoring

1. Check the oven temperature at the start and end of each drying cycle using the independent thermometer
2. Do not open the oven door unnecessarily during drying, as this introduces cool air and extends drying time
3. For multi-day drying, check the oven temperature at least once daily

7.5. Removing Samples

1. Wear heat-resistant gloves or oven mitts
2. Use metal tongs for small containers (aluminum tins, crucibles)
3. Transfer hot containers directly into a desiccator to cool to room temperature. Do not weigh hot samples; thermal convection currents around hot objects cause unstable balance readings (Harris & Lucy, 2020).
4. Allow samples to cool in the desiccator for 20–30 minutes before weighing
5. Close the oven door after removing samples

7.6. Verifying Dryness (Constant Mass)

For analytical applications (soil moisture, LOI), drying is complete only when the sample reaches constant mass:

1. After the initial drying period, remove the sample, cool in the desiccator, and weigh
2. Return the sample to the oven for an additional 2–4 hours
3. Cool and re-weigh
4. If the mass has changed by less than 0.01 g between successive weighings, constant mass has been achieved
5. If the mass has decreased by more than 0.01 g, return to the oven and repeat until constant mass is reached

7.7. Shutdown

1. If the oven will not be used for several days, turn it off using the power switch or controller
2. Leave the door slightly ajar to prevent moisture buildup and odors inside the chamber
3. Ensure no samples remain in the oven before shutting down

8. Quality Control and Acceptance Criteria

- Verify oven temperature against an independent thermometer at least monthly; record in the oven calibration log
- Acceptable temperature accuracy: +/- 2 C of setpoint at 105 C
- If the oven temperature drifts beyond +/- 5 C, remove the oven from service and contact the lab manager
- Ensure the desiccator contains active (indicating) desiccant; regenerate or replace when the indicator changes color
- For analytical drying, always confirm constant mass (Section 6.6) rather than relying on a fixed drying time alone

8.1. Interferences

- Overcrowding the oven can create temperature gradients and uneven drying, leading to inconsistent results
- Opening the oven door frequently extends drying time and introduces moisture
- Drying samples containing volatile compounds at 105 C will cause mass loss beyond water content, biasing moisture determinations

9. Waste Disposal

Waste Type	Disposal Method
Dried soil samples	Standard trash or return to field site
Aluminum tins	Clean and reuse, or recycle
Spent desiccant	Regenerate at 210 C or dispose as non-hazardous solid waste

10. References

Gardner, W. H. (1986). Water content. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 493–544). Soil Science Society of America.

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research.* Oxford University Press.

11. Training Record

Before using the drying oven independently, personnel must:

1. Read this SOP and SOP-L002 Laboratory Safety in full
2. Demonstrate proper use of heat-resistant gloves and tongs
3. Demonstrate setting the oven temperature and verifying it with an independent thermometer
4. Demonstrate loading and unloading samples, including transfer to the desiccator
5. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Oven does not heat	Power switch off, unplugged, or blown fuse	Check power connections; replace fuse if needed
Temperature overshoots setpoint	Faulty thermostat or controller	Remove from service; contact lab manager
Uneven drying across samples	Overcrowded shelves or blocked vent	Redistribute samples; check vent is clear
Samples not reaching constant mass after 48 hours	Very wet or clay-rich samples; temperature too low	Verify setpoint is 105 C; extend drying time; check oven seal
Unusual odor during drying	Contaminated samples or residue from previous use	Turn off oven; ventilate; clean interior

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L106

Refrigerators and Freezers

Standard Operating Procedure

SOP Number	SOP-L106
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, sample storage protocols, and inventory management for the laboratory refrigerator (4 C), freezer (approximately –30 C), and ultra-low freezer (approximately –80 C). Proper cold storage is essential for preserving sample integrity for chemical, biological, and molecular analyses (APHA et al., 2012).

2. Scope

- **Applies to:** All use of the laboratory refrigerator (4 C), freezer (–30 C), and ultra-low freezer (–80 C) for sample preservation and reagent storage
- **Does not apply to:** Food storage (prohibited), field coolers, or cryogenic storage (liquid nitrogen)
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Cold chain:** The unbroken sequence of refrigerated storage and transport that maintains samples at the required temperature from collection through analysis; a break in the cold chain may compromise sample integrity.
- **Cryogenic storage:** Storage at temperatures below -80 °C, achieved with ultra-low freezers or liquid nitrogen dewars; required for DNA, RNA, enzymes, and some biological samples.
- **Freezer burn:** Dehydration and oxidation of samples stored improperly in a freezer, caused by sublimation of ice crystals; prevented by airtight sealing of containers.
- **Holding temperature:** The target storage temperature specified by the analytical method or regulatory protocol for a given sample type (e.g., 4 °C for water samples, -20 °C for soil extracts, -80 °C for nucleic acids).
- **Temperature excursion:** An event in which the storage temperature falls outside the acceptable range; must be documented and the potential impact on sample integrity evaluated.
- **Ultra-low freezer (-80 °C):** A mechanical freezer capable of maintaining temperatures between -70 °C and -86 °C; used for long- term storage of DNA, RNA, microbial cultures, and enzyme preparations.

4. Responsibilities

Role	Responsibility
Lab PI	Approve storage assignments, ensure training compliance
Lab Manager	Monitor temperatures, coordinate power outage response
Trained Personnel	Store and retrieve samples following this SOP, maintain inventory records
New Students	Complete training before independent use

4.1. Acknowledgements

We thank Aparna Chintapalli for setting up the –80 C ultra-freezer map and inventory system.

5. Safety and Hazards

⚠ Hazards

- **Physical:** The –80 C freezer poses a risk of cold burns (frostbite) upon contact with surfaces or samples. Heavy drawers and racks can cause crush injuries if dropped.
- **Chemical:** No flammable materials may be stored in the laboratory refrigerator or freezers. These units are not explosion-proof and can generate significant static electricity, which can cause an explosion in the presence of flammable fumes.
- **Electrical:** Power outages can compromise stored samples (see Section 7).

❗ Important

No food or beverages shall be stored in the laboratory refrigerator or freezers under any circumstances.

5.1. Required PPE

☐ Insulated cryogenic mittens or gloves (required for –80 C freezer)
☐ Lab coat
☐ Safety glasses (when handling chemical reagents from cold storage)
☐ Closed-toed shoes

5.2. Emergency Procedures

For cold burns, warm the affected area gradually with lukewarm water; do not rub. Seek medical attention if blistering occurs. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Laboratory refrigerator	Lab, SGM 133	Set to 4 C
Laboratory freezer	Lab, SGM 133	Set to approximately –30 C
Ultra-low freezer (–80 C)	Lab, SGM 133	*TBD – confirm manufacturer and model number*
Thermometer / temperature logger	Inside each unit	For monitoring; check daily

6.2. Consumables

Item	Notes
Cryoboxes (cardboard or plastic)	For organizing samples in the –80 C freezer
Cryogenic labels / markers	Must withstand –80 C without peeling
Sample tubes (cryovials, microcentrifuge tubes)	Must be rated for the target storage temperature

7. Procedure

7.1. Refrigerator (4 C)

1. Store samples that require short-term cold storage (soil extracts, water samples awaiting analysis, reagents with refrigeration requirements)
2. Label all containers with: sample ID, date, researcher name, and project
3. Do not overcrowd the refrigerator; allow air circulation around samples
4. Remove expired samples and reagents during monthly cleanouts
5. *TBD – define shelf assignment system for the refrigerator*

7.2. Freezer (–30 C)

1. Store samples requiring medium-term frozen storage (archived soil samples, tissue samples, reagents requiring –20 C or colder)
2. Label all containers with: sample ID, date, researcher name, and project
3. Organize samples by project; do not mix projects within the same shelf area
4. *TBD – define shelf assignment and inventory system for the –30 C freezer*

7.3. Ultra-Low Freezer (–80 C)

The –80 C freezer is organized using a hierarchical location system. Every sample must be documented in the Freezer Inventory Google Sheet and in your laboratory notebook.

7.3.1. Loading Protocol

1. When loading the freezer for the first time or after defrosting, install a few drawers at a time from top to bottom, allowing the freezer to stabilize between additions
2. Only open the freezer when you are ready to place or remove samples. Minimize the time the door is open.
3. Wear insulated cryogenic mittens and a lab coat when accessing the freezer

7.3.2. Location Hierarchy

The freezer inventory uses the following hierarchy:

- **Shelf:** Each shelf is contained behind an inner door, numbered from top to bottom. (Note: Shelf 1 is currently empty.)
- **Divider:** Each divider is a column of drawers within the shelf, labeled A through E from left to right
- **Drawer:** Drawers are numbered sequentially from top to bottom within each divider
- **Box:** Each drawer contains five boxes, labeled 1 through 5 from front to back
- **Box position:** Each box is divided into 100 positions. Numbering runs from front-left (position 1) to back-right (position 100), counting along rows from left to right

7.3.3. Required Inventory Fields

Record the following in the Google Sheet and in your lab notebook each time you store or remove a sample:

Field	Description
Shelf	Shelf number (top to bottom)
Divider	Column letter (A–E, left to right)
Drawer	Drawer number (top to bottom)
Box	Box number (1–5, front to back)
Box position	Position number (1–100)
Project	Project name or code
Date collected	Date sample was collected in the field
Sample ID	Unique sample identifier
Sample description	Description including any relevant analytical results (e.g., NanoDrop concentration)

> **Tip**
>
> Before opening the freezer, identify the exact shelf, divider, drawer, box, and position you need. Have all your samples labeled and ready to go. This minimizes the time the door is open and protects the integrity of all stored samples.

8. Quality Control and Acceptance Criteria

8.1. Temperature Monitoring

- Check and record the temperature of each unit daily (or use a continuous temperature logger)
- Acceptable ranges: refrigerator 2–6 C; freezer –25 to –35 C; ultra-low freezer –75 to –85 C
- If temperature is outside the acceptable range, investigate immediately (door left ajar, power outage, compressor failure)
- *TBD – establish an alarm system or temperature logging protocol with automatic notifications*

8.2. Power Outage Protocol

! Power Outage Response

If the power goes out, it is imperative that valuable samples in either freezer be moved to a location where power is currently available to maintain freezing conditions. The laboratory generators do not provide sufficient power to keep any of the refrigerators or freezers at their appropriate temperatures.

1. Contact the lab manager and PI immediately
2. Do not open freezer doors unless moving samples to a backup location
3. A closed –80 C freezer can maintain temperature for approximately 24 hours if the door remains shut
4. If power is not expected to be restored within 4 hours, arrange to transfer critical samples to a backup freezer on campus
5. *TBD – identify backup freezer locations on campus and establish agreements for emergency sample transfer*

8.3. Inventory Maintenance

- Conduct a full inventory audit of the –80 C freezer at least once per semester
- Remove samples from completed or abandoned projects promptly
- Ensure the Google Sheet is current; discrepancies between the sheet and actual contents must be resolved immediately

9. Waste Disposal

Waste Type	Disposal Method
Expired or abandoned samples	Dispose per the relevant SOP for the sample type (e.g., biohazard, chemical waste, standard trash)
Broken cryovials	Sweep up carefully; treat as sharps if broken glass
Used cryoboxes	Standard trash (if uncontaminated)

10. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

11. Training Record

Before using the laboratory cold storage independently, personnel must:

1. Read this SOP and SOP-L002 Laboratory Safety in full
2. Demonstrate proper use of cryogenic PPE
3. Demonstrate the ability to locate a sample using the freezer hierarchy and Google Sheet
4. Successfully store and retrieve a sample under supervision, updating the inventory in both the Google Sheet and lab notebook
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L107

Hot Plates and Dry Bath

Standard Operating Procedure

SOP Number	SOP-L107
Revision	0.2
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the safe operation, maintenance, and hazard mitigation for laboratory hot plates, stirring hot plates, and digital dry bath heaters used within the environmental analysis laboratories.

2. Scope

- **Applies to:** Hot plates, stirring hot plates, and digital dry bath (heat block) heaters used for sample heating, digestion, reagent preparation, and enzymatic or thermal incubations
- **Does not apply to:** Muffle furnaces (see SOP-L111), water baths, autoclaves, or microwave digestion systems
- **Location:** Environmental analysis laboratories

3. Definitions

- **Dry bath (heating block):** An aluminum block with drilled wells that fits standard tubes (1.5 mL, 15 mL, 50 mL); heated by a resistive element to provide uniform, conductive temperature control without the hazard of a liquid bath.
- **Flash point:** The lowest temperature at which a liquid's vapor can ignite in air; flammable solvents must never be heated above their flash point on an open hot plate.
- **Hot plate:** An electrically heated flat surface used to heat beakers, flasks, or evaporating dishes; may or may not include an integrated magnetic stirrer.
- **Hot plate / stirrer:** A combination unit providing both heating and magnetic stirring; the most common configuration in the EA Program laboratory.
- **Magnetic stir bar:** A PTFE-coated magnetic rod placed in a vessel; rotated by the stirrer magnet beneath the hot plate surface to provide mixing.
- **Set temperature vs. actual temperature:** The temperature displayed on the hot plate controller may differ substantially from the actual liquid temperature; always verify with a calibrated thermometer.
- **Surface temperature limit:** The maximum safe surface temperature of the hot plate; organic solvents should never be heated on an open hot plate above their boiling point or flash point.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Thermal:** Heating surfaces and metal blocks can exceed 300 °C and remain dangerously hot long after power is disconnected. Contact causes severe thermal burns.
- **Fire and Explosion:** Heating volatile, flammable solvents on an open-bench hot plate can ignite vapors. Standard hot plates are not explosion-proof and may generate sparks from mechanical switches or internal cycling.
- **Electrical:** High current draw can overload circuits. Liquid boil-overs or spills create immediate electrical short risks.

5.1. Required PPE

☐ Safety glasses or chemical splash goggles
☐ Laboratory coat (fully buttoned)
☐ Heat-resistant thermal gloves (when handling active or cooling vessels, blocks, or glassware)
☐ Nitrile gloves (underneath thermal gloves if chemical handling is simultaneous)

5.2. Emergency Procedures

In case of burn, spill, or equipment failure, refer to SOP-L002 Laboratory Safety or call campus safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Hot plate / stirring hot plate	Lab bench	Ceramic, aluminum, or silicone-coated top plate
Digital dry bath (heat block)	Lab bench	Interchangeable aluminum blocks for various tube sizes
PTFE-coated stir bars	Supply drawer	Various sizes
External thermocouple probe	Instrument drawer	For independent temperature verification

6.2. Consumables

Item	Notes
Borosilicate glassware	Pyrex or Kimax; certified for thermal stress

Item	Notes
Interchangeable aluminum blocks	Sized for 1.5 mL, 15 mL, or other tube formats

7. Procedure

7.1. Pre-Operational Inspection

1. Inspect the power cord and plug for fraying, exposed wires, or chemical degradation. Do not use if damaged.
2. Ensure the cord does not drape across or contact the heating surface.
3. Verify that the heating surface and aluminum blocks are free of residual chemical films, crusts, or signs of pitting. Clean with a damp cloth if necessary (unit must be unplugged and cool).

7.2. Sample and Vessel Preparation

1. **Glassware inspection:** For hot plates, use only borosilicate glassware (e.g., Pyrex, Kimax) certified for high thermal stress. Inspect for star cracks, hairline fractures, or deep scratches. Discard compromised glassware.
2. **Dry bath alignment:** Ensure the interchangeable aluminum block profile matches your specific sample tubes (e.g., 1.5 mL microcentrifuge tubes vs. 15 mL conical tubes). Incorrect sizing limits thermal contact and degrades temperature control accuracy.

! Sealed Containers

Never heat completely sealed containers on a hot plate. Pressure buildup can cause catastrophic vessel failure.

7.3. Operation and Parameter Adjustment

1. Plug the unit directly into a grounded wall outlet. Avoid extension cords or daisy-chained power strips due to high wattage requirements.
2. Toggle the main power switch to the `ON` position.
3. **Temperature control:** Set the target temperature via the digital interface or analog dial.

External Temperature Probes

If using an external temperature probe (thermocouple), ensure it is securely immersed in the solution and not resting directly against the bottom or side walls of the glassware.

4. **Stirring control (if applicable):** Place the stir bar flat inside the container. Turn the RPM dial slowly from zero to the target velocity. Sudden adjustments can cause the stir bar to decouple (spin erratically), requiring the dial to be reset to zero to re-engage.

7.4. Shutdown and Post-Run Monitoring

1. Turn off the heating and stirring functions by switching controls to `0` or pressing `Stop`.
2. Turn the main power switch to `OFF`.
3. **Hot Top warning:** If the unit features a "Hot Top" indicator light, leave the unit plugged in until the surface drops below 50 °C and the warning light turns off.
4. Once completely cool, wipe down any condensed moisture or accidental spills.

8. Quality Control and Acceptance Criteria

- Verify the set temperature against an independent calibrated thermometer or thermocouple before use with critical samples.
- If the measured temperature deviates by more than ±5 °C from the set point, remove the unit from service and arrange calibration or repair.

9. Waste Disposal

Waste Type	Container	Disposal Method
Chemical residues	Appropriate waste jug	Per chemical-specific SDS
Damaged glassware	Sharps container	Standard glass waste

10. Maintenance

- **Monthly:** Wipe aluminum dry blocks with a mild laboratory detergent to remove oxidation or mineral buildup. Never use steel wool or abrasive pads.
- **Annually:** Calibrate temperature readouts using a calibrated surface thermometer or reference thermocouple.

11. References

Consult the manufacturer's manual for model-specific temperature calibration procedures and replacement parts. No external peer-reviewed references apply to this general equipment SOP.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Flammable solvent handling
L111	Muffle Furnace	Higher-temperature heating

SOP	Title	Relationship
L409	Microbial Biomass Carbon	Uses dry bath at 56 °C
L315	eDNA Processing	Uses dry bath for extraction

13. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L109

Thermo Scientific Sorvall ST8R Centrifuge

Standard Operating Procedure

SOP Number	SOP-L109
Revision	0.2
Author(s)	Micah Maglasang
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe setup, operation, and maintenance of the Thermo Scientific Sorvall ST8R centrifuge. The centrifuge is used to separate substance mixtures of different densities and is integral to sample preparation workflows across multiple laboratory SOPs (Thermo Fisher Scientific, 2016).

2. Scope

- **Applies to:** Thermo Scientific Sorvall ST8/8R centrifuge in the EA Program laboratory
- **Does not apply to:** Clinical centrifuges (see *SOP-L108*), microcentrifuges (see *SOP-L26*), or field-portable centrifuges
- **Location:** EA Program laboratory, Pomona College

3. Definitions

- **Balancing:** The process of pairing tubes of equal mass (within 0.1 g) in opposite rotor positions; required to prevent rotor imbalance, vibration, and bearing damage; failure to balance is the leading cause of centrifuge damage and injury (Thermo Fisher Scientific, 2016).
- **Fixed-angle rotor:** A rotor that holds tubes at a fixed angle (typically 20–45° from vertical); particles pellet against the side and bottom of the tube.
- **g-force (relative centrifugal force, RCF):** The centrifugal acceleration expressed as a multiple of gravitational acceleration (9.81 m s^{-2}); calculated as $\text{RCF} = 1.118 \times 10^{-5} \times r \times N^2$ where r is the rotor radius in cm and N is speed in rpm (Thermo Fisher Scientific, 2016).
- **Pellet:** The solid material sedimented to the bottom (or side-bottom) of a centrifuge tube after centrifugation.
- **Supernatant:** The liquid layer remaining above the pellet after centrifugation; the fraction of interest in many extraction procedures.
- **Swinging-bucket rotor:** A rotor whose buckets pivot to a horizontal position during spinning; particles pellet at the base of the tube, producing a well-defined pellet and cleaner supernatant.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Schedule maintenance, verify rotor condition
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Rotating rotor stores significant kinetic energy; rotor or bucket failure can eject debris. Maintain a 30 cm clear zone around the centrifuge during operation. Never open the door until the rotor has completely stopped and the display confirms it (Thermo Fisher Scientific, 2016).
- **Chemical:** Corrosive samples can damage rotors and accessories if not cleaned promptly

after use.
- **Biological:** Pathogenic or biohazardous samples require sealed tubes or biosafety rotor lids. The centrifuge is not aerosol-tight in the event of mechanical failure (World Health Organization, 2020).

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling samples)

5.2. Emergency Procedures

In the event of a hazardous spill or rotor failure:

1. Turn off the power supply immediately; pull the power plug if necessary.
2. Leave the area and do not attempt to open the centrifuge.
3. Contact the lab manager and refer to SOP-L003 Hazardous Materials Handling.
4. The emergency door release may be used only to recover samples during a power failure, not during mechanical failure (Thermo Fisher Scientific, 2016).

6. Equipment and Materials

Item	Notes
Thermo Scientific Sorvall ST8/8R centrifuge	(Thermo Fisher Scientific, 2016)
Power supply cable	Grounded outlet required
Rotors and buckets	Must be approved for this centrifuge
Instruction manual	Keep accessible near the instrument
Balance tubes	For balancing samples before runs

7. Procedure

7.1. Site Selection and Setup

1. Operate the centrifuge indoors only.
2. Place on a horizontally leveled, rigid surface with adequate load-bearing capacity.
3. Maintain a safety zone of at least 30 cm around the centrifuge; do not place any hazardous substances within this zone (Thermo Fisher Scientific, 2016).
4. Ensure the setup location is well-ventilated and not exposed to heat or direct sunlight.
5. Plug only into properly grounded sockets.
6. Keep the main plug freely accessible at all times.

7.2. Preparation

1. Inspect the rotor, buckets, and accessories for signs of corrosion, cracks, or removed protective coating; do not use damaged components (Thermo Fisher Scientific, 2016).
2. Do not modify the mechanical components of the rotor or alter any electronic components of the centrifuge.
3. Load the rotor properly: always balance samples symmetrically.
4. Never overload the rotor beyond its rated capacity.
5. Verify that the rotor is locked securely into place before closing the door.
6. When working with corrosive samples, ensure tubes are fully sealed.

! Hazardous Substances

- Do not centrifuge explosive or flammable materials.
- Do not centrifuge toxic, radioactive, or pathogenic materials without appropriate safety precautions and sealed rotor lids (World Health Organization, 2020).
- The centrifuge is neither inert nor explosion-protected; never operate in an explosion-prone environment.
- Use fully sealed tubes for highly corrosive substances that could impair rotor mechanical stability.

7.3. Operation

1. Close the centrifuge door before starting a run.
2. Set the desired speed, temperature (8R model), and time using the control panel.
3. Press START to begin the run.
4. Do not move, lean on, or place anything on top of the centrifuge while it is running.
5. Minimize time spent near the centrifuge during operation (Thermo Fisher Scientific, 2016).
6. Wait until the rotor has come to a complete stop and the display confirms it before opening the door.
7. Never open the centrifuge while the rotor is spinning.

Emergency Stop

Press the STOP key to shut down the centrifuge at any time. In an emergency, pull the power supply plug to disconnect power immediately (Thermo Fisher Scientific, 2016).

7.4. Shutdown and Cleanup

1. Remove samples promptly after the run is complete.
2. Inspect the rotor and chamber for spills or condensation.
3. If corrosive samples were centrifuged, clean the rotor, buckets, and chamber thoroughly with deionized water and dry before storage (Thermo Fisher Scientific, 2016).
4. If toxins or pathogenic substances have contaminated the centrifuge, carry out appropriate disinfection before further use (World Health Organization, 2020).

5. Wipe down the exterior of the centrifuge.
6. Leave the door slightly ajar to allow the chamber to dry (if no hazardous residues are present).

7.5. Cover Panel and Door Safety

1. Never operate the centrifuge if cover panels are damaged or missing.
2. Do not attempt to bypass the door interlock mechanism.
3. If mechanical failure occurs (rotor or bucket crash), the centrifuge is not aerosol-tight; leave the room and contact the lab manager (World Health Organization, 2020).

8. Quality Control and Acceptance Criteria

- Visually inspect the rotor and accessories before each use for cracks, corrosion, or wear (Thermo Fisher Scientific, 2016).
- Verify that samples are properly balanced before every run.
- Confirm the door closes and latches securely.
- Report any unusual vibrations, noises, or error messages to the lab manager immediately.
- Follow the manufacturer's maintenance schedule in the instruction manual (Thermo Fisher Scientific, 2016).

9. Waste Disposal

Waste Type	Disposal Method
Contaminated cleaning materials	Dispose per SOP-L003 if hazardous chemicals were involved
Damaged rotors or accessories	Contact lab manager for proper disposal
Biohazardous spill materials	Autoclave before disposal

10. References

- Thermo Fisher Scientific (2016)
- World Health Organization (2020)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Thermo Fisher Scientific. (2016). *Sorvall ST8 and ST8R centrifuge: Instruction manual*. https://www.thermofisher.com/us/en/home/life-science/lab-equipment/centrifuges/benchtop-centrifuges/sorvall-st8.html

World Health Organization. (2020). *Laboratory biosafety manual* (4th ed.). World Health Organization. https://www.who.int/publications/i/item/9789240011311

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Chemical and biological spills
L403	KCl Extraction	Uses centrifuge for soil extraction
L409	Microbial Biomass Carbon	Uses centrifuge for extract clarification

12. Training Record

Before operating the Sorvall ST8R centrifuge independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the manufacturer's instruction manual (Thermo Fisher Scientific, 2016)
4. Observe a trained operator perform rotor loading, a centrifuge run, and post-run cleanup
5. Demonstrate proper sample balancing, rotor installation, and emergency shutdown under supervision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L111

Muffle Furnace Operation

Standard Operating Procedure

SOP Number	SOP-L111
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe operation, temperature programming, and maintenance of the muffle furnace used in the EA Program laboratory. The muffle furnace provides controlled high-temperature combustion (up to 1,100 C) for loss-on-ignition analysis (SOP-L406), pre-combustion of crucibles and glass fiber filters, ashing of organic materials, and flux fusion preparations.

2. Scope

- **Applies to:** All use of muffle furnaces in EA Program laboratories, including routine ignition (550 C for SOM), high- temperature ashing (up to 1,000 C), and pre-combustion of labware
- **Does not apply to:** Drying ovens operating below 300 C (see SOP-L105); tube furnaces for elemental analysis (integral to analytical instruments); kilns
- **Location:** EA Program laboratory, SGM 134

3. Definitions

- **Ashing:** High-temperature combustion of organic matter in an open crucible in the muffle furnace, leaving only the inorganic (mineral) residue; the basis of loss-on-ignition analysis.
- **Crucible:** A heat-resistant container (ceramic, porcelain, or platinum) used to hold samples during furnace ignition; must be pre-ignited to constant mass before use.
- **Loss on ignition (LOI):** The mass loss when a dried sample is ignited at 550 °C for 4 hours, expressed as a percentage of dry mass; used as a proxy for soil organic matter content.
- **Muffle:** The interior chamber of the furnace, thermally isolated from the heating elements; provides a controlled oxidizing atmosphere for combustion.
- **Pre-combustion:** Heating glassware, crucibles, or glass fiber filters to 450–550 °C to destroy any organic contamination before use in carbon or organic matter analyses.
- **Ramping:** Programming the furnace to increase temperature at a controlled rate (e.g., 5 °C min^{-1}) to prevent thermal shock to crucibles and to avoid rapid oxidation that can cause sample loss.
- **Set point temperature:** The target temperature programmed into the furnace controller; verify with a calibrated thermocouple if precise temperature is critical.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize furnace use
Lab Manager	Schedule maintenance, verify thermocouple calibration
Trained Personnel	Operate the furnace per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

Hazards

- **Thermal:** The furnace exterior, door, and chamber reach extremely high temperatures. Crucibles removed from the furnace at 550 C or above can cause severe burns on contact. The furnace remains hot for hours after shutdown.
- **Fire:** Combustible materials (paper, wood, plastic) placed on or near the furnace can ignite. Keep a 30 cm clear zone around the furnace on all sides.
- **Fumes:** Combustion of organic matter produces CO_2, water vapor, and potentially odorous or irritating fumes. Ensure adequate room ventilation or connect the furnace exhaust to a fume hood or duct if available.
- **Electrical:** The furnace draws high current. Do not use extension cords. Inspect the power cord for damage before each use.

5.1. Required PPE

- ☐ Heat-resistant gloves (rated to at least 500 C)
- ☐ Safety glasses or face shield
- ☐ Lab coat (cotton preferred; synthetic fabrics melt)
- ☐ Closed-toed shoes

5.2. Emergency Procedures

For burns, apply cool running water immediately and seek first aid. If the furnace produces unusual smoke, odors, or sparks, shut it off at the circuit breaker and evacuate the room. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

Item	Notes
Muffle furnace (programmable)	SGM 134; confirm maximum temperature rating
Crucible tongs (long-handled, heat-rated)	For placing and retrieving crucibles
Ceramic crucibles or porcelain boats	Pre-combusted before first use
Heat-resistant pad or ceramic tile	For resting hot crucibles during transfer to desiccator
Desiccator with active desiccant	For cooling crucibles before weighing
Thermocouple or secondary thermometer	For periodic temperature verification

7. Procedure

7.1. Pre-Use Inspection

1. Inspect the furnace chamber for debris, cracked insulation, or damage to the heating elements. Do not operate if damage is visible; report to the lab manager.
2. Verify that the area around the furnace is clear of combustible materials (minimum 30 cm clearance on all sides and top)
3. Confirm that the furnace is plugged directly into a dedicated wall outlet (no extension cords or power strips)

7.2. Loading

1. Arrange crucibles or boats inside the chamber so they do not touch each other or the heating elements. Leave space for air circulation.
2. Close the furnace door gently; do not slam (the refractory lining is fragile)

7.3. Temperature Programming

1. Set the target temperature and ramp rate per the analytical SOP:
   - Loss on ignition (SOP-L406): ramp to 550 C, hold 4 hours
   - Pre-combustion of glass fiber filters: ramp to 450 C, hold 4 hours
   - Pre-combustion of crucibles: ramp to 550 C, hold 1 hour
   - Ceramic boat preparation (SSM, SOP-L407): ramp to 900 C, hold 30 minutes
2. If the furnace is programmable, set the ramp rate to approximately 10 C per minute unless the analytical SOP specifies otherwise
3. Start the program and verify that the displayed temperature begins to rise

> **Tip**
>
> Avoid placing samples into a furnace that is already at full temperature unless the analytical SOP explicitly requires it. Rapid thermal shock can crack crucibles and cause spattering.

7.4. During Operation

1. Do not leave the furnace unattended during the initial ramp-up (first 30 minutes). Check that the temperature is tracking the set point.
2. Post a “Furnace in Use” sign on the furnace and the lab door, noting the expected completion time
3. Do not open the furnace door during the hold period unless troubleshooting

7.5. Unloading

1. After the hold period, turn off the furnace or allow the programmed cooldown to proceed
2. Allow the furnace to cool to below 200 C before opening the door. Opening at higher temperatures causes rapid thermal shock to samples and exposes the operator to radiant heat.
3. Using crucible tongs and heat-resistant gloves, transfer crucibles to a heat-resistant pad, then immediately to the desiccator
4. Allow crucibles to cool to room temperature in the desiccator (approximately 30–45 minutes) before weighing

7.6. Shutdown

1. Verify that the furnace is off and the temperature is decreasing
2. Remove the "Furnace in Use" sign
3. Leave the furnace door slightly ajar (1–2 cm) after it has cooled below 100 C to prevent moisture buildup inside the chamber

8. Maintenance

Task	Frequency
Visual inspection of chamber and elements	Before each use
Clean furnace floor of spilled material	After each use
Thermocouple verification (compare to NIST-traceable thermometer)	Annually or after suspected malfunction
Replace heating elements	As needed (contact manufacturer or lab manager)
Check door seal and gasket	Every 6 months

> **!** Important
>
> Never use water or wet cloths to clean the furnace interior. Allow the chamber to cool completely, then brush out ash and debris with a dry, soft brush.

9. Quality Control

- Verify furnace temperature with an independent thermocouple or NIST-traceable thermometer at least annually. The furnace set point and the verified temperature should agree within +/- 10 C at 550 C.
- Record the verification date and result in the instrument logbook.
- If temperature uniformity is critical (e.g., for large batches), place thermocouples at the front, center, and back of the chamber and record any gradient.

10. Waste Disposal

Waste Type	Disposal Method
Combusted soil ash	Standard trash (inert mineral residue)
Crucibles	Clean and reuse
Failed heating elements	E-waste; coordinate with campus facilities

11. References

- Furnace manufacturer's operating manual (consult for model-specific instructions and maximum temperature rating)
- Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments. *Journal of Paleolimnology*, 25, 101–110.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L105	Drying Ovens	Low-temperature companion
L200	Balance Calibration	Pre- and post-ignition weighing
L406	Loss on Ignition	Primary application SOP
L407	Total Carbon	Ceramic boat preparation

13. Training Record

Before operating the muffle furnace independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper use of heat-resistant gloves and crucible tongs
4. Load, program, and unload the furnace under supervision for one complete cycle
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L112

Autoclave and Steam Sterilization

Standard Operating Procedure

SOP Number	SOP-L112
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe operation and maintenance of the laboratory autoclave for steam sterilization of glassware, media, reagent water, and biohazardous waste. Autoclaving uses saturated steam under pressure (typically 121 C at 15 psi for 15–30 minutes) to achieve sterilization

by denaturing proteins and nucleic acids in microorganisms. In the EA Program, the autoclave is used to sterilize equipment for microbiological analyses (e.g., SOP-L305 Colilert), to decontaminate foreign soil samples (SOP-L015), and to sterilize waste before disposal.

2. Scope

- **Applies to:** All use of the laboratory autoclave in EA Program laboratories for sterilization of liquids, solids, glassware, and biohazardous waste
- **Does not apply to:** Dry heat sterilization (use muffle furnace or drying oven); chemical sterilization (e.g., bleach, ethylene oxide); filter sterilization (see SOP-L114)
- **Location:** *TBD* (confirm autoclave location; shared facility or SGM)

3. Definitions

- **Autoclave:** A pressurized vessel that uses saturated steam to achieve sterilization by heating materials to 121 °C at 15 psi (103 kPa) for a defined exposure time.
- **Biological indicator:** A standardized preparation of resistant bacterial spores (typically *Geobacillus stearothermophilus*) used to verify that the autoclave cycle achieved sterilization; must be run periodically to validate cycle effectiveness.
- **Chemical indicator (autoclave tape):** Tape printed with a dye that changes color when exposed to steam and heat; indicates that the item was exposed to the cycle but does not confirm sterilization.
- **Gravity displacement cycle:** A standard autoclave cycle in which steam enters from the top, displaces cooler air downward and out through a drain; used for wrapped instruments, glassware, and liquid media.
- **Liquid cycle:** An autoclave cycle with a slow exhaust phase to prevent superheated liquids from boiling violently when pressure is released; required for all liquid loads.
- **Sterilization:** The complete elimination of all viable microorganisms, including bacterial endospores; achieved by saturated steam at 121 °C for at least 15 minutes at 15 psi.
- **Wrapped load:** Items wrapped in autoclave paper or placed in autoclave pouches before sterilization; wrapping maintains sterility after the cycle.

4. Responsibilities

Role	Responsibility
Lab PI	Authorize autoclave use, approve waste protocols
Lab Manager	Schedule maintenance, verify biological indicators
Trained Personnel	Operate the autoclave per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Thermal/Pressure:** The autoclave operates at 121 C and 15 psi. Pressurized steam causes severe burns. Never attempt to open the door while the chamber is pressurized.
- **Scalding liquids:** Autoclaved liquids are superheated and can boil violently (bumping) when disturbed. Allow liquids to cool inside the chamber for at least 10 minutes after the cycle completes, and open the door slowly.
- **Glassware failure:** Cracked or starred glassware may shatter under pressure. Inspect all glassware before loading.
- **Biological:** Biohazardous waste must be contained in autoclave bags. Assume the exterior of bags is contaminated until the cycle is verified.

5.1. Required PPE

☐ Heat-resistant gloves (autoclave gloves, rated for steam)
☐ Safety glasses or face shield
☐ Lab coat
☐ Closed-toed shoes

5.2. Emergency Procedures

For steam burns, flush with cool running water for at least 20 minutes and seek medical attention. If the autoclave fails to depressurize, do not force the door; shut off power and contact the lab manager. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

Item	Notes
Laboratory autoclave (gravity or pre-vacuum)	Confirm model and location with lab manager
Autoclave-safe trays (stainless steel or polypropylene)	For containing loads
Autoclave bags (clear or orange, printed with biohazard symbol)	For waste sterilization
Autoclave tape (indicator tape)	External indicator; changes color at 121 C
Biological indicator ampoules (e.g., *Geobacillus stearothermophilus* spore strips)	For periodic verification
Chemical integrator strips (Class 5)	Internal indicator in each load
Aluminum foil	For covering flask openings
Heat-resistant secondary containers	For liquids (to contain boil-over)

7. Procedure

7.1. Pre-Use Inspection

1. Check the water level in the autoclave reservoir; refill with distilled or deionized water if below the minimum mark
2. Inspect the door gasket for cracks, brittleness, or debris; clean if needed
3. Verify that the drain screen/strainer is clear of debris
4. Ensure the pressure gauge reads zero and the chamber is at ambient temperature

7.2. Loading

1. Select the appropriate cycle for the load type:

Load Type	Cycle	Temperature	Time	Notes
Wrapped instruments, glassware	Gravity	121 C	30 min	Loosely pack for steam penetration
Liquids (media, water)	Liquid / Slow exhaust	121 C	15–30 min	Leave caps loose or cover with foil; use slow exhaust to prevent boil-over
Biohazardous waste	Gravity	121 C	60 min	Double-bag in autoclave bags; do not seal bags tightly
Foreign soil (SOP-L015)	Gravity	121 C	30 min	Per SOP-L015 requirements

2. Place items in autoclave-safe trays. Do not overload; leave space for steam circulation.
3. For liquids: loosen caps or cover with foil. Place containers in a secondary tray to catch boil-over.
4. For waste bags: leave bags slightly open or use bags with steam-permeable patches. Place bags upright in a tray.
5. Place a chemical integrator strip inside the center of the load (the coldest point)
6. Apply autoclave indicator tape to the outside of each package or bag

7.3. Running the Cycle

1. Close and latch the autoclave door securely
2. Select the appropriate cycle (see table above)
3. Start the cycle and record the start time in the autoclave logbook
4. Monitor the temperature and pressure gauges during the initial pressurization to confirm normal operation
5. Do not leave the area until the autoclave has reached operating temperature and pressure

7.4. Unloading

1. When the cycle is complete, verify that the chamber pressure has returned to zero before opening the door
2. Crack the door open 1–2 cm and wait 2–5 minutes to allow residual steam to vent (prevents steam flash and liquid bumping)
3. Wearing autoclave gloves and face shield, open the door fully
4. Allow liquids to sit in the chamber for an additional 10 minutes after opening the door before removing
5. Remove trays using autoclave gloves; place on a heat-resistant surface away from foot traffic
6. Check the chemical integrator strip: it should show a complete color change indicating adequate steam exposure. If incomplete, the load must be re-processed.
7. Record the cycle completion time, temperature, pressure, and integrator result in the autoclave logbook

7.5. Waste Handling After Sterilization

1. After a successful waste cycle, seal the autoclave bags
2. Sterilized biohazardous waste can be disposed in the regular trash **only if** the institution's waste policy permits this and the biological indicator program confirms efficacy
3. Label the sealed bag with "Autoclaved" and the date
4. If the chemical integrator did not pass, do not dispose; re-run the cycle or contact the lab manager

7.6. Shutdown

1. After the final cycle of the day, drain the chamber if the manufacturer recommends it
2. Leave the door slightly ajar to allow the chamber to dry and prevent odor buildup
3. Wipe the door gasket with a damp cloth to remove debris

8. Verification and Quality Control

- **Chemical indicators:** Include a Class 5 integrator strip in every load. This confirms that the load reached the target temperature for the required duration.
- **Biological indicators:** Run a *Geobacillus stearothermophilus* spore test at least monthly (or per campus EH&S policy). Incubate the exposed ampoule per the manufacturer's instructions. A positive (growth) result means the autoclave failed; quarantine all loads processed since the last successful test and contact the lab manager.
- **Autoclave logbook:** Record every cycle: date, operator, load description, cycle type, temperature, pressure, duration, and indicator results.
- **Annual maintenance:** Schedule a professional inspection and calibration of the pressure gauge, safety valve, and temperature sensor at least annually.

9. Maintenance

Task	Frequency
Check water level in reservoir	Before each use
Clean door gasket	Weekly
Clean drain screen/strainer	Weekly
Run biological indicator test	Monthly
Professional inspection and calibration	Annually
Replace door gasket	As needed (when cracked or leaking)

10. Waste Disposal

Waste Type	Disposal Method
Successfully autoclaved biohazardous waste	Standard trash (if permitted by campus policy)
Failed autoclave loads (indicator did not pass)	Re-process or contact EH&S
Used chemical/biological indicator strips	Standard trash
Autoclave water (drain)	Drain (non-hazardous unless contaminated)

11. References

- CDC/HICPAC. (2008). *Guideline for Disinfection and Sterilization in Healthcare Facilities.* Centers for Disease Control and Prevention.
- Autoclave manufacturer's operating manual (consult for model- specific instructions, cycle parameters, and maintenance schedules)

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L006	Waste Disposal	Waste classification and pickup
L015	Foreign Soil Handling	Autoclave requirement for imported soil
L101	Glassware Cleaning	Pre-sterilization cleaning
L305	Colilert Quanti-Tray	Sterilized glassware requirement

13. Training Record

Before operating the autoclave independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator run a complete cycle (loading through unloading and indicator verification)
4. Demonstrate proper loading for liquids and for waste (bag preparation, indicator placement, cycle selection)
5. Run one complete cycle under supervision with a passing chemical integrator result
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L113

Laminar Flow Cabinet Operation

Standard Operating Procedure

SOP Number	SOP-L113
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, decontamination, and maintenance of the ESCO laminar flow cabinet (clean bench) used in the EA Program laboratory. The cabinet provides an ISO Class 5 (Class 100) particle-free work environment by passing HEPA-filtered air across the work surface in a unidirectional (laminar) flow pattern. It is used for aseptic work that requires protection of the *sample or product* from airborne contamination, including eDNA extraction setup, PCR master mix preparation, sterile media preparation, and filter handling for microplastics analysis.

> **!** Important
>
> A laminar flow cabinet (clean bench) protects the **work** from contamination. It does **not** protect the **operator** from hazardous aerosols, pathogens, or chemical vapors. For work involving biohazardous materials (BSL-2 organisms, human pathogens), a biological safety cabinet (BSC, Class II) is required. The EA Program does not currently work with BSL-2 materials, but if the scope changes, consult the PI and campus biosafety officer.

2. Scope

- **Applies to:** All use of the ESCO laminar flow cabinet in SGM 134, including eDNA and PCR work, sterile reagent preparation, and microplastics filter handling
- **Does not apply to:** Fume hoods (for chemical vapor protection); biological safety cabinets (BSC, Class II); field work
- **Location:** SGM 134

3. Definitions

- **Biosafety cabinet (BSC):** A ventilated enclosure that protects both the user and the environment from aerosols; classified as Class I, II, or III depending on the level of protection. Not the same as a laminar flow cabinet.
- **HEPA filter (High-Efficiency Particulate Air):** A filter rated to remove at least 99.97% of particles 0.3 µm or larger; used in laminar flow cabinets to produce particle-free air over the work surface.
- **Horizontal laminar flow cabinet:** A clean bench in which HEPA- filtered air flows horizontally from the back of the cabinet toward the user; protects samples from particles but does not protect the user from aerosols (not suitable for hazardous biological agents).
- **Laminar flow:** Smooth, parallel flow of air in one direction at uniform velocity; prevents turbulence that would re-introduce particles to the work surface.
- **UV germicidal lamp:** A short-wave ultraviolet lamp installed in some cabinets to reduce surface contamination between work sessions; effective only on exposed surfaces; never operate with personnel present.
- **Vertical laminar flow cabinet:** A clean bench in which HEPA- filtered air flows downward from the top onto the work surface; the standard configuration in the EA Program lab.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, schedule HEPA filter replacement
Lab Manager	Coordinate annual certification, maintain UV lamp log
Trained Personnel	Operate the cabinet per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **UV radiation:** The cabinet may include a UV germicidal lamp for surface decontamination. UV-C (254 nm) causes skin burns and eye damage. Never operate the UV lamp while hands or face are inside the cabinet. The UV lamp must be off and the sash open before working.
- **Electrical:** The cabinet uses high-voltage blower motors. Do not modify electrical components.
- **Chemical:** If using ethanol or bleach for surface decontamination, follow standard chemical safety precautions.

5.1. Required PPE

☐ Nitrile gloves
☐ Lab coat (clean; lint-free preferred for molecular work)
☐ Safety glasses (if UV lamp has been recently active, as a precaution during the transition)

6. Equipment and Materials

Item	Notes
ESCO laminar flow cabinet	SGM 134; horizontal or vertical flow
70% ethanol spray bottle	For surface decontamination
10% bleach solution (for eDNA work)	Destroys residual DNA on surfaces
Lint-free wipes (Kimtech or equivalent)	For wiping surfaces
UV germicidal lamp (if installed)	Built into cabinet; timer-controlled or manual

7. Procedure

7.1. Startup

1. Ensure the UV lamp is off
2. Raise or open the sash to the working position
3. Turn on the blower fan and allow it to run for at least 15 minutes before beginning work. This purges the cabinet interior and establishes stable laminar flow.
4. While the cabinet purges, wipe down the entire work surface, side walls, and interior of the sash with 70% ethanol using lint-free wipes. For eDNA work, follow the ethanol wipe with a 10% bleach wipe, then a final ethanol wipe to remove bleach residue.
5. Place only the materials needed for the immediate task inside the cabinet. Minimize clutter; objects block airflow and create turbulence.

> **Tip**
>
> Arrange items so that clean (sterile) materials are upstream (closer to the HEPA filter) and used/contaminated items are downstream (closer to the operator). This prevents contaminated air from passing over clean surfaces.

7.2. Working in the Cabinet

1. Wear clean nitrile gloves; spray gloves with 70% ethanol before reaching into the cabinet
2. Work at least 10 cm from the front edge to stay within the laminar flow zone
3. Move slowly and deliberately; rapid arm movements disrupt laminar flow and pull room air into the work zone
4. Do not block the air intake grilles (front and/or rear) with equipment, paper, or notebooks
5. Do not use open flames (Bunsen burners) inside a laminar flow cabinet; they disrupt airflow and can damage the HEPA filter
6. Keep the cabinet running continuously while working; do not turn the blower off between tasks during a session

7.3. Shutdown

1. Remove all materials from the cabinet
2. Wipe the work surface with 70% ethanol (and bleach for eDNA work)
3. Lower the sash if applicable
4. Optionally, turn on the UV lamp for 15–30 minutes for surface decontamination. Ensure no personnel are exposed to UV light during this period. Post a "UV Lamp Active" sign.
5. After UV exposure, turn off the UV lamp
6. Turn off the blower (or leave it running on low if lab policy requires continuous operation to maintain cabinet cleanliness)

7.4. UV Lamp Use

1. The UV lamp is a supplemental decontamination tool, not a substitute for chemical wipe-down
2. Run the UV lamp for 15–30 minutes after each use session
3. Record UV lamp use in the UV lamp log (posted on the cabinet). UV lamps lose efficacy over time; replace after the manufacturer's recommended hours (typically 5,000–8,000 hours).
4. Do not rely solely on UV decontamination for eDNA work; chemical wipe-down with bleach is more effective at destroying DNA.

8. Maintenance

Task	Frequency
Wipe interior with 70% ethanol	Before and after each use

Task	Frequency
Bleach wipe (eDNA/PCR work)	Before and after each use
Check blower operation and airflow	Monthly (hold a tissue at the intake to confirm airflow direction)
Replace pre-filter (if present)	Every 6 months or when visibly dirty
HEPA filter certification (by qualified technician)	Annually
Replace UV lamp	Per manufacturer hours or when annual certification shows reduced germicidal output

9. Quality Control

- Annual HEPA filter certification must confirm that the cabinet meets ISO Class 5 (less than 100 particles per ft^3 at 0.5 um). If it fails, the cabinet must not be used for aseptic work until the filter is replaced and the cabinet re-certified.
- For eDNA/PCR work: periodically swab the work surface and run a PCR negative control to verify that the decontamination procedure is effective
- Log all UV lamp hours to track lamp aging

10. Waste Disposal

Waste Type	Disposal Method
Used wipes (ethanol/bleach)	Standard trash
Used gloves	Standard trash
Spent HEPA or pre-filters	Standard trash (non-hazardous unless contaminated with biohazardous material)
Spent UV lamp	Mercury-containing waste; coordinate with campus EH&S for recycling

11. References

- ESCO Corporation. *Laminar Flow Cabinet User Manual.* (Consult for model-specific instructions.)
- NSF/ANSI 49. *Biosafety Cabinetry: Design, Construction, Performance, and Field Certification.* (Reference standard for BSCs; laminar flow cabinets are distinct but share some maintenance practices.)

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L700	DNA Extraction	eDNA work requiring clean bench
L703	PCR and Gel Electrophoresis	PCR master mix preparation
L308	Microplastics in Water	Filter handling under clean air

13. Training Record

Before operating the laminar flow cabinet independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper startup, surface decontamination, and shutdown
3. Demonstrate correct working technique (arm position, airflow awareness, material arrangement)
4. For eDNA/PCR users: demonstrate the bleach decontamination protocol
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L114

Vacuum Filtration Apparatus

Standard Operating Procedure

SOP Number	SOP-L114
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the assembly, operation, and maintenance of vacuum filtration systems used in the EA Program laboratory. Vacuum filtration separates suspended solids from liquids by drawing the liquid through a filter membrane or glass fiber filter under reduced pressure. It is used across many analytical methods, including soil and water extract filtration (SOPs L403, L409, L418), microplastics analysis (SOP-L308), chlorophyll extraction (SOP-L310), and eDNA concentration (SOP-L700).

2. Scope

- **Applies to:** All vacuum filtration in EA Program laboratories using filter flasks, filter funnels (Buchner, glass frit, analytical, or Nalgene disposable units), and Fisher Scientific Maxidry vacuum pumps or building vacuum lines
- **Does not apply to:** Syringe filtration (push-through, no vacuum); centrifugal ultrafiltration; field filtration of eDNA samples (covered in SOP-F700)
- **Location:** SGM 133 and SGM 134

3. Definitions

- **Filter funnel:** The upper component of a vacuum filtration assembly that holds the filter membrane and directs liquid onto it; typically borosilicate glass or polysulfone.
- **Filtrate:** The liquid that passes through the filter membrane and collects in the side-arm flask; the fraction of interest in most dissolved-analyte analyses.
- **Glass fiber filter (GFF):** A depth filter composed of borosilicate glass microfibers; commonly 0.7 µm nominal pore size (GF/F); used for chlorophyll, particulate carbon, and TSS analyses.
- **Membrane filter:** A thin polymeric film with a defined pore size (typically 0.45 µm); used to separate dissolved from particulate fractions in water samples.
- **Nominal pore size:** The manufacturer-stated pore size of a filter; actual retention varies by manufacturer and depends on the particle size distribution of the material being filtered.
- **Retentate:** The material retained on the filter membrane; the fraction of interest for TSS, chlorophyll, and particulate analyses.
- **Side-arm (vacuum) flask:** A thick-walled borosilicate flask with a side arm for connection to the vacuum line; designed to withstand the pressure differential during filtration.
- **Vacuum trap:** A secondary flask or bottle interposed between the filtration funnel and the vacuum pump to catch filtrate overflow and protect the pump.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain pumps, replace tubing

Role	Responsibility
Trained Personnel	Operate filtration systems per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Glassware under vacuum:** Filter flasks (Erlenmeyer with sidearm) are under reduced pressure during operation. Cracked or starred flasks can implode. Inspect all glassware before each use; reject any with visible damage.
- **Chemical:** Filtrates may contain hazardous chemicals (acids, solvents). Handle filtrate according to the analytical SOP.
- **Biological:** Samples may contain pathogens. Wear gloves.
- **Sharp edges:** Broken glass fiber filters and fritted glass funnels can cut skin. Handle with forceps.

5.1. Required PPE

☐ Safety glasses (mandatory; protects against glass implosion)
☐ Nitrile gloves
☐ Lab coat

5.2. Emergency Procedures

If a filter flask implodes, do not touch the broken glass. Sweep up fragments with a brush and dustpan (not hands) and dispose in a sharps container or labeled "Broken Glass" box. If contaminated with hazardous chemicals, follow SOP-L004 Chemical Spill Response.

6. Equipment and Materials

6.1. Filtration Hardware

Item	Qty	Location	Notes
Fisher Scientific Maxidry vacuum pump	4	SGM 134	Oil-free diaphragm pump
Filter flask (sidearm Erlenmeyer, 500 mL or 1 L)	Multiple	SGM 134	Borosilicate glass; inspect for cracks
Rubber stopper with hole (matched to funnel stem)	Multiple	SGM 134	Connects funnel to flask
Vacuum tubing (thick-wall rubber or silicone)	–	SGM 134	Check for cracks and kinks

Item	Qty	Location	Notes
In-line vacuum trap (flask or bottle between the filter flask and pump)	1 per pump	SGM 134	Prevents liquid from reaching the pump

6.2. Funnel Types

Funnel Type	Application
Buchner funnel (porcelain or polypropylene)	General filtration with flat filter paper or GF filters
Glass frit funnel (sintered glass, various porosities)	Fine filtration; reusable; requires careful cleaning
Nalgene analytical filter unit (disposable, sterile, 0.45 um PES)	Sterile filtration for microbiological and eDNA work
Millipore glass filter holder (47 mm)	Membrane filtration for water quality (TSS, chlorophyll, microplastics)

6.3. Filter Media

Filter Type	Pore Size	Application
Whatman GF/A (glass fiber)	1.6 um	General particulate filtration
Whatman GF/F (glass fiber)	0.7 um	Chlorophyll, DOC
Whatman 934-AH (glass fiber)	1.5 um	TSS, microplastics (SOP-L308)
Cellulose nitrate membrane	0.45 um	Bacterial enumeration, general
PES membrane (polyethersulfone)	0.45 um	Sterile filtration, eDNA
PVDF membrane	0.45 um	DOC filtration (low carbon blank)
PTFE membrane	0.45 um	Organic solvent filtration

> **Note**
>
> Select the filter type and pore size specified in the relevant analytical SOP. Using the wrong filter can bias results (e.g., cellulose acetate filters leach DOC and are unsuitable for carbon analysis).

7. Procedure

7.1. Assembly

1. Inspect the filter flask for cracks or stars. Do not use damaged flasks.
2. Connect vacuum tubing from the flask sidearm to the vacuum trap inlet, and from the vacuum trap outlet to the pump
3. Place the rubber stopper into the neck of the flask

4. Seat the appropriate funnel into the stopper
5. If using a 47 mm filter holder: assemble the base, place the filter on the fritted support using forceps (avoid touching the filter with fingers), and clamp the funnel top in place

7.2. Pre-Wetting or Pre-Combustion (If Required)

- For gravimetric analyses (TSS): pre-dry and pre-weigh the filter per the analytical SOP
- For DOC filtration: pre-rinse the filter with 50–100 mL of sample or DI water and discard the first rinse (removes filter-leached carbon)
- For pre-combusted GF/F filters (chlorophyll, DOC): filters should have been fired at 450 C for 4 hours per SOP-L111 and stored in a clean container

7.3. Filtration

1. Turn on the vacuum pump. Verify suction by briefly covering the funnel opening with a gloved hand (you should feel resistance).
2. Pour the sample into the funnel slowly and steadily. For particulate-laden samples, add in small aliquots to avoid overloading the filter.
3. Allow the liquid to pass through completely
4. Rinse the funnel walls with a small volume of DI water (or the matrix specified in the analytical SOP) to transfer any adhering particles to the filter
5. Continue vacuum for 10–30 seconds after the last visible liquid has passed through to remove residual moisture (do not over-dry if the filter will be extracted for chlorophyll or DOC)
6. Turn off the vacuum pump
7. Carefully disconnect the funnel from the stopper

7.4. Filter Handling

1. Remove the filter from the funnel using clean forceps
2. Transfer the filter to the appropriate container per the analytical SOP:
    - Aluminum tin (for drying/weighing: TSS)
    - Amber vial with extraction solvent (chlorophyll)
    - Extraction tube (eDNA, microplastics)
    - Petri dish (if storing before analysis)
3. Label the container immediately

7.5. Filtrate Handling

1. If the filtrate is the analyte of interest (e.g., dissolved nutrients, DOC), transfer it from the filter flask to a labeled sample vial using a clean graduated cylinder or by pouring
2. Acidify or preserve the filtrate per the analytical SOP
3. If the filtrate is waste, dispose per SOP-L006

7.6. Cleanup

1. Discard used disposable funnels and filters per the analytical SOP
2. Rinse reusable funnels, stoppers, and filter flasks with DI water immediately after use
3. For glass frit funnels: back-flush with DI water, then soak in an appropriate cleaning solution (acid bath for inorganic work, detergent for general use) per SOP-L101
4. Coil vacuum tubing loosely (do not kink) and store

8. Vacuum Pump Maintenance

Task	Frequency
Check vacuum tubing for cracks/kinks	Before each use
Empty and clean the vacuum trap	After each use session
Verify pump suction (should reach at least 20 in Hg / 500 mmHg)	Monthly
Replace vacuum tubing	Annually or when degraded
Service pump (diaphragm replacement)	Per manufacturer schedule or when suction declines

> **!** Important
>
> Always use a vacuum trap (secondary flask or bottle) between the filter flask and the pump. If liquid reaches the pump, it can damage the diaphragm and contaminate the pump internals.

9. Quality Control

- For analytical methods requiring pre-weighed filters (TSS), follow the weighing QC in the analytical SOP
- For DOC filtration, verify filter blanks by filtering DI water and analyzing the filtrate; blank DOC should be less than 0.5 mg/L
- Inspect all glassware for cracks before every use

10. Waste Disposal

Waste Type	Disposal Method
Used filters (non-hazardous)	Standard trash
Used filters (hazardous matrix)	Chemical waste per SOP-L006
Filtrate (hazardous)	Appropriate waste stream per SOP-L006
Filtrate (non-hazardous)	Drain with water if approved by EH&S
Broken glassware	Sharps container or “Broken Glass” box

11. References

- Fisher Scientific. *Maxidry Vacuum Pump User Manual.*
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater.* 22nd ed. Section 2540: Solids.

12. Related SOPs

SOP	Title	Relationship
L101	Glassware Cleaning	Cleaning funnels and flasks
L111	Muffle Furnace	Pre-combustion of GF filters
L308	Microplastics in Water	Filtration application
L310	Chlorophyll Extraction	Filtration application
L403	KCl Extraction	Extract filtration
L409	Microbial Biomass C	Extract filtration
L700	DNA Extraction	eDNA filter processing

13. Training Record

Before operating vacuum filtration systems independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper assembly of the filtration system (flask, stopper, funnel, trap, pump, tubing)
3. Demonstrate correct filter handling with forceps
4. Perform one filtration under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L115

Sonicator – Ultrasonic Bath and Probe

Standard Operating Procedure

SOP Number	SOP-L115
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation and maintenance of the ultrasonic bath and probe sonicator used in the EA Program laboratory. Sonication uses high-frequency sound waves (typically 20–40 kHz) to generate cavitation bubbles in a liquid medium. The collapse of these bubbles produces localized shear forces that disaggregate particles, disperse samples, degas solutions, enhance extraction, and clean labware. In the EA Program, sonication is used for soil particle dispersion, glassware cleaning, sample homogenization, and cell lysis in DNA extraction workflows.

2. Scope

- **Applies to:** The Fisher Scientific ultrasonic bath (SGM 134) and any probe-tip sonicators available in EA Program laboratories
- **Does not apply to:** Vortex mixing (see future SOP-L104); bead beating for DNA extraction (integral to the extraction protocol in SOP-L700); industrial-scale ultrasonics
- **Location:** SGM 134

3. Definitions

- **Amplitude:** The peak displacement of the sonicator probe tip, expressed as a percentage of maximum; higher amplitude delivers more energy per unit time to the sample.
- **Cavitation:** The formation and violent collapse of microscopic bubbles in liquid exposed to ultrasound; the primary mechanism by which ultrasound disrupts cells and disperses particles.
- **Duty cycle:** For pulsed sonication, the fraction of each cycle during which the probe is active (e.g., 0.5 s on / 0.5 s off = 50% duty cycle); used to limit heat input to sensitive samples.
- **Probe sonicator (ultrasonic processor):** A device that converts electrical energy to ultrasonic vibrations (typically 20 kHz) via a piezoelectric transducer; the probe tip vibrates in direct contact with the sample.
- **Sonication bath:** An ultrasonic cleaning bath in which the sample container is immersed in water through which ultrasound is transmitted; less intense than probe sonication; used for dispersing particles or aiding extraction.
- **Specific energy ($J\ mL^{-1}$):** Total ultrasonic energy delivered per unit volume; the parameter most predictive of cell disruption yield for a given sample type; increases with amplitude, time, and volume.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain sonicator, replace bath water
Trained Personnel	Operate the sonicator per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Hearing:** Ultrasonic baths produce audible noise (buzzing/ humming) during operation. Probe sonicators at high power can produce high-intensity noise. Wear hearing protection if exposure exceeds a few minutes at high power settings.
- **Aerosol generation:** Probe sonication of open samples generates aerosols. If the sample contains hazardous biological or chemical material, sonicate inside a fume hood or biological safety cabinet.
- **Heat:** Extended sonication heats the bath water and the sample. Monitor temperature if heat-sensitive analytes are involved.
- **Skin contact:** Do not immerse hands in the bath while it is operating. Cavitation can damage skin tissue.
- **Glassware:** Thin-walled or cracked glassware may fail under sonication. Use containers rated for ultrasonic use.

5.1. Required PPE

☐ Safety glasses
☐ Nitrile gloves
☐ Lab coat
☐ Hearing protection (for probe sonication at high power or extended bath sonication)

5.2. Emergency Procedures

For chemical spills from broken containers, follow SOP-L004 Chemical Spill Response. If the sonicator sparks, smokes, or produces unusual odors, unplug immediately and report to the lab manager.

6. Equipment and Materials

Item	Location	Notes
Fisher Scientific ultrasonic bath	SGM 134	With lid, timer, and heater (if equipped)
Probe sonicator (if available)	SGM 134	With microtip and standard tip
Rack or beaker holder for bath	SGM 134	Keeps sample containers off the bath floor for uniform sonication
Deionized water	–	For filling the bath
Detergent solution (for cleaning applications)	–	Per SOP-L101

7. Procedure

7.1. Ultrasonic Bath

7.1.1. Setup

1. Fill the bath with deionized water to the manufacturer's recommended level (typically 2–3 cm above the transducer surface or to the fill line). Do not operate the bath without water; this damages the transducer.
2. If using the bath for cleaning, add the appropriate detergent concentration per SOP-L101
3. Degas the bath water by running the sonicator for 5 minutes without a load (degassed water transmits ultrasonic energy more effectively)

7.1.2. Operation

1. Place sample containers (tubes, beakers, flasks) in the bath using a rack or holder. Containers should be partially submerged but not resting on the bath floor (direct contact with the floor can cause hot spots and uneven sonication).
2. Set the timer for the duration specified in the analytical SOP (typical ranges: 1–15 minutes for sample dispersion, 15–30 minutes for glassware cleaning)
3. If the bath has a heater, set the temperature as required (common: room temperature for samples, 40–50 C for cleaning)
4. Start the sonicator. Verify cavitation by observing fine bubbles rising from the bath surface.
5. Do not open the lid or reach into the bath during operation
6. When the timer stops, remove containers using tongs or gloves

7.1.3. Post-Use

1. Turn off the sonicator
2. If the bath water is visibly contaminated, drain, rinse, and refill with fresh DI water
3. Replace the bath water at least weekly even if it appears clean
4. Wipe the exterior with a damp cloth

7.2. Probe Sonicator

7.2.1. Setup

1. Select the appropriate tip (microtip for volumes less than 10 mL; standard tip for 10–50 mL)
2. Attach the tip securely; a loose tip reduces energy transfer and can damage the probe
3. Clamp the probe in a ring stand so the tip is immersed in the sample to approximately one-third of the liquid depth. Do not touch the container walls or bottom with the tip.

7.2.2. Operation

1. Set the power level and pulse settings per the analytical SOP (common: 20–40% amplitude, 5 seconds on / 5 seconds off pulsing to minimize heating)

2. If the sample is hazardous, work inside a fume hood or biological safety cabinet with the sash lowered
3. Start sonication. A faint hissing and visible agitation confirm cavitation.
4. Monitor sample temperature; if overheating is a concern, place the sample container in an ice bath during sonication
5. After the prescribed duration, stop the sonicator

7.2.3. Post-Use

1. Immediately wipe the probe tip with a lint-free tissue dampened with 70% ethanol or DI water
2. For molecular biology work (eDNA, PCR), decontaminate the tip with 10% bleach, rinse with DI water, then wipe with 70% ethanol
3. Inspect the tip for pitting or erosion; a heavily pitted tip reduces performance and should be replaced

8. Maintenance

Task	Frequency
Replace bath water	Weekly
Clean bath interior (drain, wipe, refill)	Weekly
Inspect probe tip for pitting	Before each use
Replace probe tip	When pitted or eroded
Check bath transducer (listen for changes in tone)	Monthly

9. Quality Control

- For soil particle dispersion, verify that the sonication time and power are sufficient by checking particle size distribution results against the analytical SOP's acceptance criteria
- For glassware cleaning, verify cleanliness by rinsing with DI water and checking for water sheeting (clean glass sheets evenly; dirty glass beads)
- If the bath fails to produce visible cavitation, contact the lab manager (the transducer may be failing)

10. Waste Disposal

Waste Type	Disposal Method
Used bath water (no hazardous chemicals)	Drain
Used bath water (with detergent or chemicals)	Dispose per SOP-L006

Waste Type	Disposal Method
Spent probe tips	Standard trash (stainless steel)

11. References

- Fisher Scientific. *Ultrasonic Bath User Manual*. (Consult for model-specific instructions.)
- Mason, T. J. & Lorimer, J. P. (2002). *Applied Sonochemistry: Uses of Power Ultrasound in Chemistry and Processing*. Wiley-VCH.

12. Related SOPs

SOP	Title	Relationship
L101	Glassware Cleaning	Ultrasonic cleaning application
L401	Soil Texture (Hydrometer)	Particle dispersion
L700	DNA Extraction	Cell lysis application

13. Training Record

Before operating the sonicator independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper bath fill level and degassing
3. For probe sonicator users: demonstrate tip attachment, immersion depth, and power/pulse settings under supervision
4. Perform one sonication task under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L116

Sample Grinding and Homogenization – Ball Mill, Mortar and Pestle

Standard Operating Procedure

SOP Number	SOP-L116
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the grinding and homogenization of dried soil, sediment, plant tissue, and other solid environmental samples to produce a fine, uniform powder suitable for chemical, elemental, and isotopic analysis. Many analytical methods require a homogeneous subsample of 0.5–20 mg (e.g., EA-IRMS capsules, SOP-L414) or 0.5–1.0 g (e.g., acid digestion, SOP-L410, SOP-L411). Without thorough grinding and homogenization, small subsamples drawn from an incompletely mixed bulk sample may not represent the true composition of that sample, introducing analytical error unrelated to the instrument or method (Bremner, 1996; Nelson & Sommers, 1996). Particle-size reduction to ≤ 150 μm also improves combustion completeness in high-temperature elemental analyzers and reduces between-capsule mass variance in EA-IRMS work (Sollins et al., 1996; Thermo Fisher Scientific, 2010).

2. Scope

- **Applies to:** Dry grinding and homogenization of air-dried or oven-dried (60–105 °C) solid samples, including mineral soils, organic soils, sediments, dried plant tissue, compost, and biochar, using agate or steel mortar and pestle, ball mill, or mechanical grinder.
- **Does not apply to:** Wet grinding or homogenization of fresh tissue (cryogenic grinding for molecular biology applications); sieving-only sample preparation (see SOP-F400 for 2 mm sieving); bead beating for DNA extraction (integral to SOP-L700); ball milling specifically for TC and EA-IRMS (see SOP-L117).
- **Location:** EA Program laboratory, SGM 134, Edmunds 017 - Rock Grinding

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain grinding equipment, replace worn components
Trained Personnel	Grind and homogenize samples per this SOP
New Students	Complete training before independent use

4. Definitions

Term	Definition
Grinding	Mechanical size reduction of solid particles by impact, compression, or abrasion (Bernhardt, 1994).

Term	Definition
Homogenization	Mixing and size reduction to produce a sample in which any subsample of a given mass has the same composition as any other subsample of that mass (Nelson & Sommers, 1996).
Particle size (d_{50})	Median particle diameter by mass; the most common target for soil analysis is 150 μm (U.S. No. 100 mesh sieve) (Nelson & Sommers, 1996).
Cross-contamination	Transfer of material from a previously processed sample to a subsequent one via residue retained on grinding equipment surfaces (Boutton, 1991).
Sacrificial subsample	A small amount of the upcoming sample ground and discarded before collecting the analytical aliquot, to purge residual material from equipment surfaces (Boutton, 1991).
Respirable crystalline silica (RCS)	Quartz and cristobalite particles $\leq$ 10 μm aerodynamic diameter released during grinding of mineral soils; an IARC Group 1 carcinogen (International Agency for Research on Cancer, 1997).
Air-dried	Dried at ambient temperature ($\leq$ 40 °C) until mass is constant; the standard pre-treatment for 2 mm sieving (Nelson & Sommers, 1996).

5. Safety and Hazards

Hazards

- **Respirable dust and silica:** Grinding dry soil and plant tissue generates fine respirable dust including crystalline silica (quartz), which is an IARC Group 1 carcinogen at particle sizes $\leq$ 10 μm (International Agency for Research on Cancer, 1997). Always perform grinding inside a fume hood or wear a NIOSH-approved N95 respirator.
- **Contaminated soils:** Soils from legacy-contaminated sites (Pb, As, Hg, pesticides, PAHs) pose additional inhalation and dermal hazards during grinding. Consult the Lab PI before processing (U.S. Environmental Protection Agency, 2007).
- **Noise:** Ball mills and mechanical grinders can exceed 85 dB. Wear hearing protection for runs $>$ 1 min (National Institute for Occupational Safety and Health, 1998).
- **Pinch/crush hazards:** Milling cups and clamps are heavy. Secure milling cups before starting the mill and keep hands clear during operation.
- **Heat**: Milling cups may get hot if samples are run for extended periods of time. Use protective gloves to handle hot items, or wait until they have cooled down enough to avoid burns.
- **Cross-contamination:** Residual sample in grinding equipment is the primary quality

risk in sequential grinding. Thorough cleaning between samples is essential (Section 6.5).

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ N95 dust mask or work in fume hood
- ☐ Hearing protection (ball mill or mechanical grinder)

5.2. Emergency Procedures

For dust inhalation, move to fresh air immediately. For cuts from broken equipment, apply first aid per SOP-L002. Report all incidents to the Lab Manager.

6. Equipment and Materials

6.1. Grinding Equipment

Item	Location	Best For
Agate mortar and pestle (various sizes)	SGM 134	Small batches (< 50 g); trace metal work (low contamination)
Ceramic (porcelain) mortar and pestle	SGM 134	General purpose; not for trace metals (contributes Al, Si)
Ball mill (planetary or mixer mill)	Edmunds 017 - Rock Grinding	Small batches (< 5g); estimated particle size
Mechanical grinder (Wiley mill, Thomas mill)	TBD	Plant tissue; coarse initial grinding

6.2. Accessories

Item	Notes
Stainless steel spatula	Transferring sample
Brush (soft bristle, nylon)	Cleaning mortar and milling cups
Lint-free wipes (Kimtech)	Wiping surfaces
70% ethanol spray bottle	Decontamination
Compressed air (can or line)	Blowing dust from crevices
Test sieves (No. 60 = 250 μm; No. 100 = 150 μm; No. 200 = 75 μm)	Verifying particle size

Item	Notes
Labeled sample vials or coin envelopes	Storing ground samples
Analytical balance	Per SOP-L200
Weighing boat/paper	Used for weighing samples

7. Procedure

7.1. Sample Selection and Drying

Samples must be thoroughly dry before grinding. Residual moisture causes particles to clump, impedes size reduction, and can promote microbial activity that alters sample chemistry during storage (Nelson & Sommers, 1996).

1. Use air-dried samples sieved to 2 mm (SOP-F400), or oven-dry per the drying requirements in the table below:

Analytical Pathway	Drying Temperature	Rationale
TC, LOI, metals, or general chemistry	105 °C to constant mass	Standard gravimetric drying; isotope fractionation not a concern (Nelson & Sommers, 1996)
δ^{15}N, δ^{13}C (EA-IRMS)	40–60 °C to constant mass	Temperatures > 60 °C volatilize ^{14}N-enriched NH_4^+, artificially elevating δ^{15}N (Kool et al., 2011; **Guntiñas2012?**)
Plant tissue (any analysis)	60 °C to constant mass	Higher temperatures degrade labile N compounds and alter tissue δ^{15}N (Kalra, 1998)

2. Cool samples to room temperature in a desiccator before grinding.
3. Record drying temperature, start and end times, and initial and final mass in the lab notebook.

7.2. Mortar and Pestle (Manual Grinding)

Manual grinding with mortar and pestle is appropriate for small batches (< 50 g), hard materials requiring careful size control, and laboratories where a ball mill is unavailable (Nelson & Sommers, 1996).

1. Place a clean, dry mortar on a stable bench surface. If working with contaminated soil or generating visible dust, work inside a fume hood.

2. Add a small amount of dried sample (5–20 g) to the mortar.
3. Grind with a circular and pressing motion, periodically scraping material from the sides back to the center.
4. Continue grinding until the sample passes through the target sieve (No. 60 [250 μm] for general chemistry; No. 100 [150 μm] for EA-IRMS (Thermo Fisher Scientific, 2010)). For plant tissue, grind to a uniform fine powder.
5. Transfer the ground sample to a labeled vial or coin envelope with a clean spatula.
6. Record the sample ID and particle size achieved.

> **Tip**
>
> For hard or fibrous samples (e.g., woody roots, calcium carbonate nodules), pre-crush coarsely with a hammer inside a thick plastic bag before transferring to the mortar. This reduces grinding time and wear on the mortar.

7.3. Ball Mill (Mechanical Grinding)

Ball milling provides more reproducible particle size reduction and higher throughput than manual grinding, and is preferred for batches > 20 samples or where particle size uniformity is critical (Bernhardt, 1994).

1. Select the appropriate milling cup and ball material (*confirm which materials we have for balls/jars*):

Material	Best Use Case	Notes
Stainless steel	General chemistry (non-metal)	Faster; may contribute Fe, Cr, Ni, Mn
Ceramic	TBD	TBD

2. Load the sample and milling balls into the milling cup. The milling cup's volume should be occupied roughly by 1/3 sample, 1/3 grinding ball(s) and 1/3 air.

> **Safe Running**
>
> - **Filling:** Sample quantity should not be below 25% of the milling cup's holding capacity.
> - **Material** Milling ball and milling cup material must always be the same.
> - **Single Running** Do not run machine with only one milling cup loaded. If you only need to use one milling cup, still load the second one completely empty (no sample nor milling balls)

3. Tightly seal milling cup and load it into the milling cup holder. Make sure to insert cups in the correct orientation, as shown below:



Figure 1: *Orientation of milling cup for left hand side holder. Cup should be mirrored for the right hand side holder.*

4. Turn locking pin into the locked position as shown in the image below and turn the handwheel until maximum clamping range is reached. Close hood.



Figure 2: *Locked and unlocked pin positions*

5. Set speed and duration per the table below or per the analytical SOP:

Sample Type	Frequency (Hz)	Duration	Target Size
Organic soil	30	2 min	≤ 75 μm
Quartz Sand	30	1 min	N/A

6. Run the mill. If the milling cup becomes warm to the touch, alternate 2-minute grinding intervals with 1-minute rest periods to prevent thermal alteration of labile organic matter and N compounds (Kool et al., 2011).
7. After run ends, open the hood and unlock the locking pin before removing milling cups. Wait 30 seconds after stopping before opening the milling cup (dust settles). Open inside the fume hood or with N95 protection.
8. Transfer the ground sample to a labeled container.
9. If required, verify particle size by passing a subsample through the target sieve.
10. Perform cleaning run with quartz sand in between sample runs. Add the same volume of quartz sand as the samples you are running, and the milling balls you are using. The quartz run will help clean the cups and remove any sample that might have gotten stuck to the cup. After the quartz run, clean cup thoroughly according to the "Cleaning Between Samples" section.

7.4. Mechanical Grinder (Plant Tissue)

A Wiley-type or Thomas-type cutting mill provides rapid coarse grinding of dried plant tissue and is generally faster than mortar and pestle for batch processing (Kalra, 1998).

1. Set the screen mesh to the target particle size (1 mm [No. 18] or 0.5 mm [No. 35]).
2. Feed dried tissue into the hopper slowly; do not overload.
3. Collect ground material from the output chamber.
4. For analyses requiring finer particle size (e.g., EA-IRMS), follow with mortar and pestle or ball mill to reduce further (Thermo Fisher Scientific, 2010).

7.5. Cleaning Between Samples

Cross-contamination is the primary quality risk in sequential grinding. For isotope work, residues as small as 0.05 mg C from a high-carbon sample can produce measurable δ^{13}C bias in a subsequent low-carbon sample (Boutton, 1991). Clean thoroughly between every sample:

1. Disassemble the mortar/pestle or milling cup and balls.
2. Brush out all visible residue with a soft-bristle brush.
3. Wipe all surfaces with a lint-free tissue dampened with 70% ethanol.
4. **Trace metal work:** rinse with 10% HNO_3 (trace-metal grade), then triple-rinse with Type I deionized water (18.2 MΩ), and air-dry (U.S. Environmental Protection Agency, 2007).
5. **Isotope work:** rinse with Type I deionized water, wipe with ethanol, and blow dry with clean compressed air.

6. **Wiley mill:** run a small amount of clean quartz sand through the mill between samples to abrade residues from the cutting chamber, then brush and wipe.
7. Allow all equipment to dry completely before grinding the next sample.

> **!** Important
>
> When transitioning between samples with very different compositions (e.g., high-carbon compost followed by low-carbon mineral soil), grind a sacrificial subsample of the next sample and discard it before collecting the analytical aliquot. This purges micro-residues that ethanol-wiping alone may not remove (Boutton, 1991). This step is mandatory for EA-IRMS work.

7.6. Storage

1. Transfer ground powder to a labeled vial (glass or polypropylene with a polyethylene-lined cap). Avoid foam-gasketed caps, which can off-gas organic vapors.
2. Label with: sample ID, date ground, equipment and milling cup material, and target particle size.
3. Store in a desiccator at room temperature. Ground samples have large surface area and are hygroscopic; even brief exposure to humid air can add 0.5–2% mass as adsorbed water (Nelson & Sommers, 1996).
4. For long-term storage (> 2 weeks), seal with Parafilm and store at $-$20 °C.
5. Record grinding date, equipment, milling cup material, speed, and duration in the lab notebook and sample tracking spreadsheet.

8. Quality Control

QC Check	Frequency	Acceptance Criterion
Particle size verification (sieve)	$\geq$ 10% of samples per batch	$\geq$ 90% by mass passes target sieve; re-grind failures
Equipment blank (clean milling cup 2 min)	Once per batch and after new milling cup	No visible powder; TC blank $<$ 0.05 mg C if applicable
Certified reference material (CRM) – trace metals	1 per batch (trace metal work)	Recovered elemental concentration within 10% of certified value (U.S. Environmental Protection Agency, 2007)
Reference standard – isotopes (USGS40, USGS41a)	1 per batch (isotope work)	δ^{13}C within $\pm$ 0.5 ‰; δ^{15}N within $\pm$ 0.5 ‰ of certified value (Brand et al., 2014)
Sample replicate (duplicate grind)	1 per 10 samples	%C or %N: CV $\leq$ 5%; isotopes: SD $\leq$ 0.3 ‰
Sacrificial subsample log	Every inter-sample cleaning (isotope work)	Recorded in lab notebook

Document grinding equipment and milling cup material for every batch; different materials introduce different trace elements and the record is essential for troubleshooting contamination (Bernhardt, 1994).

9. Waste Disposal

Waste Type	Disposal Method
Grinding residue (non-hazardous soil/plant)	Standard solid waste or return to sample stock
Grinding residue (contaminated soil)	Hazardous solid waste per SOP-L006
Quartz sand (cleaning aid)	Standard solid waste
Used ethanol wipes	Standard solid waste (non-halogenated)
Worn grinding balls or milling cups (agate, ceramic)	Standard solid waste
Worn grinding components (stainless steel)	Metal recycling
HNO_3 rinse waste	Acid waste per SOP-L006

10. References

Bernhardt, C. (1994). *Zerkleinerungstechnik* (3rd ed.). Deutscher Verlag für Grundstoffindustrie.

Boutton, T. W. (1991). *Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis*. 155–171.

Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M., & Prohaska, T. (2014). Assessment of international reference materials for isotope-ratio analysis (IUPAC technical report). *Pure and Applied Chemistry*, *86*(3), 425–467. https://doi.org/10.1515/pac-2013-1023

Bremner, J. M. (1996). Nitrogen – total. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 1085–1121). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c37

International Agency for Research on Cancer. (1997). *Silica, some silicates, coal dust and para-aramid fibrils* (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 68). IARC. https://publications.iarc.fr/81

Kalra, Y. P. (Ed.). (1998). *Handbook of reference methods for plant analysis*. CRC Press. https://doi.org/10.1201/9781420049398

Kool, D. M., Dolfing, J., Wrage, N., & Van Groenigen, J. W. (2011). Nitrification and denitrification as sources of sediment nitrous oxide production: A minireview. *Journal of Integrative Plant Biology*, *53*(1), 7–16. https://doi.org/10.1111/j.1744-7909.2010.01009.x

National Institute for Occupational Safety and Health. (1998). *Criteria for a recommended standard: Occupational noise exposure* (Nos. 98-126). NIOSH. https://www.cdc.gov/niosh/docs/98-126/

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 961–1010). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c34

Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter: Mechanisms and controls. In *Geoderma* (Vol. 74, pp. 65–105). https://doi.org/10.1016/S0016-7061(96)00036-5

Thermo Fisher Scientific. (2010). *Flash IRMS EA: Operating manual*.

U.S. Environmental Protection Agency. (2007). *Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils*. U.S. EPA. https://www.epa.gov/sites/default/files/2015-12/documents/3051a.pdf

11. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	2 mm sieving before grinding
F608	Plant Tissue Sampling	Dried tissue requiring grinding
L105	Drying Ovens	Pre-grinding drying
L117	Ball Mill Prep for TC and EA-IRMS	Instrument-specific ball mill protocol
L200	Balance Calibration	Weighing ground subsamples
L406	Loss on Ignition	Downstream analysis
L410	Hot-Plate Acid Digestion	Requires ground sample
L411	MARS Microwave Digestion	Requires ground sample
L414	EA-IRMS Combustion	Requires finely ground sample
L415	Carbonate GasBench-IRMS	Requires ground carbonate sample

12. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP in full and sign the SOP acknowledgment form.
2. Complete SOP-L002 Laboratory Safety training.
3. Demonstrate proper mortar-and-pestle technique including grinding motion and inter-sample cleaning.
4. If using the ball mill, demonstrate proper milling cup loading, speed/time selection, and milling cup cleaning per Section 7.5.
5. Grind at least 5 samples under supervision with acceptable particle size verification (all material passes the target sieve).
6. Be signed off by the PI or Lab Manager.

Trainee	Trainer	Date	Initials

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L200

Analytical Balance Calibration and Operation – Mettler Toledo

Standard Operating Procedure

SOP Number	SOP-L200
Revision	1.0
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the routine calibration, verification, and proper operation of Mettler Toledo analytical balances used in the EA Program laboratory. Accurate mass measurements are fundamental to nearly every quantitative laboratory procedure, including soil moisture determination (SOP-L400), loss on ignition (SOP-L406), DNA extraction (SOP-L700), and chemical reagent preparation. Regular calibration ensures that balance readings are traceable to national standards and within acceptable tolerance for analytical work (Harris & Lucy, 2020; Mettler Toledo, 2023).

2. Scope

- **Applies to:** All Mettler Toledo analytical balances (0.1 mg readability) and precision balances (0.01 g or 0.001 g readability) in the EA Program laboratory
- **Does not apply to:** Portable field balances, top-loading balances used only for rough weighing (greater than 1 g tolerance), or microbalances (0.001 mg readability) requiring specialized calibration procedures
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Accuracy:** The closeness of a measurement to the true (reference) value; assessed by weighing a certified reference weight and comparing the result to the certified mass.
- **Calibration:** Comparison of a balance's readings against reference weights of known mass to establish a correction curve or verify performance within tolerance.
- **Certified reference weight:** A weight whose mass is traceable to national or international measurement standards (NIST or equivalent); classified by tolerance class (OIML E1 through M3 or ASTM 0 through 7).
- **Drift:** Slow, systematic change in balance readings over time, caused by temperature fluctuation, vibration, or electronic aging.
- **External calibration:** Calibration using calibrated weights placed manually on the pan; required on balances without internal weights.
- **Internal calibration (AutoCal):** Automatic calibration using a built-in reference weight that is mechanically engaged by the balance; the Mettler Toledo XPE205 performs internal calibration automatically in response to temperature changes.
- **Linearity:** The degree to which balance readings are proportional to applied mass across the full weighing range; assessed by weighing multiple reference weights spanning the range.
- **Repeatability:** The standard deviation of repeated measurements of the same weight under identical conditions; a key indicator of short-term balance precision.
- **Tare:** The function that zeroes the displayed mass with a container on the pan, so that subsequent readings show only the mass of material added.

4. Responsibilities

Role	Responsibility
Lab PI	Ensure annual professional calibration is scheduled
Lab Manager	Perform monthly verification checks, maintain calibration log
Trained Personnel	Perform daily checks before use, report malfunctions
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Calibration weights are dense metal objects; dropping them can cause injury to feet or damage to the balance pan. Handle with forceps only.
- **Chemical:** Some samples weighed on the balance may be hazardous. Refer to the relevant SOP for the material being weighed.

5.1. Required PPE

☐ Lab coat
☐ Nitrile gloves (when handling calibration weights or chemical samples)
☐ Safety glasses (when weighing chemical reagents)

5.2. Emergency Procedures

If a chemical spill occurs on or near the balance, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use. If the balance pan or draft shield is damaged, remove the balance from service and contact the lab manager.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Mettler Toledo analytical balance	Lab bench, SGM 133	*TBD – confirm model number (e.g., ME204, MS205DU, or XPE205)*
Anti-vibration table or stone slab	Lab bench	Balance must sit on a stable, vibration-free surface
Spirit level (built into balance)	On balance base	For leveling verification

6.2. Calibration Weights and Supplies

Item	Notes
ASTM Class 1 or OIML Class E2 calibration weight set	Traceable to NIST; includes 1 mg, 10 mg, 100 mg, 1 g, 10 g, 50 g, 100 g, 200 g (or as appropriate for balance capacity)
Weight handling forceps	Never touch weights with bare hands
Lint-free wipes (Kimwipes)	For cleaning the pan
Compressed air canister (filtered)	For removing dust from the chamber
Calibration log binder or digital log	For recording all checks

7. Procedure

7.1. Balance Placement and Environment

Proper placement is essential for accurate weighing. The balance should remain in the same location and should not be moved unless absolutely necessary (Mettler Toledo, 2023).

1. Place the balance on a dedicated anti-vibration table or stone slab, away from doors, windows, air vents, and foot traffic
2. Keep the balance away from direct sunlight and heat sources (ovens, hot plates), which cause thermal drift
3. Verify the balance is level using the built-in spirit level. Adjust the leveling feet until the bubble is centered.
4. Allow the balance to warm up for at least 30 minutes after being powered on before performing any measurements or calibration (Harris & Lucy, 2020)

7.2. Internal Calibration (FACT/iSOCAL)

Most Mettler Toledo analytical balances are equipped with an internal calibration function (Fully Automatic Calibration Technology, FACT, or iSOCAL) that uses a built-in reference weight (Mettler Toledo, 2023).

1. Ensure the balance is unloaded and the draft shield doors are closed
2. Press the internal calibration button or navigate to **Menu > Calibration > Internal Calibration** (exact path varies by model)
3. The balance will automatically perform the calibration sequence. Do not touch the balance, lean on the bench, or create air currents during this process.
4. When the display reads “Calibration Done” or returns to the weighing screen, record the date and result in the calibration log
5. If the internal calibration fails, repeat the process. If it fails again, remove the balance from service and contact the lab manager.

> **Note**
>
> If the balance is set to automatic FACT mode, it will self-calibrate whenever it detects a temperature change of 1.5 C or more. However, manual internal calibration should still be performed monthly as part of the verification schedule.

7.3. External Calibration Verification (Monthly)

External verification uses traceable reference weights to confirm that the balance is reading accurately across its range. This check does not replace the annual professional calibration but provides ongoing confidence in measurement quality (**aac_guide2019?**).

1. Perform an internal calibration first (Section 6.2)
2. Allow the balance to stabilize with an empty pan and closed draft shield. Tare to read 0.0000 g (for a 0.1 mg balance).
3. Using forceps, place the reference weights on the center of the pan one at a time, starting with the smallest. Record the displayed mass for each weight.
4. Test at a minimum of three points spanning the expected working range of the balance (e.g., low, mid, and high):

Check Point	Reference Weight	Acceptance Criterion (analytical, 0.1 mg)
Low	1 g	Displayed value within +/- 0.5 mg of nominal
Mid	50 g	Displayed value within +/- 0.5 mg of nominal
High	200 g	Displayed value within +/- 1.0 mg of nominal

5. If any reading falls outside the acceptance criterion, re-zero, repeat the measurement, and clean the pan and weight with a lint-free wipe. If the reading is still out of tolerance, perform an external calibration (Section 6.4) or remove the balance from service.
6. Record all results, the date, and the operator initials in the calibration log.

> **Tip**
>
> Always place weights in the center of the pan. Off-center loading (corner loading) can introduce errors, especially on high-resolution balances. Periodically test corner loading by placing a single weight at each corner and comparing readings.

7.4. External Calibration (As Needed)

If the internal calibration or monthly verification indicates the balance is out of tolerance, perform a full external calibration using a traceable reference weight.

1. Navigate to **Menu > Calibration > External Calibration**

2. When prompted, place the specified calibration weight (the balance will display the required mass, typically its full capacity or a specific test weight)
3. Place the weight on the center of the pan using forceps
4. Wait for the calibration to complete and record the result
5. Repeat the verification checks in Section 6.3 to confirm the balance is now within tolerance
6. *TBD – confirm external calibration weight value for the specific Mettler model in the lab*

7.5. Annual Professional Calibration

1. Schedule an annual on-site calibration by a Mettler Toledo service technician or an ISO 17025-accredited calibration laboratory
2. The technician will perform a comprehensive calibration including linearity, eccentricity (corner loading), repeatability, and hysteresis tests
3. Retain the calibration certificate in the lab's quality records binder
4. *TBD – record the current service contract details and contact information for scheduling*

7.6. Daily Use and Best Practices

Follow these practices every time you use the balance to ensure accurate and reproducible results (Harris & Lucy, 2020; Mettler Toledo, 2023):

1. Check that the balance is level before use
2. Close all draft shield doors before taring or reading a mass
3. Allow the reading to stabilize fully (stability indicator appears) before recording a value
4. Use appropriate weighing vessels (aluminum tins, ceramic crucibles, weighing paper, or glass vials depending on the application)
5. Tare the vessel before adding sample material
6. Handle objects with forceps, spatulas, or gloves; fingerprints add mass and oils that cause drift
7. Never place chemicals directly on the pan
8. Clean up any spills immediately with a lint-free wipe or brush
9. Leave the draft shield closed and the balance powered on between uses to maintain thermal equilibrium
10. Record the balance ID, date, and any anomalies in the lab notebook

7.7. Cleaning and Maintenance

1. Wipe the pan and interior of the draft shield with a lint-free wipe after each use
2. Use filtered compressed air to remove dust from hard-to-reach areas inside the weighing chamber
3. If the pan becomes stained or corroded, remove it and clean with mild soap and water; dry thoroughly before replacing
4. Do not use solvents to clean the draft shield (they may damage acrylic or glass)
5. Inspect the draft shield gaskets periodically for wear; replace if they no longer seal properly

8. Quality Control and Acceptance Criteria

- Internal calibration (FACT): at minimum monthly, or after any balance relocation or significant temperature change
- External verification with traceable weights: monthly, recorded in the calibration log
- Annual professional calibration: required; certificate on file
- Daily pre-use level check: required
- Acceptance tolerance for routine analytical work (0.1 mg readability): displayed value within +/- 0.5 mg of the nominal weight for loads up to 100 g, and within +/- 1.0 mg for loads above 100 g
- If tolerance is exceeded after re-calibration, tag the balance "OUT OF SERVICE" and notify the lab manager

8.1. Calibration Log

Maintain a calibration log (paper binder or spreadsheet) with the following columns:

Date	Operator	Check Type	Weight Used (g)	Displayed Value (g)	Pass/Fail	Notes

9. Waste Disposal

No waste is generated by this procedure. Cleaning materials (Kimwipes) can be disposed of in standard trash unless they contacted hazardous chemicals, in which case follow SOP-L003.

10. References

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Mettler Toledo. (2023). *Good weighing practice: The SOP for balances and scales*. https://www.mt.com/us/en/home/library/guides/laboratory-weighing/good-weighing-practice.html

11. Training Record

Before using the analytical balance independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform internal calibration and external verification
3. Demonstrate proper leveling, taring, weighing, and draft shield use
4. Successfully complete a verification check with reference weights under supervision
5. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Reading drifts continuously	Air currents or vibration	Close draft shield doors; check placement away from vents and traffic
Reading drifts slowly in one direction	Temperature change or warm sample	Allow balance to equilibrate; cool samples to room temperature before weighing
Balance does not tare to zero	Residue on pan or overloaded tare	Clean pan; remove vessel and re-tare
Internal calibration fails	Significant temperature change or mechanical issue	Allow 30 min warm-up; retry. If persistent, contact lab manager.
Displayed value differs from reference weight by more than tolerance	Calibration drift	Perform external calibration (Section 6.4). If still out, remove from service.
"Overload" message	Sample exceeds balance capacity	Use a balance with higher capacity
Balance will not power on	Power supply disconnected or fuse blown	Check power connection; contact lab manager
Unstable reading with small masses (less than 10 mg)	Static charge on sample or vessel	Use anti-static device or ionizer; ground yourself before weighing

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L203

Trilogy Laboratory Fluorometer

Standard Operating Procedure

SOP Number	SOP-L203
Revision	0.5
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Turner Designs Trilogy Laboratory Fluorometer, a compact, multifunctional instrument used for fluorescence, absorbance, and turbidity measurements with interchangeable snap-in optical modules. The Trilogy is the primary instrument in the EA Program laboratory for chlorophyll *a* determination and turbidity analysis of water samples (Arar & Collins, 1997; Turner Designs, 2014).

2. Scope

- **Applies to:** Turner Designs Trilogy Laboratory Fluorometer equipped with fluorescence, absorbance, and turbidity optical modules
- **Does not apply to:** Field fluorometers, in-vivo chlorophyll measurements with multiprobes (see SOP-F200), or benchtop spectrophotometers (Hach DR3900; see SOP-L303)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure calibration standards are current
Lab Manager	Maintain optical modules, replace light sources as needed
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Blank correction:** Subtraction of the fluorescence signal from a blank (solvent only, no sample) from sample readings to remove background fluorescence from the solvent and optical components.
- **Chlorophyll *a*:** The primary photosynthetic pigment in phytoplankton and algae; quantified by the Trilogy using a chlorophyll *a* module (excitation ~430 nm, emission ~685 nm); expressed in µg L^{-1} (water) or µg g^{-1} (sediment) (Arar & Collins, 1997).

- **Emission wavelength:** The wavelength at which the fluorophore emits light after excitation; detected by the fluorometer's photodetector.
- **Excitation wavelength:** The wavelength of light used to illuminate the sample; chosen to match the absorption maximum of the target fluorophore.
- **Fluorometer:** An instrument that measures fluorescence intensity emitted by a sample after excitation; more sensitive than spectrophotometry for low-concentration analytes such as chlorophyll *a* and CDOM (Turner Designs, 2014).
- **Linear dynamic range (LDR):** The concentration range over which instrument response is linear; for the Trilogy chlorophyll module, approximately 0–1000 µg/L (Turner Designs, 2014).
- **Module (Trilogy):** An interchangeable optical block that sets the excitation and emission wavelengths for a specific analyte; available modules include chlorophyll *a* (acidification and non-acidification), CDOM, rhodamine WT, and turbidity.
- **Pheophytin *a*:** A degradation product of chlorophyll *a* formed by loss of the central magnesium ion; contributes to total fluorescence and is distinguished from chlorophyll *a* by the acidification step of EPA Method 445.0 (Arar & Collins, 1997).
- **Quinine sulfate unit (QSU):** A fluorescence unit based on the fluorescence of a standard quinine sulfate solution; used for CDOM and some other modules.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Chlorophyll extraction uses 90% acetone, which is flammable and an irritant. Handle in a fume hood. Acidification with 0.1 N HCl is required for EPA 445.0. See SOP-L003.
- **Physical:** Glass test tubes used for extracted chlorophyll measurements can break. Handle with care.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (especially when handling acetone or standards)

5.2. Emergency Procedures

For acetone spills, extinguish all ignition sources, ventilate the area, and clean up per SOP-L003 Hazardous Materials Handling. For skin or eye contact with acetone or acid, flush with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Turner Designs Trilogy Fluorometer	With color touch screen (Turner Designs, 2014)
Fluorescence optical module(s)	Application-specific (e.g., chlorophyll *a*, rhodamine WT)
Absorbance optical module	With interchangeable filter paddles (560/10, 600/10, 750/10 nm)
Turbidity optical module	Infrared LED at 850 nm (ISO 7027)
Solid secondary standard	For verifying instrument stability
Printer or PC connection	For data export (USB or serial)

6.2. Consumables

Item	Notes
Methacrylate cuvettes (10 × 10 mm)	Minimum 2 mL; for ammonium and general fluorescence
Polystyrene cuvettes (10 × 10 mm)	For turbidity measurements
Glass cuvettes (10 × 10 mm)	Minimum 1.5 mL; for absorbance
Glass test tubes (12 × 35 mm)	For extracted chlorophyll measurements

6.3. Reagents and Standards

Item	Notes
Chlorophyll *a* standard	Pure chlorophyll *a* in 90% acetone; Turner Designs or equivalent
90% acetone (reagent grade)	For chlorophyll extraction
0.1 N HCl	For acidification step (EPA 445.0) (Arar & Collins, 1997)
Formazine turbidity standards	4000 NTU primary standard; valid 4 weeks after preparation
Ultrapure water	For blanks

7. Procedure

7.1. Startup

1. Turn on the Trilogy using the power switch on the back of the instrument.

2. Allow the instrument to warm up for approximately 10 minutes before taking measurements (Turner Designs, 2014).
3. Connect the Trilogy to a printer or PC if data export is needed.

7.2. Installing an Optical Module

1. Select the appropriate optical module for the analysis:
   - **Fluorescence module** for chlorophyll *a*, rhodamine WT, or other fluorescent analytes
   - **Absorbance module** for colorimetric assays
   - **Turbidity module** for nephelometric turbidity measurements
2. Open the sample compartment lid.
3. Align the module with the slot and press firmly until it clicks into place.
4. The Trilogy will automatically detect the installed module and display the corresponding menu on the touch screen.
5. If using the absorbance module, install the appropriate filter paddle for the target wavelength.

7.3. Selecting the Measurement Mode

Mode	When to Use
Raw mode	Qualitative measurements; no calibration required; results in RFU, raw absorbance, or NTU
Direct Concentration mode	Quantitative measurements in concentration units; calibration required

7.4. Fluorescence Module: Chlorophyll *a* (EPA Method 445.0)

EPA Method 445.0 is the standard fluorometric method for measuring extracted chlorophyll *a* and pheophytin *a* in marine and freshwater algae (Arar & Collins, 1997).

1. Calibrate the fluorescence module in Direct Concentration mode using a known concentration of pure chlorophyll *a* in 90% acetone (see §7.3).
2. Insert the glass test tube containing the extracted sample.
3. Touch **Measure Fluorescence** to obtain the pre-acidification reading.
4. Add the prescribed volume of 0.1 N HCl, mix gently, and wait 90 seconds.
5. Measure again to obtain the post-acidification reading.
6. Calculate chlorophyll *a* and pheophytin *a* per EPA 445.0 (Arar & Collins, 1997).

> **Standard Stability**
>
> Recalibrate with a fresh chlorophyll *a* standard every few months. The standard degrades over time, especially if exposed to light or warmth (Turner Designs, 2014).

7.5. Single-Sample Measurements

1. Open the lid and insert the appropriate cuvette or test tube.
2. Close the lid.
3. Optionally, touch **Sample ID** to name the sample; touch **Save**.
4. Touch the **Measure** button corresponding to the installed module.
5. The Trilogy measures the sample for 6 seconds and reports the average reading.
6. Results for the most recent 20 measurements are displayed on the home screen.

> **!** Important
>
> The Trilogy stores a maximum of 20 measurements at a time. Older readings are overwritten. Measurements are not retained between power cycles. Always export data to a printer or PC before turning off the instrument or exceeding 20 readings.

7.6. Shutdown

1. Export all data to a printer or PC.
2. Remove the cuvette or test tube from the sample compartment.
3. Clean the cuvette holder if any liquid was spilled (wipe with 70% ethanol and a lint-free tissue).
4. Close the sample compartment lid.
5. Turn off the instrument using the rear power switch.

8. Quality Control and Acceptance Criteria

8.1. When to Calibrate

1. Before running a new batch of samples
2. After a temperature change of ±5 °C in the lab
3. After changing to a different optical module or analyte
4. When verification readings deviate unacceptably from the expected value

8.2. Calibration Overview

Calibration converts the raw instrument signal to concentration units. The Trilogy supports single-point and multi-point calibrations (up to five standards plus a blank). Multi-point calibrations provide superior accuracy across a wide concentration range (Turner Designs, 2014).

8.3. Direct Concentration Calibration Procedure

1. Turn on the Trilogy and confirm the correct module is installed.
2. Touch **Calibrate** on the home screen.
3. Select **Run New Calibration**.

4. Select the desired unit of measurement.
5. Insert the blank (ultrapure water or 90% acetone, as appropriate) and touch **OK**.
6. Enter the concentration of the first standard. For multi-point calibrations, begin with the lowest concentration.
7. Insert the standard and touch **OK**.
8. After the reading, either select **Proceed with Current Calibration** or **Enter More Standards**.
9. Optionally, save the calibration for future use.
10. Verify calibration by measuring the same standard used in step 6; the displayed concentration should match the known value within the acceptable tolerance (Turner Designs, 2014).

8.4. Verification

- After calibration, re-read a mid-range standard to confirm accuracy.
- Re-read the standard every few hours during extended sessions to detect drift.
- Optionally, read the solid secondary standard and record its value for instrument stability tracking.
- If verification readings drift beyond acceptable limits, recalibrate before continuing.

8.5. QC Acceptance Criteria

- Laboratory reagent blank (LRB) every 10–15 samples; must read below the lower detection limit.
- QC standards must recover within ±20% of the known value (Arar & Collins, 1997).
- Relative standard deviation (RSD) of replicate measurements must be within ±20%.
- Field duplicates must agree within ±20% RPD.
- Samples, standards, LRBs, and QCs must all be at ambient room temperature; fluorescence is temperature-dependent (APHA et al., 2012).

9. Waste Disposal

Waste Type	Disposal Method
Acetone extracts	Collect in organic waste carboy in fume hood; EH&S pickup
Acidified samples (HCl)	Neutralize and dispose per SOP-L003
Used cuvettes (non-hazardous)	Standard trash (polystyrene, methacrylate) or recycle (glass)
Formazine standards	Collect in aqueous waste; EH&S pickup

10. References

- Turner Designs (2014)
- Arar & Collins (1997)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll textita and pheophytin textita in marine and freshwater algae by fluorescence*. U.S. Environmental Protection Agency, Office of Research; Development, National Exposure Research Laboratory. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417

Turner Designs. (2014). *Trilogy laboratory fluorometer: User's manual*. https://www.turnerdesigns.com/trilogy-laboratory-fluorometer

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Acetone and HCl handling
L310	Chlorophyll Extraction	Primary application of this instrument

12. Training Record

Before operating the Trilogy Fluorometer independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Turner Designs Trilogy User's Manual (Turner Designs, 2014)
4. Observe a trained operator perform module installation, calibration, and sample measurement
5. Perform a calibration and measure a set of standards with acceptable results under supervision
6. Be signed off by the PI or lab manager

13. Appendix A: Troubleshooting – Fluorescence

Symptom	Probable Cause	Corrective Action
"Bad Calibration" error	Blank brighter than standard	Compare blank and standard in Raw mode; ensure standard is more concentrated than blank
Erratic readings	Incorrect standard value entered	Review the standard value entered; must match actual concentration
Negative values	Sample near zero	Normal if near blank; blank value is automatically subtracted

Symptom	Probable Cause	Corrective Action
Low readings	Wrong optical module	Verify that module excitation/emission wavelengths match the analyte
High background signal	Contaminated cuvette holder	Clean holder with 70% ethanol and a lint-free tissue

14. Appendix B: Troubleshooting – Turbidity

Symptom	Probable Cause	Corrective Action
Readings disagree with other meters	Different light sources (visible vs IR)	Calibrate with the same standard; color interference affects visible-light meters but not the Trilogy
Readings vary between replicates	Particle movement in sample	Normal; particles shift and scatter light differently each time
Readings change after recalibration	Formazine standard has aged	Use fresh formazine (valid for 4 weeks per ISO 7027)



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L204

Shimadzu TOC-L Analyzer with TNM-L and SSM-5000A

Standard Operating Procedure

SOP Number	SOP-L204
Revision	2.1
Author(s)	Marc Los Huertos
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Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the general operation, calibration, maintenance, and shutdown of the Shimadzu TOC-L total organic carbon analyzer and its companion modules: the TNM-L Total Nitrogen Module and the SSM-5000A Solid Sample Module. The TOC-L uses a 680 C combustion catalytic oxidation method, which is one of two principal approaches to TOC analysis (the other being wet chemical oxidation with persulfate); the high-temperature combustion method is preferred for environmental samples because it more completely oxidizes refractory organic matter such as humic substances (Sharp, 1993; Sugimura & Suzuki, 1988).

All instrument control is performed through the LabSolutions TOC software on the dedicated computer.

This SOP covers the instrument itself. For application-specific sample preparation, measurement modes, and calculations, refer to the relevant analytical SOPs:

- SOP-L311 Dissolved Organic Carbon in Water (NPOC)
- SOP-L407 Total Carbon (Soils) (SSM)
- SOP-L409 Microbial Biomass Carbon (CFE extracts)
- SOP-L418 Water-Extractable DOC (soil extracts)

2. Scope

- **Applies to:** All use of the Shimadzu TOC-L analyzer, TNM-L module, SSM-5000A module, and ASI-L autosampler in the EA Program laboratory, operated through LabSolutions TOC software
- **Does not apply to:** Manual or standalone operation without computer control; instruments at other institutions; application- specific sample preparation or data interpretation (see analytical method SOPs)
- **Location:** EA Program analytical laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Authorize instrument access, approve maintenance
Instrument Manager	Perform preventive maintenance, replace consumables, manage software updates
Trained Personnel	Operate the instrument per this SOP, log all runs
New Students	Complete training before independent use

4. Definitions

- **Catalytic combustion:** Oxidation of organic carbon in the sample at 680 °C over a platinum catalyst in the presence of oxygen; the standard method used by the Shimadzu TOC-L for liquid samples.
- **Dissolved organic carbon (DOC):** Organic carbon in a water or soil extract sample that passes through a 0.45 µm filter; the NPOC method is the standard approach for DOC measurement.
- **IC (inorganic carbon):** Carbon present as carbonate, bicarbonate, and dissolved CO_2; removed by acidification and sparging before NPOC measurement.
- **NDIR detector (non-dispersive infrared detector):** A detector that measures CO_2 concentration by its absorption of infrared light at ~4.3 µm; the detection element in the Shimadzu TOC-L.
- **NPOC (non-purgeable organic carbon):** The organic carbon fraction remaining in a sample after acidification and sparging to remove inorganic carbon and purgeable volatile organic carbon; the operationally defined DOC measured by the Shimadzu TOC-L.
- **SSM-5000A:** The Shimadzu Solid Sample Module that combusts dried, ground solid materials (soils, sediments, plant tissue) directly in ceramic boats at up to 900 °C for total carbon analysis.
- **TC (total carbon):** The sum of all carbon forms (organic and inorganic) in a sample; measured by direct combustion without acidification.
- **TOC (total organic carbon):** TC minus IC; in practice, usually determined as NPOC for environmental samples.

- **TON (total organic nitrogen):** Total nitrogen minus inorganic nitrogen; measured simultaneously by the TNM-L module using chemiluminescence detection of NO produced during combustion.

5. Safety and Hazards

⚠ Hazards

- **Thermal:** The combustion furnace operates at 680 C in liquid analysis mode and up to 900 C in SSM mode. Ceramic boats, combustion tubes, and furnace surfaces can cause severe burns.
- **Chemical:** Hydrochloric acid (2 M HCl) is used for acidification and inorganic carbon sparging. Phosphoric acid (25%) is used as the IC reagent. Avoid skin and eye contact with both.
- **Compressed gas:** The instrument requires high-purity carrier gas (air or oxygen) supplied from a pressurized cylinder at approximately 200 kPa. Follow all compressed gas safety procedures (National Research Council, 2011).
- **Electrical:** The instrument operates on high-voltage mains power. Do not open instrument panels or attempt internal repairs.
- **Ozone:** The TNM-L chemiluminescence detector uses an ozone generator. The instrument vents ozone through an internal decomposer, but ensure that the exhaust line is connected and unobstructed. If an ozone odor is detected, shut down the TNM-L and ventilate the area.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling acid, standards, or samples)
☐ Heat-resistant gloves (when handling ceramic boats from the SSM)

5.2. Emergency Procedures

For acid splashes, flush the affected area with water for at least 15 minutes. For burns from hot surfaces, apply cool running water and seek first aid. If ozone is detected, shut down the TNM-L, open windows, and leave the room until the odor dissipates. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Instrument Description

6.1. System Overview

The Shimadzu TOC-L is a combustion catalytic oxidation analyzer that quantifies carbon (and, with the TNM-L, nitrogen) in liquid samples (Shimadzu Corporation, 2019b). The system in the

EA Program laboratory consists of four main components:

- **TOC-L analyzer:** Combusts liquid samples at 680 C over a platinum catalyst. The resulting CO_2 is measured by a non-dispersive infrared (NDIR) detector. The analyzer supports multiple measurement modes (TC, IC, TOC, NPOC, POC).
- **TNM-L Total Nitrogen Module:** Measures total nitrogen (TN) simultaneously with carbon analysis. Nitrogen compounds in the combustion gas are converted to nitric oxide (NO), which is detected by a chemiluminescence detector after reaction with ozone (Shimadzu Corporation, 2019a). The TNM-L operates in parallel with the NDIR carbon detector, so carbon and nitrogen data are acquired from the same injection.
- **SSM-5000A Solid Sample Module:** Accepts solid samples in ceramic boats for direct combustion at up to 900 C. Connects to the TOC-L for CO_2 detection. The TNM-L is not used during SSM operation.
- **ASI-L Autosampler:** Holds up to 93 sample vials (8 mL or 40 mL) for unattended sequential analysis of liquid samples. Includes an automated rinse function between injections.

6.2. Measurement Modes (Liquid Samples)

Mode	Full Name	What It Measures
TC	Total Carbon	All carbon (organic + inorganic) by direct combustion
IC	Inorganic Carbon	Carbonate/bicarbonate carbon by acidification and sparging only
TOC	Total Organic Carbon (by difference)	TOC = TC - IC; requires two sequential measurements
NPOC	Non-Purgeable Organic Carbon	Sample is acidified and sparged to remove IC, then combusted; the most common mode for routine analysis (APHA et al., 2012, Section 5310B)
POC	Purgeable Organic Carbon	Volatile organic carbon recovered during the sparging step; POC = TC - (NPOC + IC)
TN	Total Nitrogen (TNM-L)	All nitrogen species measured simultaneously with any carbon mode

6.3. Detection Principles

The TOC-L and TNM-L rely on two detectors operating in series on the same combustion gas stream:

- **NDIR detector (carbon):** Infrared absorption by CO_2 is proportional to the mass of carbon injected. The detector is selective for CO_2 and insensitive to other combustion gases.
- **Chemiluminescence detector (nitrogen, TNM-L only):** NO in the combustion gas reacts with ozone (O_3) to produce excited NO_2*, which emits light as it returns to the ground state. The emitted light intensity is proportional to the mass of nitrogen injected (Walsh, 1989).

7. Required Materials

7.1. Instrument Components

Component	Notes
Shimadzu TOC-L analyzer	With combustion furnace and NDIR detector
TNM-L Total Nitrogen Module	Chemiluminescence detector for TN
SSM-5000A Solid Sample Module	For direct combustion of solid samples
ASI-L Autosampler	93-position; 8 mL or 40 mL vials
Dedicated computer	Running LabSolutions TOC software
Carrier gas (high-purity air)	Regulated to 200 kPa (or per manual)
Exhaust tubing	Connected to fume hood or building exhaust

7.2. Consumables

Item	Notes
TC catalyst (Pt on alumina beads)	Replace when blanks rise or sensitivity drops; typical life 3,000–5,000 injections
IC reagent (25% phosphoric acid)	For IC/NPOC acidification; check level weekly
Combustion tubes (quartz)	Inspect monthly; replace if cracked or discolored
Ceramic sample boats	For SSM; pre-combust at 900 C before first use
Halogen scrubber	Replace every 6–12 months or per manufacturer schedule
Dehumidifier / moisture trap	Drain weekly; more often in humid weather
Autosampler vials and septa	Acid-washed for trace-level work; use pre-combusted glass vials for NPOC
Syringe needle (instrument syringe)	Inspect for bends or clogs; replace as needed

7.3. Calibration Standards

Standard	Use	Preparation
Potassium hydrogen phthalate (KHP)	Primary TC/NPOC calibration	2.125 g/L = 1,000 mg C/L stock (APHA et al., 2012)
Sodium bicarbonate / sodium carbonate	Primary IC calibration	See Shimadzu manual for concentrations
Potassium nitrate (KNO_3)	Primary TN calibration (TNM-L)	7.218 g/L = 1,000 mg N/L stock
Deionized water (18.2 MOhm)	Blanks, dilutions, and standard preparation	From Milli-Q; verify TOC less than 0.05 mg/L

8. Procedure

> **!** Computer Control Only
>
> All operations described below are performed through LabSolutions TOC. Do not adjust furnace temperature, gas flow, or other parameters using the instrument front panel unless directed by the instrument manager during troubleshooting.

8.1. Instrument Startup (Cold Start)

A cold start applies when the instrument has been fully powered down (furnace off, gas closed). Allow approximately 60 minutes from power-on to running samples.

1. Open the carrier gas cylinder valve. Verify the regulator output pressure is set to 200 kPa at the instrument inlet. Check for leaks at all fittings (listen for hissing; apply leak-detection solution if uncertain).
2. Verify that the exhaust line from the TNM-L ozone decomposer is connected and routed to the fume hood or building exhaust
3. Check consumable levels:
   - IC reagent bottle (25% phosphoric acid): refill if below the minimum line
   - Dehumidifier / moisture trap: drain if water is visible
   - Rinse water reservoir on the ASI-L: fill with fresh deionized water
4. Power on the TOC-L main unit using the switch on the right side panel
5. If the TNM-L is needed, power on the TNM-L using the switch on its rear panel
6. Power on the ASI-L autosampler
7. Turn on the dedicated computer and launch LabSolutions TOC
8. In LabSolutions TOC, verify that communication with all modules is established. The status bar should show “Warming Up”
9. **Wait for the furnace** to reach 680 C (approximately 20–30 minutes). The software status changes from “Warming Up” to “Ready”
10. **Wait for the TNM-L** ozone generator and chemiluminescence detector to stabilize (approximately 30–45 minutes after power-on; the TNM-L status indicator changes to “Ready”)
11. Run 3–5 injections of deionized water as system conditioning blanks to flush the injection port, syringe, and combustion tube. Discard these results.
12. Run 3 additional deionized water blanks and verify baseline stability: TC blank values should be below 0.1 mg/L C; TN blank values should be below 0.1 mg/L N

8.2. Instrument Startup (From Standby/Sleep)

When the instrument is in standby mode (furnace at temperature, gas flowing, software idle), startup is faster:

1. Launch LabSolutions TOC if not already open
2. Verify that the status bar shows “Ready” for the TOC-L and (if applicable) the TNM-L

3. Check the IC reagent level and drain the moisture trap
4. Fill the ASI-L rinse reservoir with fresh deionized water
5. Run 3 deionized water conditioning blanks. If blanks are below 0.1 mg/L C and 0.1 mg/L N, proceed to calibration.

8.3. Standby and Sleep Modes

Choose the appropriate idle mode based on how soon the instrument will be used again:

Mode	Furnace	Carrier Gas	TNM-L	When to Use
Active standby	680 C (on)	Flowing	On	Next run within 24 hours
Reduced standby	680 C (on)	Flowing	Off	Next run within 2–3 days; no TN needed
Sleep	Off (cooling)	Closed	Off	No runs planned for 3+ days
Full shutdown	Off (cold)	Closed	Off	Extended break, maintenance, or relocation

8.3.1. Entering Active Standby

1. After the final sample, run 3–5 deionized water blanks to flush the system
2. Leave the furnace at 680 C and the carrier gas flowing
3. Leave the TNM-L powered on
4. Empty the autosampler rack and cover with a dust cover
5. The instrument manager may set the software to a low carrier gas flow rate overnight to conserve gas (consult the manual for the minimum flow that maintains a positive purge of the combustion tube)

8.3.2. Entering Reduced Standby

1. Flush the system with deionized water blanks as above
2. Power off the TNM-L (rear panel switch). This extends the ozone decomposer lifetime.
3. Leave the TOC-L furnace at 680 C and gas flowing
4. Cover the autosampler

8.3.3. Entering Sleep Mode

1. Flush the system with deionized water blanks
2. Power off the TNM-L
3. In LabSolutions TOC, initiate a furnace cooldown (or simply power off the TOC-L)
4. Once the furnace temperature drops below 200 C (approximately 90 minutes), close the carrier gas cylinder valve
5. Power off the ASI-L
6. Close LabSolutions TOC and shut down the computer

8.3.4. Full Shutdown

Follow the Sleep Mode steps, then:

1. Allow the furnace to cool to room temperature
2. Disconnect the carrier gas line if the instrument will be moved or serviced
3. Empty the IC reagent bottle if the shutdown will exceed 30 days (phosphoric acid can corrode the reagent lines over long idle periods)
4. Empty the ASI-L rinse reservoir to prevent microbial growth
5. Cover the instrument with a dust cover

8.4. Software Navigation

LabSolutions TOC organizes work into the following areas:

- **Instrument Monitor:** Real-time display of furnace temperature, carrier gas flow, detector signal (NDIR and chemiluminescence), and system status
- **Method Editor:** Define measurement mode (TC, IC, NPOC, TN, etc.), injection volume, number of replicate injections, sparge time (for NPOC), acidification volume, and calibration curve parameters
- **Sample Table:** Enter sample IDs, vial positions, dilution factors, and measurement method for each sample
- **Calibration:** Build, view, and edit calibration curves. The software fits curves by least-squares regression and reports r^2 and residuals.
- **Data Browser:** Review completed runs, view peak integrations, export results to CSV, and reprocess data

8.5. Calibration

Calibrate at the start of each analytical session or whenever the method, measurement mode, or concentration range changes. Carbon and nitrogen calibrations are built independently but can be run simultaneously if both detectors are active.

8.5.1. Carbon Calibration

1. Prepare a multi-point calibration series from KHP dissolved in deionized water. Choose concentrations that bracket the expected sample range (e.g., 0, 1, 5, 10, 25, 50, 100 mg/L C). Prepare fresh daily or from a refrigerated stock per SOP-L020 (APHA et al., 2012).
2. For IC calibration, prepare standards from sodium bicarbonate/sodium carbonate in deionized water
3. In LabSolutions TOC, open the Method Editor and select the measurement mode (e.g., NPOC, TC, or TC-IC)
4. Enter calibration standard concentrations in the Calibration window
5. Load standards into the autosampler in order of increasing concentration
6. Run the calibration sequence. The software will perform replicate injections (typically 3) of each standard and fit a regression

7. Verify that $r^2 \geq 0.999$ and that each standard back- calculates to within 10% of its nominal value (15% at the lowest standard is acceptable)
8. Save the calibration curve

8.5.2. Nitrogen Calibration (TNM-L)

1. Prepare a multi-point calibration series from KNO_3 dissolved in deionized water (e.g., 0, 0.5, 1, 5, 10, 25, 50 mg/L N)
2. In the Method Editor, confirm that TN measurement is enabled alongside the carbon mode
3. Enter TN calibration concentrations in the Calibration window
4. The TN calibration runs simultaneously with the carbon calibration if both are loaded in the same sample table; otherwise, run the TN standards as a separate sequence
5. Verify that $r^2 \geq 0.999$ for the nitrogen curve
6. Save the calibration curve

8.6. Setting Up an Analytical Run (Liquid Samples)

1. In the Method Editor, confirm the measurement mode, injection volume, number of replicate injections, and (for NPOC mode) sparge time and acid volume
2. In the Sample Table, enter the run sequence:
    a. Calibration verification standard (mid-range) as the first sample
    b. Instrument blanks (deionized water) at the beginning, every 10–15 samples, and the end of the run
    c. Method blanks appropriate to the application (e.g., 0.5 M K_2SO_4 for soil extracts; consult the analytical method SOP)
    d. Sample vials in the order they are loaded in the autosampler
    e. A final calibration verification standard
3. Set the dilution factor for each sample if applicable
4. Verify that autosampler vial positions in the Sample Table match the physical rack positions
5. Confirm that the rinse reservoir on the autosampler is filled with deionized water
6. Click **Start** to begin the sequence

> **Replicate Injection Settings**
>
> The software default is 3 replicate injections per sample. If the coefficient of variation (CV) among replicates exceeds 2%, the software can be configured to add up to 2 additional injections automatically (set in Method Editor under "Maximum Injections"). The outlier replicate is excluded from the reported mean.

8.7. Setting Up a Solid Sample Run (SSM-5000A)

1. In LabSolutions TOC, switch to SSM mode
2. Pre-combust ceramic boats at 900 C in a muffle furnace (SOP-L111) to remove residual carbon. Allow boats to cool in a desiccator.

3. Weigh the solid sample into a clean ceramic boat. Record the mass to 0.01 mg.
4. In the Sample Table, enter the sample ID, boat number, and sample mass
5. Place the ceramic boat on the SSM sample port and push it into the furnace when prompted by the software
6. The SSM combusts the sample; evolved CO_2 flows to the TOC-L NDIR detector
7. The software reports carbon content in mg C or as a percentage of sample mass

> **TNM-L and SSM**
>
> The TNM-L chemiluminescence detector is not used during SSM solid sample analysis. Total nitrogen in solid samples requires a separate instrument (e.g., the Flash EA; see SOP-L208).

8.8. Monitoring a Run in Progress

1. Use the Instrument Monitor to watch real-time peak shapes and detector baselines
2. If a peak appears saturated (flat top), the sample exceeds the calibration range. Pause the run, dilute the sample, and re-queue.
3. If the CV among replicate injections exceeds 2%, investigate (air bubble, particulates, insufficient sample volume) and consider re-running.
4. If the system blank rises above 0.5 mg/L C or 0.5 mg/L N during a run, the combustion tube or catalyst may need replacement. Complete the current run, note affected samples, and consult Section 9.

8.9. Data Export

1. After the run, open the Data Browser and review all results
2. Check that calibration verification standards recovered within 90–110% of their nominal values
3. Export results to CSV: **File > Export**, including sample ID, mode, mean concentration, SD, CV, and number of valid injections
4. Save the LabSolutions TOC run file (.toc) to the instrument computer and back up to the lab network drive
5. Record the run date, analyst, method, and file name in the instrument logbook

9. Quality Control

QC procedures are tailored to each analytical method and are specified in the relevant method SOP. The following instrument-level QC applies to all runs (US Environmental Protection Agency, 2009):

- System blanks (deionized water) at the start of every session must read below 0.1 mg/L C and 0.1 mg/L N
- A calibration verification standard (mid-range) must be run at the beginning and end of every sample sequence, with recovery within 90–110%
- Replicate injection CV must be below 2% for liquid samples

- All QC results are recorded in the instrument logbook and in the exported data file
- For additional QC requirements (method blanks, duplicates, matrix spikes, continuing calibration verification frequency), refer to SOP-L802 Quality Control

10. Maintenance

10.1. Routine Maintenance Schedule

Task	Frequency	Notes
System blank check	Before each analytical session	
Drain moisture trap / dehumidifier	Weekly (more often in humid conditions)	Prevents water from reaching the NDIR detector
Check IC reagent level	Weekly	Refill with fresh 25% H_3PO_4 when below the minimum line
Inspect injection syringe needle	Weekly	Replace if bent, corroded, or clogged
Check autosampler rinse reservoir	Before each session	Refill with fresh DI water
Inspect combustion tube	Monthly or every 500 injections	Look for discoloration, cracks, or catalyst settling
Replace TC catalyst	Every 3,000–5,000 injections or when blanks rise	Pt on alumina; record replacement date
Replace halogen scrubber	Every 6–12 months	Prevents halide damage to the NDIR detector
Replace TNM-L ozone decomposer	Annually	Degraded decomposer leaks ozone into the room
Replace SSM combustion tube	As needed	Inspect after each batch of solids
Carrier gas cylinder replacement	As needed	Monitor regulator pressure; order when below 500 psi
Software and firmware updates	As released by Shimadzu	Coordinate with instrument manager

10.2. Catalyst Replacement Procedure

1. Allow the furnace to cool to room temperature (power off the TOC-L and wait at least 3 hours, or use the forced-air cooling function if available)
2. Wearing heat-resistant gloves and safety glasses, remove the combustion tube from the furnace per the manufacturer's procedure
3. Dispose of the spent catalyst (Pt on alumina) as hazardous waste (precious metal content; collect for EH&S pickup per SOP-L006)

4. Fill the combustion tube with fresh catalyst to the manufacturer's specified depth (approximately 12 cm)
5. Add a thin layer of quartz wool above and below the catalyst bed
6. Reinstall the combustion tube. Reconnect all tubing and fittings.
7. Restart the furnace and allow it to reach 680 C
8. Run 20–30 deionized water conditioning injections to stabilize the new catalyst. Blanks should decrease progressively to below 0.1 mg/L C.
9. Run a calibration verification standard to confirm sensitivity before resuming sample analysis
10. Record the replacement date, catalyst lot number, and conditioning results in the instrument logbook

10.3. Combustion Tube Inspection and Replacement

1. Remove the combustion tube as described in Section 9.2, step 2
2. Inspect for cracks, discoloration, or accumulation of non-volatile residue on the inner walls
3. If the tube is damaged, replace with a new quartz combustion tube
4. Repack with catalyst and condition as in Section 9.2

10.4. Syringe and Needle Maintenance

1. Remove the syringe from the autosampler or manual injection port
2. Inspect the needle for bends, burrs, or corrosion
3. Flush the syringe barrel with deionized water, then with dilute HCl (0.1 M), then with deionized water again
4. If the syringe plunger binds or the needle is damaged, replace the entire syringe assembly
5. Reinstall and run 3 deionized water blanks to verify proper function

11. Troubleshooting

Symptom	Probable Cause	Corrective Action
Blank values increasing over time	Catalyst degradation; contaminated combustion tube	Replace catalyst; inspect and replace combustion tube
Poor replicate precision (CV > 2%)	Air bubble in syringe; particulates in sample; insufficient volume	Check syringe for air leaks; re-filter sample; verify vial volume
NDIR signal saturated (flat-top peaks)	Sample exceeds calibration range	Dilute sample and re-run
TNM-L "Not Ready" status persists	Ozone generator not warmed up; decomposer exhausted; exhaust blocked	Allow more warm-up; replace decomposer; check exhaust tubing
No communication with instrument	Cable disconnected; software crash; instrument not powered on	Check cables; restart software; verify power

Symptom	Probable Cause	Corrective Action
Autosampler skips vials	Position mismatch; septa not pierced	Verify positions; check that septa are intact
SSM signal noisy or irreproducible	Boat not fully inserted; sample mass too large; residual carbon on boat	Confirm position; reduce mass; pre-combust boats longer
Carrier gas pressure alarm	Cylinder empty or regulator set incorrectly	Replace cylinder; adjust regulator
Moisture trap fills rapidly	High humidity; furnace tube leak	Drain more frequently; inspect tube fittings for leaks
IC reagent not dispensing	Reagent bottle empty; tubing kinked or clogged	Refill bottle; inspect and replace tubing

12. Waste Disposal

Waste Type	Disposal Method
Acidified liquid waste (HCl/H_3PO_4)	Neutralize and dispose per SOP-L006
Spent TC catalyst (Pt on alumina)	Collect for EH&S hazardous waste pickup (precious metal)
Used combustion tubes (quartz)	Standard glass waste (broken-glass box) if not contaminated
Used ceramic boats (SSM)	Clean and reuse, or standard trash
Autosampler rinse water	Drain
Calibration standard waste	Aqueous waste; drain if dilute

13. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

National Research Council. (2011). *Prudent practices in the laboratory: Handling and management of chemical hazards* (Updated). National Academies Press. https://doi.org/10.17226/12654

Sharp, J. H. (1993). The dissolved organic carbon controversy: An update. *Oceanography*, *6*(2), 45–50. https://doi.org/10.5670/oceanog.1993.12

Shimadzu Corporation. (2019a). *TNM-L total nitrogen module: User manual*.

Shimadzu Corporation. (2019b). *TOC-L series total organic carbon analyzer: User manual*.

Sugimura, Y., & Suzuki, Y. (1988). A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Marine Chemistry*, *24*(2), 105–131. https://doi.org/10.1016/0304-4203(88)90043-6

US Environmental Protection Agency. (2009). *Method 415.3, rev. 1.1: Determination of total organic carbon and specific UV absorbance at 254 nm in source water and drinking water* (EPA/600/R-09/122). Office of Research; Development, US EPA.

Walsh, T. W. (1989). Determination of dissolved nitrogen in estuarine waters using high-temperature combustion and chemiluminescence detection. *Marine Chemistry*, *26*(4), 295–311. https://doi.org/10.1016/0304-4203(89)90036-4

14. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials Handling	Chemical safety for acids
L006	Waste Disposal	Catalyst and chemical waste disposal
L020	Reagent and Standard Preparation	KHP and KNO_3 standard prep
L111	Muffle Furnace	Pre-combustion of ceramic boats
L311	Dissolved Organic Carbon (Water)	NPOC application SOP
L407	Total Carbon (Soils)	SSM application SOP
L409	Microbial Biomass Carbon (CFE)	DOC extract application SOP
L418	Water-Extractable DOC	Soil extract application SOP
L802	Quality Control	QC requirements for all runs

15. Training Record

Before operating this instrument independently, personnel must:

1. Read this SOP in full and the relevant sections of the Shimadzu TOC-L, TNM-L, and SSM-5000A user manuals
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Observe a trained operator perform a complete session: cold start, calibration (carbon and nitrogen), sample analysis, data export, and transition to standby
4. Demonstrate competence preparing calibration standards (KHP and KNO_3) per SOP-L020
5. Perform a supervised analytical run with acceptable QC results (blanks below 0.1 mg/L, calibration verification recovery 90–110%, replicate CV below 2%)
6. Demonstrate entering and exiting standby and sleep modes
7. Be signed off by the instrument manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L205

X-Ray Fluorescence (XRF) Sample Preparation and Analysis

Standard Operating Procedure

SOP Number	SOP-L205
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for preparing geological rock samples and analyzing their major-element oxide composition by wavelength-dispersive X-ray fluorescence (WD-XRF). The procedure covers sample powdering, flux fusion bead preparation, bead polishing, and instrument operation on the PANalytical XRF spectrometer using the SuperQ software with the "Geology Beads" application (Claisse, 2003; Norrish & Hutton, 1969; PANalytical, 2012).

2. Scope

- **Applies to:** Rock, mineral, and geological samples analyzed as lithium tetraborate fusion beads for major-element oxide determination (e.g., SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O, TiO_2, P_2O_5, MnO)
- **Does not apply to:** Pressed-pellet trace-element analysis, handheld (portable) XRF measurements, or non-geological materials
- **Locations:** Rock preparation lab (powdering and polishing); analytical lab / wet lab (weighing, fusion); XRF lab (instrument operation)

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, ensure training compliance
Lab Technician	Maintain instrument, assist with troubleshooting
Trained Personnel	Follow this SOP, maintain sample tracking, clean up
New Students	Complete training before independent use

Contact the lab technician, Jade Star, or Bob with any questions.

4. Definitions

- **Certified reference material (CRM):** A geological standard with certified major-element oxide concentrations from an accredited producer (e.g., NIST, USGS, BCR); used to verify XRF accuracy during each analytical session (Norrish & Hutton, 1969).

- **Detection limit (MDL):** The lowest concentration of an element reliably detected above background; varies by element, matrix, and instrument settings; typically 1–50 mg/kg for common elements by WD-XRF.
- **Flux (dilithium tetraborate, $Li_2B_4O_7$):** A reagent mixed with the rock powder in a 2:1 (flux:sample) ratio; lowers the melting point and produces a homogeneous glass bead that eliminates mineralogical matrix effects during analysis (Claisse, 2003).
- **Fluorescence (X-ray):** The emission of characteristic X-rays from a sample excited by primary X-rays; each element emits at unique energies that identify and quantify it (Norrish & Hutton, 1969).
- **Fundamental parameters (FP) method:** An XRF calibration approach that uses known physical constants to calculate element concentrations from measured fluorescence intensities without matrix-matched standards for every sample type.
- **Fusion bead:** A glass disc produced by melting the powdered rock sample with lithium tetraborate flux at 1000 °C; the homogeneous glass eliminates mineralogical matrix effects (Claisse, 2003).
- **Geology Beads:** The SuperQ application/archive configured for major-element oxide analysis of fusion beads on the PANalytical XRF spectrometer (PANalytical, 2012).
- **K-alpha and K-beta lines:** The most commonly measured characteristic X-ray emission lines for elements; produced when an electron is ejected from the K shell and replaced from the L or M shell, respectively.
- **Loss on ignition (LOI):** The mass percentage lost when a pre-weighed rock powder is heated to 1000 °C; reflects H_2O, CO_2, and organic matter content; used as a correction in major-element summation (Norrish & Hutton, 1969).
- **Matrix effects:** Absorption and enhancement of X-ray signals by other elements in the sample; minimized by the flux fusion method (Claisse, 2003).
- **Shatterbox:** A vibratory ring-and-puck mill used to grind rock chips to a fine powder suitable for fusion.
- **SuperQ:** The PANalytical software used to control the XRF spectrometer, define measurement sequences, and retrieve results (PANalytical, 2012).
- **WD-XRF (wavelength-dispersive XRF):** An XRF configuration in which characteristic X-rays are separated by a crystal monochromator before detection; provides higher spectral resolution and lower detection limits than energy-dispersive (ED-XRF) systems.
- **X-ray fluorescence (XRF):** A non-destructive elemental analysis technique in which a sample excited by a primary X-ray beam emits characteristic fluorescent X-rays that are measured to determine elemental composition (Norrish & Hutton, 1969).

5. Safety and Hazards

⚠ Hazards

- **High-temperature furnace:** The muffle furnace operates at 1000 °C. Crucibles and tiles removed from the furnace are extremely hot. Do not touch them, even with gloves, until fully cooled.
- **Compressed air:** Loud; wear ear protection when blowing out equipment.
- **Rock chipper:** Flying rock fragments are common. Always wear safety glasses. Never look into the chipper while it is running.

- **Shatterbox:** The unit vibrates intensely and can travel across the floor. Get help repositioning it if needed.
- **Chemical reagents:** Dilithium tetraborate flux is a fine powder; avoid inhalation. Ethanol is flammable.
- **X-ray radiation:** The XRF instrument contains an X-ray source. Follow all posted radiation safety procedures. Do not open the instrument lid while the arm is moving or the flap is up.

5.1. Required PPE

- ☐ Safety glasses (required in the rock prep lab at all times)
- ☐ Laboratory coat
- ☐ Nitrile gloves (powder handling and polishing)
- ☐ Ear protection (when using the shatterbox or compressed air)
- ☐ Heat-resistant gloves (when handling crucibles or the tile from the furnace)

5.2. Emergency Procedures

Do not work in the lab without letting someone know your plans and estimated return time. Phones and safety showers are in all labs. Refer to SOP-L002 Laboratory Safety for all emergency procedures.

6. Prerequisites and Required Reading

- SOP-L002 Laboratory Safety
- Furnace operating instructions (posted above the furnace)
- This SOP in full

! Important

Minimize contamination at every step. Wear gloves, clean equipment thoroughly between samples, and assume any shared equipment is dirty unless you just cleaned it. Leave the lab and equipment cleaner than you found it.

7. Required Materials

7.1. Equipment

Item	Location / Notes
Small rock chipper	Rock prep lab
Rocklabs shatterbox (tungsten carbide head)	Rock prep lab

Item	Location / Notes
Analytical balance	Analytical lab, corner by XRD room
Vortex mixer	Counter left of weigh station
Deionizing bar	Right of the Vortex mixer
Muffle furnace (1000 °C)	Far left corner, wet lab
Grinding/polishing laps (4 stages)	Darkroom / grinding lab
Ultrasonic cleaner (sonicator)	Grinding lab or XRF lab
Diamond-tipped pen or rotary tool	Darkroom
PANalytical XRF spectrometer	XRF lab (PANalytical, 2012)

7.2. Consumables

Item	Notes
Dilithium tetraborate flux	Stored in desiccator, left of weigh station
Graphite crucibles (9 per batch)	Drawer under the furnace
Plastic cups with lids	Cabinet left of weigh station
Brown butcher paper	Shelves, back wall, rock prep lab
White printer paper	For transferring powder
Kimwipes	General use
Ethanol	Bottle on shatterbox table; bulk jug on side counter
Sand (for cleaning shatterbox)	Bowl in center of table, rock prep lab
Green Brillo pads	Drawer, rock prep lab
Sample bags and labels	Drawer under counter, XRF lab
XRF sample sheets	Drawer left of weigh station
SiC sandpaper	For removing saw marks

8. Procedure

8.1. Step 1: Powdering the Sample

A complete fusion batch is 9 samples. If you plan fusions, turn on the furnace first (see §6.3) since it takes about 1 hour to reach 1000 °C.

8.1.1. Chipping

1. Use the rock chipper to reduce each sample to pieces smaller than approximately 1 cm. Wear safety glasses. Clean the chipper jaws thoroughly between samples with compressed air and an ethanol wipe.
2. Weigh out approximately 20–30 g of chipped material into a labeled sample bag.

8.1.2. Shatterbox Milling

3. Place a piece of brown butcher paper under the shatterbox head assembly to catch any spillage.
4. Wipe all shatterbox bowl components (ring, puck, and bowl) with an ethanol-dampened Kimwipe. Allow to dry completely.
5. Load the chipped sample into the tungsten carbide bowl. Do not overfill (maximum ~two-thirds full).
6. Lock the lid and run the shatterbox for 60–90 seconds. Longer milling times reduce particle size further.
7. Unlock the lid and carefully transfer the powder onto white printer paper, keeping all components on the paper edge so exterior metal surfaces do not contact the sample. Funnel the powder into the labeled container. Use a fresh sheet of paper for every sample.

8.1.3. Cleaning the Shatterbox

8. If powder is stuck, scrub with a green Brillo pad. If that is insufficient, run a scoop of sand for 15 seconds and discard.
9. Detail all pieces with compressed air (hold pieces in the airflow; scrub with the Brillo pad). **Do not let the metal hose tip touch any bowl surface.** Use ear protection.
10. Finish with an ethanol wipe on a Kimwipe. After the last sample, clean up, recycle paper, replace the bowl, and turn off the shatterbox.

8.2. Step 2: Weighing and Mixing

1. Bring your powdered samples to the analytical lab. Get an XRF sample sheet from the drawer left of the weigh station, fill out the top portion, and keep it on a wooden tray next to the balance.
2. Retrieve a small cup of flux from the desiccator.
3. For each sample:
    a. Label a plastic cup lid with the sample number.
    b. Place the cup (no lid) in the cardboard holder on the balance, close the sliding door, and zero.
    c. Weigh 3.5000 g (±0.0004 g) of sample powder. Record the exact mass. Zero the balance.
    d. Add 7.0000 g (±0.0004 g) of flux (2:1 flux-to-sample ratio) (Claisse, 2003). Record the mass. If you add too much flux, remove the excess but **do not return it to the reagent container**.
    e. Seal the lid tightly.
4. Mix each cup on the Vortex mixer for about one minute. If powder clings to the sides, pass the deionizing bar over the cup.

8.3. Step 3: First Fusion

8.3.1. Furnace Startup

1. Flip the black switch on the front of the furnace.

2. Press the T1 temperature button and confirm T = 1000 °C.
3. Set a duration (e.g., 24, 48, or 72 hours). Press Enter and Start. It takes roughly 1 hour to reach 1000 °C.

> **Caution**
>
> Do not spill glass in the furnace or introduce liquids. Do not use this furnace for temperatures below 500 °C – use a regular drying oven instead. Do not shut off the furnace mid-cycle with the power button; let it run out its programmed time and shut off automatically.

8.3.2. Loading Crucibles

4. Select 9 graphite crucibles from the drawer under the furnace. Clean each inside by gently wiping with a Kimwipe and blowing out with compressed air.
5. Back at the analytical lab, carefully empty each cup into the corresponding crucible. **Maintaining the correct order is critical – once the powder is in the crucible, there is no way to tell samples apart.**
6. Place the empty cups (lids on) in the box labeled "Dirty Cups for Blowing Out."

8.3.3. Fusing

7. In the wet lab, place a tile on the brick stand (small hole facing toward you) and crank the stand up until the tile is about halfway up the side of the furnace.
8. Put on both long heat-resistant gloves. Using the tongs, slide the tile into the furnace. Close the door as quickly as possible.
9. Wait 20–30 minutes for the furnace to recover to 1000 °C, then leave the samples for 10–15 minutes. Longer fusion times help carbon-rich samples degas fully.

The tile and crucibles are extremely hot when removed from the furnace. Do not touch them, even with gloves, until fully cooled.

10. Wearing gloves, open the furnace and carefully pull the tile out using the tongs and the tile hole. Move the tile to the hot plate and close the furnace.

8.4. Step 4: Regrinding and Second Fusion

1. Once the crucibles are cool enough to handle, pour the first-fusion glass back into the shatterbox bowl. Re-grind for 60 seconds.
2. Return the powder to the crucible and re-fuse at 1000 °C for 15–20 minutes to remove any remaining bubbles and produce a homogeneous bead.
3. Once cooled, inspect each bead visually. Beads should be clear, bubble-free glass covering the full crucible bottom. If a bead has a dark carbon pellet at the bottom, fuse for an additional 15 minutes.

8.5. Step 5: Polishing the Bead

Polish the flat (analytical) surface of each bead on the 4-stage grinding/polishing laps to remove surface scratches and contamination that would scatter X-rays.

1. Use the coarsest lap first (e.g., 600 grit SiC), working through progressively finer stages.
2. After each stage, clean the bead in the ultrasonic cleaner with distilled water (3 minutes).
3. After the final lap, inspect the flat surface under the lab light; it must be mirror-smooth with no visible scratches.
4. Label each bead on the curved (non-analytical) side using a diamond-tipped pen or rotary tool.

8.6. Step 6: XRF Analysis

1. Open a "Measure and Analyze" window from the SuperQ Manager. Wear gloves and avoid touching the flat (analytical) surface of the bead (PANalytical, 2012).
2. Click the multi-cup icon to open the Sample Changer. Double-click position A1. Enter the sample name; set type to "Routine," application to "Geology Beads," priority to "Normal," and verify weights are correct. Click **Add**. Available positions are A1–A8 and B5–B8.
3. Once all samples are entered, click **Measure**.

> **Note**
>
> If the XRF has not been running recently, the instrument needs to warm up until the front-panel readouts display 60 and 50. Run the first bead until those values are reached, then click Abort. Wait for the arm to return the sample, then restart. Running samples before warm-up can compromise the analysis (PANalytical, 2012).

8.7. Step 7: Retrieving Results

1. Open a "Results Evaluation" window. Set the archive to "Geology Beads" and adjust the date range. Use the "Name Mask" field to filter results. Click on a sample and then click "View."
2. To export data: open a "Results Quantitative" window. Adjust date and name mask. Click the Print icon, then "Delimited Values Report" to clipboard. Paste into a spreadsheet.

9. Quality Control

- Verify the 2:1 flux-to-sample ratio for every sample; record exact masses on the XRF sample sheet (Claisse, 2003).
- Visually inspect beads before loading; reject cracked, broken, or bubble-compromised beads.
- Run at least one CRM alongside unknowns to monitor instrument drift and accuracy (Norrish & Hutton, 1969).
- If a bead required a third fusion, note this on the sample sheet.

10. Cleanup Checklist

- ☐ All paper recycled (butcher paper, white paper)
- ☐ Shatterbox bowl cleaned and replaced; shatterbox off
- ☐ Chipper cleaned (ethanol wipe on all contact surfaces)
- ☐ Crucibles returned to the drawer (or discarded if degraded)
- ☐ Plastic cups blown out and restocked (or discarded after 6 uses)
- ☐ Polishing laps spun dry
- ☐ Sonicator drained if not in regular use
- ☐ XRF sample sheet completed and filed
- ☐ All personal items removed from the lab
- ☐ Work surfaces wiped down

11. Troubleshooting

Symptom	Possible Cause / Action
Glass does not cover crucible bottom	Insufficient fusion time; re-fuse longer
Bead cracked or has deep air bubbles	Air trapped during fusion; re-fuse briefly
Carbon pellet at bottom of bead	Carbon not fully degassed; fuse longer next time
Powder clings to plastic cup walls	Pass the deionizing bar over the cup
XRF front-panel numbers not at 60/50	Instrument not warmed up; run a bead until ready
XRF data appear anomalous	Sample run before warm-up; re-run

12. References

- Norrish & Hutton (1969)
- Claisse (2003)
- PANalytical (2012)

Claisse, F. (2003). *Fusion and fluxes for XRF sample preparation.* Corporation Scientifique Claisse.

Norrish, K., & Hutton, J. T. (1969). An accurate X-ray spectrographic method for the analysis of a wide range of geological samples. *Geochimica Et Cosmochimica Acta*, *33*(4), 431–453. https://doi.org/10.1016/0016-7037(69)90126-2

PANalytical. (2012). *SuperQ software: User manual.*

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L111	Muffle Furnace	Furnace operation details

14. Training Record

Before performing XRF sample preparation and analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review furnace safety instructions posted above the furnace
4. Observe a trained operator perform a complete batch (powdering through analysis)
5. Prepare and analyze a batch under supervision with acceptable CRM recovery
6. Be signed off by the lab technician or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L208

ThermoFisher Delta V IRMS – General Instrument Use

Standard Operating Procedure

SOP Number	SOP-L208
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the general operation, care, and policies governing the ThermoFisher Delta V Isotope Ratio Mass Spectrometer (IRMS) and its four front-end peripherals in the Oxtoby Environmental Isotope Lab (Coplen, 2011; Werner & Brand, 2001). It is the single instrument-level reference for all IRMS work. Individual application SOPs (e.g., SOP-L414 for soil CN analysis, SOP-L415 for carbonate isotopes) build on this SOP and should be read in conjunction with it.

2. Scope

- **Applies to:** The Delta V IRMS and all peripherals (ConFlo IV, Flash IRMS EA, TC/EA, GasBench II) in the Oxtoby Isotope Lab, SGM Rm 135, Pomona College.
- **Does not apply to:** Application-specific sample preparation, data reduction, or QC criteria. See the relevant application SOP.
- **Location:** David W. and Claire B. Oxtoby Environmental Isotope Lab (SGM Rm 135). Sample preparation: wet lab (SGM Rm 133).

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP; authorize new users
Lab Manager / Super-User	Certify new users; maintain reactors and gas supply
Certified User	Operate instrument per this SOP; log all sessions
Student Researcher	Operate only under supervision until certified

4. Definitions

- **ConFlo IV:** Interface hub connecting peripherals, reference gases, and the IRMS; handles reference-gas dilution and peak-center switching.
- **Delta V IRMS:** The ThermoFisher Delta V Isotope Ratio Mass Spectrometer housed in the Oxtoby Isotope Lab, SGM Rm 135.
- **Flash IRMS EA:** Elemental analyzer peripheral that combusts samples at 1020 °C to produce N_2, CO_2, and (optionally) SO_2 for simultaneous $\delta^{15}N$ and $\delta^{13}C$ analysis.
- **GasBench II:** Low-flow peripheral for headspace gas sampling from sealed vials; used for carbonate CO_2, DIC, and water $\delta^{18}O$.
- **H_3^+ Factor:** Correction applied to δ^2H data for H_3^+ ions formed in the source that interfere with HD^+ at m/z 3.
- **Isodat:** ThermoFisher software used to design analytical sequences, control the instrument, and export raw isotope data.
- **Linearity:** The dependence of measured δ on signal amplitude; a well-tuned instrument shows minimal linearity offset across the working signal range (Werner & Brand, 2001).
- **Super-User:** Staff or faculty member certified for IRMS operation and minor maintenance (gas replacement, reactor exchange).
- **TC/EA:** Thermal Conversion/Elemental Analyzer peripheral; pyrolyzes samples at ~1450 °C to produce H_2 and CO for δ^2H and $\delta^{18}O$ analysis.
- **User:** Student, staff, or faculty member certified to prepare samples and operate the IRMS without direct supervision.
- **Zero-Enrichment Test:** Series of reference-gas on/off pulses run before analysis to verify the IRMS produces consistent δ values independent of signal amplitude (Coplen, 2011).

5. Safety and Hazards

⚠ Hazards

- **Compressed gases:** He, O_2, CO_2, N_2, SO_2 (where used). Cylinders must be chained; O_2 must be stored away from flammables.
- **High-voltage electronics:** The IRMS source operates at high voltage. Do not open the source housing without authorization.
- **High temperatures:** Flash EA reactor operates at 1020 °C; TC/EA at ~1450 °C. Allow to cool before maintenance.
- **Chemical:** Tin capsules contain sample material; some samples may be hazardous. Handle per the sample-specific SOP.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (during sample loading and reactor maintenance)

5.2. Required Training

- SOP-L002 Laboratory Safety
- This SOP (Section on User Certification) before any independent use

6. Equipment and Materials

Item	Location	Notes
Delta V IRMS	SGM Rm 135	Do not power off without authorization
ConFlo IV interface	SGM Rm 135	Always on when IRMS is on
Flash IRMS EA	SGM Rm 135	Application-specific reactor config
GasBench II	SGM Rm 135	Requires dedicated Isodat method
TC/EA	SGM Rm 135	High-temperature; Super-User only for maintenance
He supply (cylinder)	SGM Rm 135	Check pressure before each session
Reference gas cylinders	SGM Rm 135	CO_2, N_2, H_2, SO_2 as needed
Tin capsules (5 x 9 mm)	SGM Rm 133	Sample prep area
Lab logbook	SGM Rm 135	Sign in/out for every session

7. Procedure

7.1. User Certification

Before operating the IRMS independently, personnel must complete the Training Record (Section 13). Contact the lab manager to schedule certification.

7.2. Pre-Analysis Checklist

1. Sign in to the lab logbook.
2. Verify He carrier gas pressure (minimum 1500 psi on the regulator).
3. Check reference gas pressures (CO_2, N_2 as applicable).
4. Confirm Isodat is running and the instrument status screen shows no error flags.
5. Verify the correct peripheral is selected in Isodat.
6. Run a Zero-Enrichment Test (3 on/off pulses) and confirm δ values are stable within $\pm 0.1‰$.
7. Run an on/off linearity check if not done within the past 48 h.

7.3. Analytical Sequence Design

1. Open the Isodat Sequence Editor.
2. Load the appropriate method file for the peripheral and analyte.
3. Build the sequence: blank(s), conditioning samples, reference materials, unknowns, check standards, blanks.
4. Reference material placement: minimum 1 RM per 10 unknowns; two-point normalization requires at least 2 different RMs.
5. Save the sequence file using the naming convention: `YYYYMMDD_Project_SeqID_User.seq`

7.4. Starting and Monitoring a Run

1. Load samples into the autosampler or carousel per the application SOP.
2. Press **Start** in the Isodat Sequence Editor.
3. Monitor the first 3–5 injections on-screen to confirm peaks are present, within expected amplitude, and baseline-resolved.
4. Do not leave the instrument unattended during the first 30 min.

7.5. Shutdown

1. After the sequence completes, export raw data per the application SOP.
2. Remove all samples and consumables from the autosampler.
3. Log gas usage and any issues in the lab logbook.
4. Leave the instrument in standby per the lab manager's current instructions (the Delta V and ConFlo IV are typically left powered on continuously).

8. Troubleshooting

Symptom	Possible Cause / Action
High m/z 28 background	Air leak; check fittings and reactor seals
High m/z 18 background	Moisture; extend bake-out time
High m/z 40 background	Air leak (Ar); check fittings
Poor standard reproducibility	Capsule sealing; exhausted reactor
No peaks in chromatogram	Gas flow interrupted; check He supply
Sequence stops mid-run	Autosampler jam; check carousel alignment
On/off tests fail repeatedly	Source needs tuning or maintenance
Red/yellow lights on IRMS panel	Check Isodat error log; contact lab manager

If you cannot resolve an issue, **stop the run** and contact the lab manager. Do not attempt reactor replacement or major maintenance unless you are a certified Super-User.

9. Quality Control

- Run at least two certified reference materials per analytical sequence for two-point scale normalization (Paul et al., 2007).
- Reference material δ values must recover within $\pm 0.2‰$ of certified values; flag sequences that exceed this threshold.
- Blanks (empty tin capsules) must produce no detectable N_2 or CO_2 peak above the noise baseline.
- Archive all raw Isodat sequence files per SOP-L801.

10. References

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, *25*(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Paul, D., Skrzypek, G., & F'orizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, *21*(18), 3006–3014. https://doi.org/10.1002/rcm.3185

Werner, R. A., & Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, *15*(7), 501–519. https://doi.org/10.1002/rcm.258

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite

SOP	Title	Relationship
L414	Soil CN by Flash EA-IRMS	Application SOP
L415	Carbonate Isotopes by GasBench-IRMS	Application SOP

12. Training Record

Before using the IRMS independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety
3. Read the application SOP for the peripheral(s) they will use
4. Observe at least one complete analysis session by an experienced user
5. Complete supervised practice runs
6. Demonstrate a start-to-finish analysis to the satisfaction of the lab technician or a Super-User
7. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L211

Echo Labs Revolve Microscope

Standard Operating Procedure

SOP Number	SOP-L211
Revision	0.1
Author(s)	Dmaia Curry, Aparna Chintapalli
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, image capture, and maintenance of the Echo Labs Revolve microscope. The Revolve is a hybrid instrument that combines an upright microscope (for prepared glass slides) and an inverted microscope (for live samples in dishes) in a single rotating platform, with an iPad retina display replacing the traditional eyepiece (Echo Labs, 2016; Murphy & Davidson, 2012). The microscope supports bright field, phase contrast, fluorescence, and dark field imaging modes. It represents a substantial investment by the EA Program and requires careful handling.

2. Scope

- **Applies to:** All use of the Echo Labs Revolve microscope in the EA Program laboratory, including bright field, phase contrast, fluorescence, and dark field modes in both upright and inverted configurations
- **Does not apply to:** Electron microscopy, confocal microscopy, dissecting/stereo microscopes, or other optical microscopes not described here
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates, maintain optics
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training (including video modules) before independent use

4. Definitions

- **Brightfield illumination:** Standard transmitted-light microscopy in which the sample is illuminated from below and absorbing or scattering structures appear dark against a bright background; the most common mode for stained biological specimens.

- **Darkfield illumination:** A transmitted-light mode in which the direct beam is blocked; only obliquely scattered light reaches the objective, making unstained transparent structures appear bright against a dark background.
- **Epifluorescence:** A fluorescence microscopy mode in which excitation light is directed through the objective onto the sample, and the emitted fluorescence is collected back through the same objective; enables fluorescent stain visualization.
- **Eyepiece (ocular):** The lens assembly through which the observer views the magnified image; typically 10x; total magnification equals eyepiece magnification times objective magnification.
- **Fluorescence filter cube:** An interchangeable optical block containing an excitation filter, dichroic mirror, and emission filter matched to a specific fluorophore (e.g., DAPI, FITC, TRITC).
- **Numerical aperture (NA):** A dimensionless number characterizing the angular range of light an objective can accept; higher NA provides better resolution and light-gathering; the resolving power is approximately $d = 0.61\lambda/NA$.
- **Objective:** The primary lens closest to the specimen; available in magnifications of 4x, 10x, 20x, 40x, and 100x (oil immersion); higher magnification objectives have shorter working distances.
- **Working distance:** The distance between the front lens of the objective and the coverslip when the specimen is in focus; decreases with increasing magnification; critical for thick samples.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The fluorescence LED produces intense illumination. Avoid looking directly into the excitation light source. The iPad display eliminates the need to view through an eyepiece, reducing direct optical exposure, but reflected excitation light can still cause eye strain.
- **Electrical:** Keep liquids away from the iPad and the microscope base. If immersion oil or sample fluid contacts the electronics, disconnect power immediately and notify the lab manager.
- **Physical:** The microscope is heavy. Use both hands when rotating the platform between upright and inverted modes. Objective lenses and condensers are precision optics; handle with care.

5.1. Required PPE

☐ Safety glasses (especially during fluorescence mode)
☐ Lab coat
☐ Nitrile gloves (when handling biological specimens or immersion oil)

5.2. Emergency Procedures

If a biological spill occurs on or near the microscope, clean and disinfect per SOP-L002 Laboratory Safety. If immersion oil contacts the iPad or electronics, power off immediately and contact the lab manager before attempting cleanup.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Echo Labs Revolve microscope	With iPad retina display
Objective lenses	4x, 10x, 40x, 100x (oil immersion)
Low-magnification condenser	For 4x through 60x objectives
High-magnification condenser	For 100x oil immersion objective
Fluorescence LED module	Excitation wavelength TBD nm
Phase contrast rings	PhL, Ph1, Ph2, Ph3 (matched to objectives)

6.2. Consumables

Item	Notes
Microscope slides	Standard 25 x 75 mm
Cover slips	No. 1.5 thickness (0.17 mm)
Immersion oil (Type A or NF)	For 100x objective only
Lens cleaning solution	Optical-grade; for objectives and condenser
Lens cleaning tissue (lint-free)	Do not use Kimwipes on coated optics
Compressed gas duster	For removing dust from the optical path
Flash drive or cloud account	For exporting captured images

7. Procedure

7.1. Microscopy Modes Overview

The Revolve supports four imaging modes. Select the mode appropriate for the specimen and analysis:

Mode	Best For	Staining Required?
Bright field	Stained specimens, gross morphology	Recommended
Phase contrast	Unstained transparent specimens, live cells	No
Fluorescence	Specimens with fluorescent labels or autofluorescent pigments (e.g., cyanobacteria)	Fluorophore-dependent

Mode	Best For	Staining Required?
Dark field	Fine structures, edges, unstained specimens	No

7.2. Starting the Microscope

1. Press the power button on the top left corner of the iPad (Echo Labs, 2016).
2. Swipe to unlock. The overhead microscope light should turn on and a live image should appear on the screen.
3. If the light does not turn on or no image appears, open the **Echo Labs** app from the home screen
4. If the screen reads "Light Off," tap **BF** at the top of the screen to enable bright field illumination
5. Load the prepared slide into the stage clips (upright mode) or place the culture dish on the stage (inverted mode)

7.3. Rotating Between Upright and Inverted Mode

> **!** Important
>
> This is the most mechanically delicate operation on the Revolve. Follow each step carefully to avoid damaging the objectives or condenser.

1. Rotate the nosepiece to move all objective lenses out of the optical path
2. Unscrew the stage and carefully lift it away from the microscope body without contacting the condenser
3. Push the iPad flat so it is out of the rotation path
4. Press the release button on the upper back of the microscope body
5. Rotate the microscope body 180 degrees counterclockwise until it clicks into place
6. Reinstall the stage and tighten the mounting knob until secure
7. Rotate the desired objective lens back into position and realign the iPad

7.4. Using Bright Field Mode

1. Confirm that **BF** is selected on the screen
2. Place the slide or specimen on the stage
3. Start with the lowest magnification objective (4x) to locate the region of interest
4. Use the outer coarse focus knob (to the right of the iPad) to bring the specimen into approximate focus, then use the inner fine focus knob to sharpen the image
5. Use the stage translation knobs to move the slide: the lower knob moves the stage left/right, the upper knob moves it toward/away from you
6. Increase magnification by rotating the nosepiece to the next objective (10x, 40x)
7. Adjust brightness, contrast, and color balance using the sliding controls on the bottom right of the screen. Adjust the physical light intensity using the flat knob on the bottom right of the microscope body.

7.5. Using Phase Contrast Mode

Phase contrast converts differences in refractive index into differences in brightness, allowing transparent, unstained specimens to be visualized.

1. Set up the specimen in bright field mode first and bring it into focus
2. Select the phase contrast ring that matches the current objective:

Objective	Phase Ring
4x	PhL
10x	Ph1
40x	Ph2
100x	Ph3

3. Tap the **PH** mode selector on the screen (if available) or manually rotate the condenser turret to the matching phase ring
4. Fine-tune focus and centering as needed

> **Note**
>
> Phase contrast images may exhibit a bright "halo" around large objects (positive phase contrast) or a dark edge (negative phase contrast). This is a normal optical artifact caused by diffracted light passing through the phase ring. A "shade-off" effect, in which homogeneous regions appear at the same intensity as the background, is also expected.

7.6. Using Fluorescence Mode

1. Tap **FL** at the top of the screen to switch to fluorescence mode
2. The fluorescence LED will illuminate automatically
3. Place the specimen on the stage and focus using the same procedure as bright field
4. Minimize illumination time to reduce photobleaching. Locate the region of interest in bright field first, then switch to fluorescence only for image capture.

> **Warning**
>
> Fluorophores lose their ability to fluoresce over time when illuminated (photobleaching). Work quickly, minimize unnecessary excitation, and consider using photoprotective mounting media when possible.

7.7. Using Dark Field Mode

Section TBD. Consult the lab manager for dark field setup procedures until this section is completed.

7.8. Capturing and Exporting Images

1. To capture an image, tap the white camera shutter button on the left side of the screen
2. Enable the **anti-shake** feature (hand icon below the shutter button; it should turn orange when active) to reduce vibration blur
3. To review captured images, tap the gallery icon (square) at the top left of the screen. Tap **Done** to return to the live view.
4. Export images via a flash drive connected to the iPad, AirDrop, or the cloud sharing option in the Echo Labs app
5. Use a consistent file-naming convention: `YYYY-MM-DD_SampleID_Objective_Mode.jpg`

7.9. Using Oil Immersion (100x Objective)

1. Focus the specimen at 40x first
2. Rotate the nosepiece to an empty position (no objective in the light path)
3. Place a single small drop of immersion oil directly on the cover slip at the point of illumination
4. Rotate the 100x objective into position. The lens front element should contact the oil without trapping an air bubble.
5. Focus carefully using the fine focus knob only
6. After use, immediately clean the 100x objective with lens cleaning solution and lint-free tissue. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.10. Replacing the Condenser

The Revolve has two condensers: one for low magnification (4x through 60x) and one for 100x oil immersion. Condenser swaps require care to avoid contaminating or scratching the optics. Consult the lab manager before performing this procedure for the first time.

Detailed replacement steps TBD; to be documented with the lab manager.

7.11. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x) or to an empty position
3. If oil immersion was used, clean the 100x objective immediately
4. Wipe any sample residue or oil from the stage with a Kimwipe dampened with lens cleaning solution
5. Close the Echo Labs app on the iPad
6. Press and hold the power button to turn off the iPad
7. Cover the microscope with the dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in captured images that do not move when the specimen is translated, the optical path may need cleaning:

1. Do not attempt to clean internal optics without the lab manager present
2. For objective lens surfaces, apply a small amount of lens cleaning solution to lint-free tissue and gently wipe in a circular motion from center to edge
3. For dust on internal surfaces, remove the arm and use short bursts of compressed gas duster. Do not allow the compressed air canister to become cold, as it will deposit a film on the optics.
4. For persistent contamination, contact Echo Labs support or the lab manager

8.2. Image Quality Checklist

Before accepting an image for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- Scale bar or magnification metadata is recorded
- Anti-shake was enabled during capture
- In fluorescence mode, confirm that photobleaching has not significantly degraded the signal

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass; standard trash if plastic
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

Echo Labs. (2016). *Revolve microscope: User guide*. https://www.echolabs.com

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. https://doi.org/10.1002/9781118382905

11. Training Record

Before using the Revolve microscope independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Watch the Echo Labs training video at https://www.youtube.com/watch?v=IcubmIsxa7w
4. Observe a trained operator demonstrate upright/inverted rotation, all four imaging modes, and oil immersion setup and cleanup
5. Demonstrate proper startup, focusing, image capture, and shutdown under supervision
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

Component	Function
iPad display (eyepiece replacement)	High-resolution live view and image capture
Arm	Connects optics to base; removable for optical path cleaning
Stage	Flat platform for specimens; translatable via X/Y knobs
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Condenser	Focuses illumination light onto the specimen
Coarse/fine focus knobs	Outer knob = coarse focus; inner knob = fine focus
Light intensity knob	Physical control on the base for illumination brightness
Phase contrast turret	PhL, Ph1, Ph2, Ph3 rings matched to objectives
Fluorescence LED	Excitation source for fluorescence imaging
Platform release button	On the upper back; allows 180-degree rotation between upright and inverted modes

13. Appendix B: Sections Requiring Completion

The following sections from the original SOP were incomplete or marked as placeholders. They should be completed with input from the lab manager:

Section	Status	Notes
Fluorescence LED wavelength	TBD	Record the excitation wavelength from the module specifications
Dark field mode procedure	TBD	Document setup and use
Condenser replacement steps	TBD	Document with the lab manager
Specific catalog numbers for consumables	TBD	Add slide, cover slip, oil, lens cleaner part numbers

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L212

NanoDrop One Spectrophotometer

Standard Operating Procedure

SOP Number	SOP-L212
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration verification, and maintenance of the Thermo Scientific NanoDrop One micro-volume UV-Vis spectrophotometer. The NanoDrop measures nucleic acid and protein concentrations from samples as small as 1–2 uL and provides absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}) used to assess sample purity. For the role of NanoDrop measurements within the DNA extraction workflow, see SOP-L700 DNA Extraction.

2. Scope

- **Applies to:** All use of the NanoDrop One spectrophotometer in the EA Program laboratory, including nucleic acid quantification, protein quantification (A_{280} method), and custom wavelength measurements
- **Does not apply to:** Cuvette-based spectrophotometers (see SOP-L201), fluorometric quantification (see SOP-L213), or Hach benchtop spectrophotometry (see SOP-L303)
- **Location:** Instrument bench, Seaver ??

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **A_{260}:** Absorbance at 260 nm; the wavelength at which double-stranded DNA and RNA absorb most strongly; used to quantify nucleic acid concentration.
- **A_{280}:** Absorbance at 280 nm; absorbed primarily by aromatic amino acids (tyrosine, tryptophan) and by phenolic contaminants; used to assess protein contamination in nucleic acid preparations.
- **A_{260}/A_{280} ratio:** The ratio of absorbance at 260 nm to 280 nm; pure dsDNA gives ~1.8; pure RNA gives ~2.0; values below 1.7 indicate protein or phenol contamination.
- **A_{260}/A_{230} ratio:** The ratio of absorbance at 260 nm to 230 nm; values of 2.0–2.2 are expected for pure nucleic acids; low values indicate contamination with chaotropic salts (guanidinium), carbohydrates, or humic acids.
- **Micro-volume spectrophotometry:** Absorbance measurement using 1–2 µL of sample held by surface tension between a pedestal and an optical fiber arm; eliminates the need for cuvettes and allows measurement of very small volumes.
- **Pedestal:** The fixed lower optic of the NanoDrop on which the sample droplet is placed; must be cleaned with a lint-free wipe between measurements to prevent carryover.
- **Pathlength normalization:** Automatic adjustment of the measured absorbance to a standardized 10 mm pathlength equivalent; allows direct comparison with traditional cuvette measurements.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The NanoDrop uses a pulsed xenon flash lamp. Do not look directly into the light source when the pedestal arm is open.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.
- **Chemical:** Cleaning solvents (e.g., 0.5 M HCl for decontamination) require appropriate handling.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For biological spills on or near the instrument, clean and disinfect per SOP-L002 Laboratory Safety. If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
NanoDrop One spectrophotometer	Instrument bench, SGM 133	Thermo Scientific
Connected laptop or NanoDrop display	Instrument bench	For data export

6.2. Consumables

Item	Notes
Lint-free wipes (Kimtech)	For pedestal cleaning between samples
Nuclease-free water	For blanking and pedestal cleaning
Elution buffer	Must match the buffer used in extraction

7. Procedure

7.1. Startup

1. Open the NanoDrop One software (or wake the integrated touchscreen)
2. Select the appropriate application (e.g., **Nucleic Acid**, **Protein A280**, or **UV-Vis**)
3. Clean the upper and lower pedestals with 2 uL of nuclease-free water; wipe with a lint-free tissue
4. Perform a blank measurement (see Section 6.2)

7.2. Blanking

1. Pipette 2 uL of the blank solution onto the lower pedestal. The blank must match the sample matrix – use the same elution buffer or solvent that the samples are dissolved in.
2. Lower the sampling arm and tap **Blank**
3. Verify that the blank spectrum is flat and the baseline absorbance at 260 nm is near zero
4. Wipe both pedestals with a lint-free tissue

> **Tip**
>
> Re-blank whenever you switch sample types, change buffers, or if the instrument has been idle for more than 30 minutes.

7.3. Measuring Samples

1. Pipette 2 uL of sample onto the lower pedestal
2. Lower the sampling arm and tap **Measure**
3. Record the following for each sample:
   - Concentration (ng/uL)
   - A_{260}/A_{280} ratio
   - A_{260}/A_{230} ratio
4. Wipe both pedestals with a lint-free tissue between every sample
5. Repeat for all samples

7.4. Interpreting Results

Parameter	Expected Range	Interpretation
A_{260}/A_{280}	1.8–2.0	Values below 1.8 suggest protein or phenol contamination
A_{260}/A_{230}	2.0–2.2	Values below 2.0 suggest humic acid, carbohydrate, salt, or guanidine contamination

> **Note**
>
> For environmental DNA extracts, A_{260}/A_{230} ratios are frequently below 2.0. If the NanoDrop concentration is more than twice the Qubit reading, the UV absorbance is likely inflated by co-extracted contaminants; use the Qubit value for planning downstream reactions.

7.5. Data Export

1. Save the session file (.tsv or .csv) to the designated project folder on the connected laptop
2. Use a consistent file-naming convention: `YYYY-MM-DD_NanoDrop_ProjectName.tsv`
3. Record results in the lab notebook and/or project data sheet

7.6. Shutdown and Daily Maintenance

1. Wipe both pedestals with 2 uL of nuclease-free water, then dry with a lint-free tissue
2. If protein or concentrated DNA residue is visible, clean with 2 uL of 0.5 M HCl, wait 10 seconds, then rinse with nuclease-free water
3. Close the software and lower the sampling arm
4. Cover the instrument with the dust cover if available

8. Quality Control and Acceptance Criteria

- Verify instrument performance at the start of each session by measuring a blank; the 260 nm absorbance should read 0.00 +/- 0.02
- If the NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; trust the Qubit value
- *TBD – establish a schedule for periodic performance verification using Thermo Scientific NanoDrop verification standards*

9. Waste Disposal

Waste Type	Disposal Method
Used lint-free wipes	Standard trash
Pipette tips	Standard trash (or biohazard if samples are hazardous)
Residual sample on pedestal	Wiped and cleaned as part of shutdown

10. References

- Thermo Fisher Scientific. *NanoDrop One/One^C User Guide*. Thermo Fisher Scientific, Waltham, MA.

- Desjardins, P. & Bhatt, T. (2010). Micro-volume quantification of nucleic acids. *Current Protocols*, A.3J.1–A.3J.16.

11. Training Record

Before using the NanoDrop independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator demonstrate blanking, measurement, and data export
4. Perform a practice session (blank plus at least 5 samples) under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L213

Qubit 4 Fluorometer

Standard Operating Procedure

SOP Number	SOP-L213
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Invitrogen Qubit 4 Fluorometer. The Qubit uses fluorescent dyes that bind selectively to the target molecule (dsDNA, ssDNA, RNA, or protein), providing more accurate quantification than UV absorbance methods, especially at low concentrations or when contaminants are present. For the role of Qubit measurements within the DNA extraction workflow, see SOP-L700 DNA Extraction.

2. Scope

- **Applies to:** All use of the Qubit 4 Fluorometer in the EA Program laboratory, including dsDNA Broad Range (BR), dsDNA High Sensitivity (HS), RNA, and protein assays
- **Does not apply to:** UV-Vis spectrophotometric quantification (see SOP-L212), Turner Designs fluorometry for chlorophyll or other environmental analytes (see SOP-L203)
- **Location:** Instrument bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Maintain reagent inventory, schedule service
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Broad Range (BR) assay:** A Qubit assay kit designed for DNA concentrations of 2–1,000 ng/µL; less sensitive than the HS assay but covers a wider dynamic range; used when high DNA yield is expected.
- **dsDNA:** Double-stranded DNA; the form quantified by most Qubit DNA assay kits; the fluorescent dye binds specifically to dsDNA, not to ssDNA, RNA, or protein contaminants.
- **Fluorophore:** A fluorescent chemical compound that absorbs light at a specific excitation wavelength and emits light at a longer emission wavelength; the Qubit dye is a fluorophore that binds selectively to dsDNA, ssDNA, or RNA depending on the kit.
- **High Sensitivity (HS) assay:** A Qubit assay kit designed for DNA concentrations of 0.2–100 ng/µL; more sensitive than BR; preferred for low-yield environmental DNA extractions from soil or water.
- **Qubit assay tube:** A thin-walled 0.5 mL PCR tube in which the working solution and sample are mixed for measurement; must be free of fluorescent contaminants; do not substitute with other tube types.
- **Working solution:** A freshly prepared mixture of Qubit reagent diluted in buffer at a ratio specified by the kit (usually 1:200); prepared fresh for each session; stable for up to 3 hours at room temperature.

5. Safety and Hazards

Hazards

- **Chemical:** Qubit reagent concentrate contains DMSO. Avoid skin contact; review the SDS before handling.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes and consult the SDS. For biological spills, clean per SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Qubit 4 Fluorometer	Instrument bench, SGM 133	Invitrogen / Thermo Fisher
USB drive or Wi-Fi connection	–	For data export

6.2. Reagents and Consumables

Item	Catalog No.	Storage
Qubit dsDNA BR Assay Kit	Q32850	Room temperature
Qubit dsDNA HS Assay Kit	Q32851	Room temperature
Qubit assay tubes (0.5 mL, thin-wall)	Q32856	Room temperature
Nuclease-free water	Various	Room temperature

Note

Use only the thin-wall 0.5 mL Qubit assay tubes (Cat. Q32856). Standard microcentrifuge tubes are not optically compatible and will produce inaccurate readings.

7. Procedure

7.1. Selecting the Assay

Choose the assay based on expected concentration (Invitrogen, 2019):

Assay	Detection Range	When to Use
dsDNA BR	2–1,000 ng	Most soil/sediment extractions
dsDNA HS	0.2–100 ng	Low-yield samples (e.g., water filters, dilute extracts)
RNA BR / HS	See kit documentation	RNA quantification
Protein	See kit documentation	Protein quantification

7.2. Preparing the Working Solution

1. Calculate the volume of working solution needed: 200 uL per assay tube (all samples plus 2 standards)
2. Dilute the Qubit reagent concentrate 1:200 in Qubit buffer. For example, for 10 tubes: 10 uL reagent + 1,990 uL buffer = 2,000 uL total.
3. Vortex briefly to mix

> **Important**
>
> Prepare the working solution fresh for each session. Do not store diluted working solution.

7.3. Preparing Standards

1. Label two Qubit assay tubes: **Std 1** and **Std 2**
2. Add 190 uL of working solution to each tube
3. Add 10 uL of Standard 1 to the first tube and 10 uL of Standard 2 to the second tube
4. Vortex each tube briefly (2–3 seconds)

7.4. Preparing Samples

1. Label one Qubit assay tube per sample
2. Add 198 uL of working solution to each tube
3. Add 2 uL of sample (DNA extract, RNA, or protein solution) to the corresponding tube
4. Vortex each tube briefly (2–3 seconds)

> **Tip**
>
> The sample volume can range from 1–20 uL as long as the total volume in each tube equals 200 uL (adjust the working solution volume accordingly). Using 2 uL of sample minimizes

consumption of precious extracts.

7.5. Incubation

1. Incubate all tubes (standards and samples) at room temperature for 2 minutes (Invitrogen, 2019)
2. Do not incubate for longer than 30 minutes; fluorescence signal decays over time

7.6. Calibration

1. On the Qubit home screen, select the appropriate assay (e.g., **dsDNA Broad Range**)
2. Tap **Read Standards**
3. Insert the Std 1 tube, close the lid, and tap **Read**
4. When prompted, insert the Std 2 tube, close the lid, and tap **Read**
5. Verify that the calibration is accepted (green checkmark). If it fails, prepare fresh standards and repeat.

7.7. Reading Samples

1. Tap **Run Samples**
2. Set the sample volume to match the volume of sample added (e.g., 2 uL)
3. Set the output units (ng/uL is standard for DNA extracts)
4. Insert the sample tube, close the lid, and tap **Read**
5. Record the concentration (ng/uL, corrected for dilution by the instrument)
6. Repeat for all samples

7.8. Data Export

1. Export results via USB or Wi-Fi to the designated project folder
2. Use a consistent file-naming convention: `YYYY-MM-DD_Qubit_ProjectName.csv`
3. Record results in the lab notebook and/or project data sheet

7.9. Shutdown and Daily Maintenance

1. Remove all tubes from the instrument
2. Wipe the sample chamber with a lint-free tissue if any liquid was spilled
3. Close the lid
4. Dispose of used assay tubes (see Section 8)

8. Quality Control and Acceptance Criteria

- Run fresh standards at the beginning of each session; do not rely on a previous calibration
- Standards must be within the acceptable range indicated by the instrument (green checkmark)

- If a sample reads "Out of Range," re-prepare it using the other assay sensitivity (e.g., switch from BR to HS for low-concentration samples)
- Acceptable DNA yield from 0.25 g of soil: typically 1–20 ng/uL in a 100 uL elution, depending on soil type
- If the NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; trust the Qubit value for downstream planning

TBD – establish a periodic performance check using Qubit DNA standards of known concentration.

9. Waste Disposal

Waste Type	Disposal Method
Used Qubit assay tubes	Standard trash (rinse if biohazardous)
Leftover working solution	Standard drain (dilute DMSO, non-hazardous at this concentration)
Pipette tips	Standard trash (or biohazard if samples are hazardous)

10. References

Invitrogen. (2019). *Qubit 4 fluorometer user guide.* Thermo Fisher Scientific. https://www.thermofisher.com/us/en/home/references/protocols/nucleic-acid-purification-and-analysis/qubit-assay-protocols.html

11. Training Record

Before using the Qubit independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator demonstrate standard preparation, calibration, sample reading, and data export
4. Perform a practice session (calibration plus at least 5 samples) under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L214

Bio-Rad QX200 Droplet Digital PCR System

Standard Operating Procedure

SOP Number	SOP-L214
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, maintenance, and troubleshooting of the Bio-Rad QX200 Droplet Digital PCR (ddPCR) system, including the QX200 Droplet Generator, the C1000 Touch Thermal Cycler with the 96-well Deep Well Reaction Module, and the QX200 Droplet Reader. The QX200 partitions each PCR reaction into approximately 20,000 nanoliter-sized droplets, enabling absolute quantification of target DNA sequences without a standard curve. For assay design, primer/probe sequences, and data analysis, see SOP-L701 ddPCR Nitrogen Cycle Primers and SOP-L702 ddPCR Data Analysis.

2. Scope

- **Applies to:** All use of the Bio-Rad QX200 ddPCR system in the EA Program laboratory, including droplet generation, thermal cycling, and droplet reading
- **Does not apply to:** Assay design, primer validation, or data analysis (see SOP-L701 and SOP-L702); conventional qPCR on other instruments
- **Location:** *TBD* (confirm room and bench assignment)

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage consumable inventory, coordinate Bio-Rad service visits
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete Bio-Rad training modules and supervised practice before independent use

4. Definitions

- **Amplitude:** The fluorescence signal intensity of a droplet; positive droplets (containing target DNA) have high amplitude; negative droplets have low amplitude; a clear separation between positive and negative populations is required for accurate quantification.
- **Droplet digital PCR (ddPCR):** A method of absolute quantification of nucleic acids in which the sample is partitioned into ~20,000 nanoliter-sized water-in-oil droplets, each containing zero or one target molecule; after PCR amplification, the fraction of fluorescent positive droplets is used to calculate target concentration using Poisson statistics.
- **FAM:** A green fluorescent dye (fluorescein amidite) used to label one probe or primer in a duplex ddPCR assay; detected in the FAM channel (channel 1) by the QX200.
- **HEX:** A yellow-orange fluorescent dye used to label a second probe in a duplex ddPCR assay, or as an alternative to VIC; detected in channel 2 by the QX200.

- **Poisson correction:** A statistical adjustment applied by the DropletQuant software to account for the probability that some droplets contain more than one target molecule; necessary when target concentrations are high.
- **Copies per microliter (copies/µL):** The absolute concentration of the target DNA in the final ddPCR reaction, as reported by DropletQuant; must be back-calculated to the original sample concentration using the appropriate dilution factor.
- **Threshold:** The fluorescence amplitude boundary separating positive from negative droplets; set manually or automatically in the analysis software; incorrect threshold setting is the most common source of ddPCR quantification error.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Droplet generation oil (Bio-Rad Cat. 1863005) is a fluorinated oil. Avoid skin contact and inhalation. Work in a well-ventilated area or fume hood when handling bulk oil. Review the SDS before first use.
- **Biological:** Samples may contain environmental DNA from unknown organisms. Treat all samples and amplified products as potentially biohazardous.
- **Physical:** The thermal cycler reaches temperatures up to 98 C. Allow the block to cool before removing plates. The Droplet Reader needle is sharp; do not reach into the reader bay.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (change between pre-PCR and post-PCR areas)

5.2. Emergency Procedures

For oil spills, absorb with paper towels and dispose as chemical waste. For thermal burns, apply cool running water and seek first aid. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
QX200 Droplet Generator	*TBD*	Generates water-in-oil emulsion

Item	Location	Notes
C1000 Touch Thermal Cycler	*TBD*	With 96-Deep Well Reaction Module
QX200 Droplet Reader	*TBD*	Reads fluorescence per droplet
Computer with QuantaSoft / QuantaSoft AP	*TBD*	For instrument control and analysis
DG8 Cartridge Holder	*TBD*	Holds cartridges during generation
Plate sealer (PX1 PCR Plate Sealer)	*TBD*	Heat-seals 96-well plates

6.2. Reagents and Consumables

Item	Catalog No.	Storage
DG8 Cartridges for QX200	1864008	Room temperature
DG8 Gaskets	1863009	Room temperature
Droplet Generation Oil for Probes	1863005	Room temperature
Droplet Generation Oil for EvaGreen	1864006	Room temperature
ddPCR 96-Well Plates	12001925	Room temperature
Pierceable Foil Heat Seals	1814040	Room temperature
ddPCR Supermix for Probes (No dUTP)	1863024	–20 C
QX200 ddPCR EvaGreen Supermix	1864034	–20 C

7. Procedure

7.1. Pre-PCR Setup (Clean Area)

> **!** Important
>
> All pre-PCR work (reaction assembly, droplet generation) must be performed in the designated pre-PCR area to prevent amplicon contamination. Use dedicated pipettes and wear clean gloves.

1. Thaw the ddPCR Supermix and DNA templates on ice
2. Prepare the reaction master mix according to the assay protocol (see SOP-L701 for primer/probe concentrations and cycling conditions)
3. Dispense 20 uL of each assembled reaction into the sample wells (middle row) of a DG8 cartridge
4. Add 70 uL of Droplet Generation Oil to the oil wells (bottom row)
5. Attach a DG8 gasket to the cartridge

7.2. Droplet Generation

1. Place the loaded cartridge into the QX200 Droplet Generator

2. Close the lid and press **Start**. Generation takes approximately 2 minutes per cartridge.
3. When complete, carefully open the lid. Do not tilt or shake the cartridge – the droplets are fragile.
4. Using a multichannel pipette set to 40 uL, gently aspirate the droplets from the top row of the cartridge and dispense into the 96-well plate. Pipette slowly (approximately 3 seconds to aspirate, 3 seconds to dispense) to avoid shearing droplets.
5. Repeat for each cartridge

7.3. Plate Sealing

1. Place the pierceable foil heat seal over the loaded 96-well plate
2. Seal using the PX1 PCR Plate Sealer at 180 C for 5 seconds
3. Visually confirm a uniform seal across all wells

7.4. Thermal Cycling

1. Place the sealed plate into the C1000 Touch Thermal Cycler with the 96-Deep Well Reaction Module
2. Program the thermal cycling protocol per SOP-L701 (typical: 95 C activation, 40 cycles of denaturation/annealing, 98 C droplet stabilization, 4 C hold)
3. Set the ramp rate to 2 C/s (critical for ddPCR; do not use the default maximum ramp rate)
4. Start the run. Cycling typically takes 2–2.5 hours.
5. After cycling, plates can be held at 4 C for up to 24 hours before reading, but immediate reading is preferred

7.5. Droplet Reading

1. Open QuantaSoft software on the connected computer
2. Set up the plate layout: assign sample names, target names, experiment type (ABS for absolute quantification), and supermix type
3. Place the sealed plate into the QX200 Droplet Reader
4. Confirm that the reader waste and buffer bottles are at appropriate levels (refill or empty as needed before starting a full plate)
5. Tap **Start** to begin reading. The reader processes each well individually (approximately 1–2 minutes per well).
6. Monitor the first few wells to confirm that droplet counts exceed 10,000 accepted events per well

7.6. Data Review

1. After reading, review the 1-D amplitude plots in QuantaSoft to verify clear separation between positive and negative droplet clusters
2. Set the fluorescence amplitude threshold manually using the NTC and positive control wells as guides
3. Check that all wells have at least 10,000 accepted droplets; flag any wells below this threshold
4. Export results as .csv for downstream analysis (see SOP-L702)

7.7. Shutdown and Daily Maintenance

1. Remove the plate from the Droplet Reader
2. Check the reader waste bottle; empty if more than 75% full
3. Check the reader buffer bottle; refill if less than 25% full
4. Wipe down the Droplet Generator cartridge holder with a lint-free tissue
5. Dispose of used cartridges and gaskets (see Section 8)
6. Close QuantaSoft and shut down instruments if no further runs are scheduled

8. Quality Control and Acceptance Criteria

- Every run must include: no-template controls (NTC, minimum 2 wells), extraction blanks (from SOP-L700), and positive controls (gBlock or plasmid at known concentration)
- Droplet count per well must exceed 10,000 accepted droplets (out of approximately 20,000 generated); wells below this threshold should be excluded
- NTC wells must show 0 or fewer than 3 positive droplets; more than 3 indicates contamination and the plate or affected target should be rerun
- Positive/negative droplet cluster separation must be unambiguous on the 1-D amplitude plot
- *TBD – define acceptable coefficients of variation for technical replicates (typically less than 15–25% for ddPCR)*
- *TBD – establish a maintenance log and schedule for Bio-Rad service visits*

9. Waste Disposal

Waste Type	Container	Disposal Method
Used DG8 cartridges and gaskets	Standard trash	Non-hazardous
Droplet generation oil waste	Chemical waste container	EH&S hazardous waste pickup
Used PCR plates and foil seals	Standard trash	Non-hazardous after thermal cycling
Pipette tips (pre- and post-PCR)	Standard trash	Non-hazardous
Reader waste fluid	Chemical waste container	EH&S hazardous waste pickup

10. References

- Bio-Rad Laboratories. *QX200 Droplet Digital PCR System Instruction Manual.* Bio-Rad Laboratories, Hercules, CA.
- Bio-Rad Laboratories. *Droplet Digital PCR Applications Guide.* Bio-Rad Bulletin 6407.
- Hindson, B. J. et al. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, 83(22), 8604–8610.

11. Training Record

Before using the QX200 system independently, personnel must:

1. Read this SOP, SOP-L701, and SOP-L003 Hazardous Materials Handling
2. Complete the Bio-Rad QX200 online training modules or equivalent manufacturer training
3. Observe a trained operator demonstrate droplet generation, thermal cycling setup, and droplet reading for a full plate
4. Perform a supervised run including reaction assembly, droplet generation, plate sealing, thermal cycling, and reading
5. Demonstrate competence in QuantaSoft plate setup and threshold setting
6. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Low droplet count (below 10,000)	Pipetting error during transfer; clogged cartridge	Pipette more slowly (3 s up, 3 s down); use a fresh cartridge
No separation between positive and negative clusters	Poor primer/probe design; degraded reagents	Review assay (SOP-L701); use fresh Supermix
Rain (droplets between clusters)	Suboptimal annealing temperature; partial inhibition	Run a temperature gradient; dilute template
Reader error or blockage	Air bubble or debris in reader line	Run a cleaning cycle per Bio-Rad instructions; contact lab manager
Positive droplets in NTC wells	Amplicon contamination	Decontaminate pre-PCR area with 10% bleach; use fresh reagents; separate pre- and post-PCR areas strictly

13. Appendix B: Sections Requiring Completion

Section	Status	Notes
Instrument location	TBD	Confirm room and bench assignment
Maintenance schedule	TBD	Establish log and Bio-Rad service intervals
Reader cleaning cycle details	TBD	Document with Bio-Rad field service engineer
Lab-specific LOD/LOQ values	TBD	Establish after primer validation (SOP-L701)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L214

QX200 Droplet Digital PCR System: Instrument Familiarization and Standard Operation

Standard Operating Procedure

SOP Number	SOP-L214
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure orients new personnel to the Bio-Rad QX200 Droplet Digital PCR (ddPCR) system and describes its safe, standard operation: the components of the system, the consumables and reagents, the four-stage workflow (droplet generation, plate sealing, thermal cycling, and droplet reading), basic use of QuantaSoft software, routine maintenance, and common troubleshooting.

It is a familiarization and operation SOP. It does not cover the science of assay or primer design (see SOP-L701) or the analysis and interpretation of results (see SOP-L702 Applications Guide). The workflow described here follows Bio-Rad's published guidance for the platform (Bio-Rad Laboratories, 2018, 2024), which itself implements the droplet-counting principle of absolute quantification (Hindson et al., 2011).

2. Scope

- **Applies to:** Operation of the QX200 ddPCR system in the lab, comprising the QX200 Droplet Generator, the PX1 PCR Plate Sealer, the C1000 Touch Thermal Cycler with 96-deep-well reaction module, and the QX200 Droplet Reader, together with QuantaSoft software.
- **Does not apply to:** Assay/primer design (SOP-L701), data analysis and interpretation (SOP-L702), or DNA extraction (SOP-L700).
- **Prerequisite:** A prepared, validated reaction mix and plate plan from SOP-L701.

> **SOP numbering**
>
> This instrument SOP is numbered L214, placing it ahead of the L700-series analysis SOPs in the lab scheme. Conceptually it precedes analysis: complete this familiarization before running samples for SOP-L702.

3. Responsibilities

Role	Responsibility
Lab PI	Approve instrument access; oversee major maintenance
Lab Manager	Maintain consumable stock, oil, and service records
Trained Personnel	Operate the system per this SOP; log each run
New Students	Complete familiarization and supervised runs before independent use

4. Definitions

- **Amplitude:** The fluorescence signal intensity of a droplet; positive droplets (containing target DNA) have high amplitude; negative droplets have low amplitude; a clear separation between positive and negative populations is required for accurate quantification.
- **Droplet digital PCR (ddPCR):** A method of absolute quantification of nucleic acids in which the sample is partitioned into ~20,000 nanoliter-sized water-in-oil droplets, each containing zero or one target molecule; after PCR amplification, the fraction of fluorescent positive droplets is used to calculate target concentration using Poisson statistics.
- **FAM:** A green fluorescent dye (fluorescein amidite) used to label one probe or primer in a duplex ddPCR assay; detected in the FAM channel (channel 1) by the QX200.
- **HEX:** A yellow-orange fluorescent dye used to label a second probe in a duplex ddPCR assay, or as an alternative to VIC; detected in channel 2 by the QX200.
- **Poisson correction:** A statistical adjustment applied by the DropletQuant software to account for the probability that some droplets contain more than one target molecule; necessary when target concentrations are high.
- **Copies per microliter (copies/µL):** The absolute concentration of the target DNA in the final ddPCR reaction, as reported by DropletQuant; must be back-calculated to the original sample concentration using the appropriate dilution factor.
- **Threshold:** The fluorescence amplitude boundary separating positive from negative droplets; set manually or automatically in the analysis software; incorrect threshold setting is the most common source of ddPCR quantification error.

5. Safety and Hazards

⚠ Hazards and precautions

- **Droplet generation oil and reader oil.** Use the oil specified for the chemistry (probe vs. EvaGreen). Avoid skin and eye contact; wear gloves and eye protection; consult the SDS for each oil. Clean spills promptly.
- **Reagents.** ddPCR supermixes and any sample DNA are handled with gloves. Environmental soil DNA is low biohazard risk, but treat all extracts with standard lab precautions.
- **Moving parts.** The droplet reader autosampler moves during a run. Keep hands clear and the lid closed while running.
- **Electrical and thermal.** Standard bench-instrument electrical safety. The thermal cycler block reaches high temperatures; do not touch the block during or immediately after a run.
- **Sharps / glass.** None in normal operation; cartridges and plates are single-use plastics.

! Consumables are not interchangeable

ddPCR requires Bio-Rad's proprietary droplet-generation reagents, cartridges, and gaskets. Substituting non-specified consumables will produce unusable droplets (Bio-Rad Laboratories, 2018). Match the oil to the supermix chemistry.

6. Equipment, Consumables, and Reagents

6.1. Instruments

Item	Role in workflow
QX200 Droplet Generator	Partitions each 20 uL reaction into ~20,000 droplets
DG8 cartridge holder	Holds the DG8 cartridge during generation
PX1 PCR Plate Sealer	Heat-seals droplets into the 96-well plate with pierceable foil
C1000 Touch Thermal Cycler (96-deep-well module)	Endpoint PCR amplification within droplets
QX200 Droplet Reader	Streams droplets single-file and reads two-color fluorescence
Instrument computer (QuantaSoft)	Plate setup, run control, thresholding, export

6.2. Consumables and reagents

Item	Notes
DG8 cartridges and gaskets	Single-use; one cartridge = 8 samples
Droplet generation oil (probes) **or** QX200 EvaGreen droplet oil	Match to supermix chemistry
Droplet reader oil	Loaded in the reader
ddPCR Supermix for Probes **or** QX200 ddPCR EvaGreen Supermix	Per assay (SOP-L701)
ddPCR 96-well plates and pierceable foil heat seals	For PX1 sealing and reading
Filtered pipette tips, low-bind tubes	Standard molecular-biology consumables

Tip

Keep a stock log for cartridges, gaskets, oils, plates, and foil. The droplet generator and reader each draw oil from onboard bottles; check levels before every session.

Ordering supermixes, oils, and assays

Order supermixes, droplet oils, cartridges, plates, and validated PrimePCR ddPCR assays from the Bio-Rad digital assay catalog: https://www.bio-rad.com/digital-assays. Confirm chemistry (probe vs EvaGreen) before ordering so the supermix and matching oil are consistent.

7. Procedure

7.1. System Overview

The QX200 system performs absolute quantification by partitioning, amplifying, and counting (Bio-Rad Laboratories, 2018; Hindson et al., 2011). A single 20 uL reaction is split into roughly 20,000 uniform water-in-oil droplets. Each droplet is a tiny independent PCR. After endpoint amplification, the reader passes droplets one at a time through a two-color optical detector (channel 1 for FAM, channel 2 for HEX or VIC), scoring each droplet as positive or negative. QuantaSoft fits the positive fraction to a Poisson model to return target concentration in copies per microliter.

The work proceeds through four instruments in sequence:

1. **Droplet Generator** (Section 6.4) makes the droplets.
2. **PX1 Plate Sealer** (Section 6.5) seals them into a plate.
3. **C1000 Touch Thermal Cycler** (Section 6.6) amplifies.
4. **Droplet Reader** (Section 6.7) counts.

7.2. Daily Startup

1. Power on the instrument computer and the instruments to be used.
2. Launch QuantaSoft and confirm it connects to the droplet generator and/or reader.
3. Check oil levels in the droplet generator and the reader; top up or replace bottles per the manual (Bio-Rad Laboratories, 2024).
4. Empty the reader's waste bottle if near full.
5. Confirm consumable stock for the planned run (cartridges, gaskets, oil, plate, foil).

7.3. Consumables Orientation

Before the first run, identify each consumable and where it loads:

- **DG8 cartridge** seats in the cartridge holder. The middle row of wells receives sample; the bottom row receives generation oil; the top row collects the generated droplets.
- **Gasket** snaps over the loaded cartridge to seal it for generation.
- **Generation oil** is chemistry-specific. Using probe oil with EvaGreen supermix (or vice versa) will fail.
- **Pierceable foil** is the only seal type compatible with the reader's autosampler; do not use hard-seal films.

7.4. Droplet Generation

1. Prepare reactions per SOP-L701 and load into the cartridge holder with a DG8 cartridge.
2. Pipette each sample into the sample wells (avoid bubbles; pipette to the first stop).
3. Pipette the matched generation oil into the oil wells.
4. Place the gasket over the cartridge.

5. Load the holder into the QX200 Droplet Generator and close the lid; the run takes about two minutes per cartridge.
6. After generation, slowly transfer the droplets from the top row of the cartridge to the corresponding wells of a ddPCR 96-well plate using a multichannel pipette. Pipette gently and at an angle; droplets are fragile and shearing them lowers the usable count.

> **Warning**
>
> Damaged or sheared droplets reduce accepted-droplet counts and can fail the QC threshold (see SOP-L702, Section 6.2). Pipette droplets slowly and avoid introducing air.

7.5. Plate Sealing (PX1)

1. Place the plate and a sheet of pierceable foil in the PX1 Plate Sealer.
2. Seal at the manufacturer-specified temperature and time (commonly 180 C for 5 s; confirm against the current manual (Bio-Rad Laboratories, 2024)).
3. Inspect the seal: the foil should be uniformly bonded with no lifted edges.

7.6. PCR Amplification

1. Transfer the sealed plate to the C1000 Touch with the 96-deep-well module.
2. Run the validated cycling program for the assay (from SOP-L701).
3. **Set the ramp rate to about 2 C/s.** This slower ramp is important for even amplification across the droplet population on this platform (Bio-Rad Laboratories, 2018).
4. Include a final hold and store at 4 C if not reading immediately; read within the window specified for the chemistry.

A generic template program is given in Appendix B; the assay-specific anneal temperature and cycle number come from SOP-L701.

7.7. Droplet Reading

1. Place the amplified, sealed plate in the QX200 Droplet Reader plate holder with the retaining clamp.
2. In QuantaSoft, set up the plate layout: sample names, assay/target names, fluorophores (FAM, HEX/VIC), experiment type (e.g., absolute quantification), and supermix/chemistry.
3. Start the run. The autosampler aspirates droplets well by well and the detector reads two channels per droplet.
4. Do not open the reader or disturb the plate while running.

7.8. QuantaSoft Orientation (Reading the Output)

This is orientation only; thresholding decisions and full analysis are covered in SOP-L702.

- **1-D plot:** fluorescence amplitude vs. droplet (event) number for one channel. Positive droplets sit above the threshold line; negatives below.
- **2-D plot:** channel 1 (FAM) vs. channel 2 (HEX/VIC) amplitude. In a duplex assay, droplets fall into four clusters: double-negative, FAM-only, HEX-only, and double-positive (Bio-Rad Laboratories, 2018).
- **Thresholding:** the boundary between positive and negative clusters. Review it for every well; adjust manually in QuantaSoft Analysis Pro where the automatic call is poor.
- **Well merging:** replicate wells of the same sample/target may be merged to increase the effective partition count before concentration is calculated.
- **Concentration:** QuantaSoft reports copies per microliter from the Poisson fit. Export as described in SOP-L702, Section 6.1.

7.9. Shutdown

1. Close the experiment and export/back up data (SOP-L702).
2. Remove and discard the plate and used cartridges/gaskets (Section 8).
3. Check and, if needed, empty the reader waste bottle.
4. Power down per the manual; leave the system in the state your lab specifies for the reader oil lines.

7.10. Maintenance and Care

- **Each session:** check oil levels and the waste bottle; inspect for leaks.
- **Routine:** keep the reader's fluidics maintained per the manual; do not let oil bottles run dry mid-run.
- **Do not:** force cartridges or gaskets, reuse single-use consumables, mix oil chemistries, or use non-pierceable seals.
- **Records:** log each run (date, operator, assay, plate, instrument notes) and any maintenance or service in the instrument logbook.

7.11. Troubleshooting

Symptom	Likely cause	Action
Low accepted-droplet count	Sheared droplets; pipetting too fast; bubbles	Pipette droplets slowly at an angle; avoid air; re-generate
Few or no droplets generated	Wrong/insufficient oil; cartridge/gasket seated poorly	Use matched oil; reseat cartridge and gasket
"Rain" (droplets between clusters)	Suboptimal anneal temperature; degraded sample	Optimize anneal (SOP-L701); check DNA quality (SOP-L700)
Reader error / clog	Low oil; debris; fluidics issue	Check oil and waste; run reader maintenance; see manual
High NTC positives	Contamination	Rerun with fresh reagents; review setup (SOP-L702, Section 6.2)

8. Quality Control and Acceptance Criteria

- Each well should yield at least 10,000 accepted droplets (target ~12,000 to 20,000); wells below 10,000 are excluded in analysis (SOP-L702).
- Run the controls defined in SOP-L702 (NTC, extraction blank, positive control) on every plate.
- Confirm uniform plate sealing and correct plate orientation before reading.
- Log instrument run metadata to support digital-PCR reporting standards (The dMIQE Group & Huggett, 2020).

9. Waste Disposal

- Used DG8 cartridges, gaskets, plates, foil, and tips: dispose as standard laboratory plastic/biohazard waste per the lab's waste plan.
- Generation and reader oil and oily waste: collect and dispose per the SDS and the lab's chemical-waste procedures; do not pour oil down the drain.
- Reagent containers: rinse and dispose per local guidance.

10. References

> **i** Finding published methods
>
> For peer-reviewed studies that used ddPCR, including environmental and microbial applications useful when validating an assay or interpreting low target concentrations, search Bio-Rad's ddPCR publication database: https://www.bio-rad.com/ddPCR/publications.

Bio-Rad Laboratories. (2018). *Droplet digital PCR applications guide*. Bio-Rad Laboratories, Inc.

Bio-Rad Laboratories. (2024). *QX200 droplet digital PCR system instruction manual*. https://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Bright, I. J., Lucero, M. Y., Hiddessen, A. L., Legler, T. C., Kitano, T. K., Hodel, M. R., Petersen, J. F., Wyatt, P. W., Steenblock, E. R., Shah, P. H., Bousse, L. J., Troup, C. B., Mellen, J. C., … Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, *83*(22), 8604–8610. https://doi.org/10.1021/ac202028g

The dMIQE Group, & Huggett, J. F. (2020). The digital MIQE guidelines update: Minimum information for publication of quantitative digital PCR experiments for 2020. *Clinical Chemistry*, *66*(8), 1012–1029. https://doi.org/10.1093/clinchem/hvaa125

11. Training Record

Before operating the QX200 system independently, personnel must:

1. Read this SOP and review the current Bio-Rad QX200 manual (Bio-Rad Laboratories, 2024) and Applications Guide (Bio-Rad Laboratories, 2018).
2. Read SOP-L701 Primer Design and SOP-L702 Applications Guide for context on what they are running and why.
3. Observe a full run (generation, sealing, cycling, reading) performed by trained personnel.
4. Complete at least one full run under supervision, including QuantaSoft plate setup and export.
5. Be signed off by the PI or lab manager.

Trainee	Date observed	Date supervised run	Signed off by	Date

12. Appendix A: Pre-Run Checklist

- ☐ Validated reaction mix and plate plan ready (SOP-L701)
- ☐ Instruments powered on; QuantaSoft connected
- ☐ Generation oil matches supermix chemistry; sufficient volume
- ☐ Reader oil level adequate; waste bottle emptied
- ☐ DG8 cartridges, gaskets, plate, and pierceable foil on hand
- ☐ Controls included on the plate (NTC, extraction blank, positive control)
- ☐ PX1 sealer at correct temperature/time
- ☐ Thermal cycler ramp rate set to ~2 C/s
- ☐ Run logged in the instrument logbook

13. Appendix B: Generic Thermal Cycling Template

Anneal/extension temperature and cycle number are assay-specific and come from SOP-L701. The structure below is a starting template for probe-based ddPCR; confirm against the supermix insert and current manual (Bio-Rad Laboratories, 2024).

Step	Temperature	Time	Cycles	Ramp
Enzyme activation	95 C	10 min	1	~2 C/s
Denaturation	94 C	30 s	39–40	~2 C/s
Anneal/extension	(assay)	1 min	39–40	~2 C/s
Enzyme deactivation	98 C	10 min	1	~2 C/s
Hold	4 C	hold	1	~2 C/s

> **Note**
>
> EvaGreen chemistry uses a different supermix, oil, and cycling profile than probe chemistry. If the lab runs EvaGreen assays, add a separate template here once the standard profile is fixed in SOP-L701.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L215

Hach DR3900 Benchtop Spectrophotometer

Standard Operating Procedure

SOP Number	SOP-L215
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the setup, operation, routine maintenance, and troubleshooting of the Hach DR3900 benchtop spectrophotometer as a laboratory instrument. The DR3900 uses a tungsten lamp (visible range) and a deuterium lamp (UV range) to measure absorbance and transmittance across 320–1100 nm, supporting both factory-stored programs for Hach reagent kits and user-defined wavelength methods (Hach Company, 2024a, 2024b).

For method-specific chemistry, reagent handling, and the analyte Method Registry (nutrients, metals, and general water quality parameters), see SOP-L303 Hach Colorimetric Analysis. For field use of the portable Hach DR900 colorimeter, see SOP-F208 Portable Spectrophotometer / Colorimeter (Field).

2. Scope

- **Applies to:** All use of the Hach DR3900 benchtop spectrophotometer in the EA Program laboratory, including stored-program colorimetric analysis, user-defined single-wavelength measurements, wavelength scans, and time-course (kinetic) measurements
- **Does not apply to:** Micro-volume UV-Vis measurements (see SOP-L212 NanoDrop); fluorometric quantification (see SOP-L203 Trilogy Fluorometer); portable field colorimetry (see SOP-F208); general spectrophotometer theory (see SOP-L201)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule lamp replacement and annual verification, manage firmware updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Absorbance (A):** The negative base-10 logarithm of transmittance: $A = -\log_{10}(T)$; directly proportional to analyte concentration (Beer-Lambert law) within the linear range of the instrument.
- **Beer-Lambert law:** The relationship between absorbance, path length, and concentration: $A = \varepsilon \cdot c \cdot l$, where ε is the molar absorptivity, c is concentration, and l is the optical path length (cell width); the basis for all colorimetric quantification.
- **Blank (reagent blank):** A sample containing all reagents except the analyte; used to zero the instrument and correct for absorbance contributed by the reagent matrix.
- **Colorimetric method:** An analytical technique in which a chemical reagent reacts with the analyte to produce a colored complex whose absorbance is proportional to analyte concentration.
- **TNT plus vial:** A Hach proprietary pre-dosed reagent vial for the DR3900; contains all required reagents sealed in a glass vial; provides consistent results without manual reagent preparation.
- **Transmittance (T):** The fraction of incident light that passes through the sample: $T = I/I_0$; related to absorbance by $A = -\log_{10}(T)$.
- **Wavelength scan:** A measurement mode in which the instrument records absorbance across a range of wavelengths to identify the absorption maximum of an unknown compound.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The deuterium lamp emits ultraviolet radiation. Do not look directly into the sample compartment when the lid is open and the lamp is on. The instrument's interlock dims the lamp when the lid is raised, but UV exposure remains possible during diagnostic modes.
- **Chemical:** Sample solutions and reagents placed in the instrument may be hazardous. Refer to the relevant method SOP (e.g., SOP-L303) and the reagent SDS for handling precautions.
- **Electrical:** Do not operate the instrument with wet hands or near standing water. If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling reagents or samples)

5.2. Emergency Procedures

For chemical spills on or near the instrument, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use. If liquid enters the instrument, unplug the power cord and do not attempt to power on until the lab manager has inspected the unit.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Hach DR3900 benchtop spectrophotometer	Lab bench, SGM 133	Wavelength range 320–1100 nm
Light shield / adapter	With DR3900	For 13 mm and 16 mm round vials (TNT plus)
USB cable or Ethernet connection	Lab bench	For data export to PC

6.2. Sample Cells

Cell Type	Path Length	Use
10 mm square glass or quartz	10 mm	General absorbance; user-defined methods
1-inch round glass	25.4 mm	Most Hach stored-program methods
16 mm round (TNT plus vials)	16 mm	TNT plus pre-dosed vial methods

6.3. Consumables

Item	Notes
Lint-free wipes (Kimwipes)	For cleaning sample cell exteriors
Silicone oil and applicator cloth	For 1-inch round cells (reduces light scattering at the glass surface)
DI water (Type II or better)	For blanking and rinsing

7. Procedure

7.1. Startup and Self-Check

1. Verify that the sample compartment is empty and the lid is closed
2. Turn on the DR3900 using the power switch on the rear panel
3. The instrument performs an automatic self-check, including lamp tests and wavelength verification. This takes approximately 30 seconds. Do not open the lid or insert samples until the home screen appears.

4. Allow the lamps to warm up for at least 15 minutes before making measurements that require high photometric accuracy (e.g., user-defined methods at low absorbance). For routine stored-program analyses, measurements may begin after the self-check completes.

7.2. Selecting an Operating Mode

From the home screen, select one of the following modes:

Mode	When to Use
Stored Programs	Pre-programmed Hach reagent kit methods; see SOP-L303 for the Method Registry
Single Wavelength	User-defined absorbance or %T at a specific wavelength
Multi Wavelength	Simultaneous measurement at 2–4 wavelengths
Wavelength Scan	Absorbance spectrum over a defined range
Time Course	Kinetic measurement at a fixed wavelength over time

7.3. Preparing and Inserting Sample Cells

1. **Select the correct cell type** for the method (see Section 5). Square cells must be oriented so the clear optical faces align with the light path (arrow on the cell pointing toward the detector).
2. **Fill the cell** to the level required by the method. Do not overfill; liquid on the outside of the cell causes erroneous readings.
3. **Clean the exterior** of the cell with a lint-free wipe. For 1-inch round cells, apply a thin film of silicone oil and wipe with the oiling cloth to eliminate fingerprints and water droplets (Hach Company, 2024a).
4. **Insert the cell** into the cell holder. Close the sample compartment lid fully before reading.

> Cuvette Selection for UV Work
>
> Standard glass and plastic cells absorb UV light below approximately 340 nm. For measurements below 340 nm in user-defined or wavelength scan modes, use quartz cells (Skoog et al., 2018).

7.4. Zeroing (Blanking) the Instrument

1. Fill a clean cell with the blank solution. The blank must match the sample matrix: DI water for simple aqueous samples, or the reagent blank specified by the Hach method.
2. Insert the blank cell and close the lid
3. Press **Zero** (stored-program mode) or **Zero/Blank** (single wavelength mode)
4. Verify that the display reads 0.000 Abs (or 100.0 %T)
5. Remove the blank cell

> **Note**
>
> Re-zero whenever you change wavelengths, switch cell types, or if the instrument has been idle for more than 30 minutes.

7.5. Measuring Samples

1. Insert the sample cell and close the lid
2. Press **Read** (or **Measure** in scan/time-course modes)
3. Record the result (absorbance, concentration, or %T depending on mode)
4. Remove the cell. Rinse and refill for the next sample, or use a fresh cell.

7.6. Wavelength Scan Mode

1. From the home screen, select **Wavelength Scan**
2. Set the start and end wavelengths and the scan increment (typically 1 nm)
3. Zero the instrument with a blank across the full scan range
4. Insert the sample and press **Start Scan**
5. The spectrum is displayed on screen. Use the cursor to read peak wavelengths and absorbance values.
6. Export the scan data via USB (see Section 6.8)

7.7. User-Defined Programs

The DR3900 allows creation of custom calibration curves for analytes not covered by the factory-stored programs:

1. Select **User Programs** from the home screen, then **New**
2. Set the measurement wavelength, units, and cell type
3. Prepare a series of standards spanning the expected concentration range (minimum 5 points plus a blank)
4. Read each standard and enter the known concentration when prompted
5. The instrument fits a calibration curve (linear, polynomial, or segmented). Review the correlation coefficient (r^2); accept only if $r^2 \geq 0.995$ for linear methods (Harris & Lucy, 2020).
6. Save the program with a descriptive name and program number

7.8. Data Export

1. Connect a USB flash drive to the USB-A port on the front panel
2. From the home screen, select **Data Log**
3. Select the readings to export and press **Send to USB**
4. Data are saved as `.csv` files that can be opened in R or Excel
5. Alternatively, connect the DR3900 to a PC via USB cable and use Hach's LINK2SC or Claros software for direct data transfer

7.9. Shutdown

1. Remove all cells from the sample compartment
2. Wipe the cell holder with a dry lint-free tissue to remove any spilled liquid
3. Close the sample compartment lid
4. Turn off the instrument using the rear power switch
5. Cover the instrument with the dust cover if one is available

8. Quality Control and Acceptance Criteria

8.1. Daily Verification

Before each analytical session:

- Run a DI water blank; the reading should be 0.000 ± 0.005 Abs at the working wavelength
- Run a Hach-supplied verification standard (e.g., StablCal formazin standard or NIST-traceable absorbance filter) at the working wavelength. Acceptance: within ± 0.01 Abs of the certified value, or ± 5%, whichever is larger.

8.2. Lamp Monitoring

The DR3900 tracks lamp hours internally. When a lamp approaches its rated lifetime (typically 2000 hours for deuterium, longer for tungsten), the instrument displays a warning.

- Record lamp hours monthly in the instrument log
- Replace lamps per the Hach service manual or contact Hach technical support
- After lamp replacement, run the full wavelength verification procedure

8.3. Annual Verification

At least once per year (or after any service event):

1. Run a multi-wavelength check using a NIST-traceable holmium oxide filter to verify wavelength accuracy (± 1 nm)
2. Run a multi-point photometric accuracy check using neutral density filters or potassium dichromate standards across the range 0.1–1.5 Abs (Skoog et al., 2018)
3. Record results in the instrument log and retain the verification report

9. Troubleshooting

Symptom	Possible Cause	Action
Self-check fails	Lamp failure or firmware error	Note the error code; contact lab manager
Blank does not zero	Dirty cell, wrong cell type, or stale blank	Clean or replace cell; prepare fresh blank
Erratic readings	Air bubbles in cell, condensation, or dirty optics	Degas sample; wipe cell; clean sample compartment
"Lamp intensity low" warning	Lamp nearing end of life	Schedule lamp replacement
Readings drift over time	Lamp not warmed up; temperature change	Allow 15 min warm-up; avoid drafts near instrument
Absorbance exceeds 2.5	Sample too concentrated	Dilute sample and re-read

10. Waste Disposal

Waste Type	Disposal Method
Rinsate from cells (DI water)	Drain
Chemical samples / reagent waste	Per method SOP (see SOP-L303)
Used lint-free wipes	Standard trash unless contaminated
Spent lamps	Hazardous waste (deuterium lamps contain UV-emitting gas); dispose through EH&S

11. References

Hach Company. (2024a). *DR3900 benchtop spectrophotometer: Procedures manual*. https://www.hach.com/dr3900-benchtop-spectrophotometer/product?id=56440352466

Hach Company. (2024b). *Water analysis guide* (7th ed.). https://www.hach.com/wag

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of instrumental analysis* (7th ed.). Cengage Learning.

12. Training Record

Before operating the DR3900 independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Read SOP-L303 if performing colorimetric analyses
4. Observe a trained operator perform a complete session: startup, self-check, zeroing, sample measurement, data export, and shutdown
5. Perform a supervised session with acceptable QC results (blank within specification, verification standard within tolerance)
6. Be signed off by the PI or lab manager

13. Appendix A: Instrument Specifications

Parameter	Specification
Wavelength range	320–1100 nm
Wavelength accuracy	± 1 nm
Wavelength resolution	1 nm
Photometric range	-0.5 to 3.5 Abs
Photometric accuracy	± 0.003 Abs at 0.5 Abs (typical)
Light sources	Tungsten (visible), deuterium (UV)
Cell types	10 mm square, 1-inch round, 16 mm round
Stored programs	200+
Data storage	500+ measurements
Connectivity	USB-A, USB-B, Ethernet
Power	100–240 V AC, 50/60 Hz

Note

Confirm specifications against the serial plate on the unit and the current Hach DR3900 product data sheet. Values above are representative of current production models (Hach Company, 2024a).

14. Appendix B: Relationship to Other SOPs

SOP	Relationship
SOP-L303	Method chemistry, reagent handling, and the analyte Method Registry for the DR3900 and DR900
SOP-F208	Field use of the portable DR900 colorimeter
	
	General spectrophotometer theory and cuvette-based UV-Vis operation
SOP-L212	Micro-volume UV-Vis (NanoDrop)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L216

Dissecting and Compound Microscope

Standard Operating Procedure

SOP Number	SOP-L216
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, image capture, and routine care of the dissecting (stereo) microscopes and compound light microscopes used in the EA Program laboratory. Dissecting microscopes provide low-to-moderate magnification (typically 7–45x) with a wide working distance and are used for sorting biological specimens, gross morphology, and sample preparation. Compound microscopes provide higher magnification (40–1000x) with transmitted illumination and are used for slide-mounted specimens requiring cellular or subcellular resolution (Murphy & Davidson, 2012).

For the Echo Labs Revolve hybrid microscope (which combines upright compound and inverted configurations with an iPad display), see SOP-L211 Revolve Microscope. The Revolve has specialized procedures that differ from the conventional microscopes described here.

2. Scope

- **Applies to:** All conventional dissecting (stereo) microscopes and compound light microscopes in the EA Program laboratory, including units with and without attached cameras
- **Does not apply to:** The Echo Labs Revolve microscope (see SOP-L211); electron microscopy; confocal microscopy; fluorescence microscopy on non-Revolve instruments
- **Location:** EA Program laboratory, SGM 131/133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage optics cleaning supplies, maintain camera software
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Brightfield microscopy:** Transmitted-light microscopy in which the specimen is illuminated from below and appears against a bright background; the standard mode for stained biological specimens.
- **Compound microscope:** A microscope with two or more lens systems (objective and eyepiece) that produce a magnified image; used for cells, microorganisms, and fine structural detail at total magnifications of 40x–1,000x.
- **Condenser:** A lens assembly below the stage that focuses and concentrates the illumination light onto the specimen; its numerical aperture and position strongly affect image contrast and resolution.
- **Dissecting microscope (stereo microscope):** A low-magnification (typically 7x–45x) binocular microscope that provides a three-dimensional image of intact specimens; used for macroinvertebrate sorting, plant tissue examination, and dissection.
- **Immersion oil:** A synthetic or natural oil with a refractive index matching glass (~1.515); applied between the 100x objective and the coverslip to eliminate the air-glass interface and maximize numerical aperture.
- **Iris diaphragm (condenser diaphragm):** An adjustable aperture in the condenser that controls the cone of illumination; reducing the diaphragm increases contrast but reduces resolution.
- **Parfocal:** The property of a set of objectives that allows switching between magnifications with only minor focus adjustment; standard on modern compound microscopes.
- **Working distance:** The distance between the front lens of the objective and the specimen surface when in focus; decreases with increasing magnification; limits the thickness of specimens that can be accommodated.

5. Safety and Hazards

⚠ Hazards

- **Optical:** Prolonged viewing through the eyepieces can cause eye strain. Take breaks during extended sorting or identification sessions. Adjust the diopter rings to match your vision before starting work.
- **Electrical:** Keep liquids away from the illumination base and power supply. If liquid enters the base, disconnect power immediately and notify the lab manager.
- **Physical:** Compound microscope objectives (especially 40x and 100x) can contact and crack the slide or cover slip if the stage is raised too quickly. Always use the coarse focus knob with caution when changing objectives.
- **Chemical/Biological:** Specimens may be preserved in ethanol, formalin, or other fixatives. Wear appropriate PPE and handle preserved material in a well-ventilated area.

5.1. Required PPE

☐ Safety glasses (when not viewing through eyepieces)

☐ Lab coat
☐ Nitrile gloves (when handling preserved specimens, stains, or immersion oil)

5.2. Emergency Procedures

For chemical spills (e.g., ethanol, formalin) on or near a microscope, clean the spill per SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling. If liquid enters the microscope body, unplug power and contact the lab manager before attempting further use.

6. Equipment and Materials

6.1. Dissecting (Stereo) Microscopes

Item	Qty	Location	Notes
Dissecting scope with illuminated base	7	SGM 131/133	7–45x zoom; incident and transmitted light
Dissecting scope with camera	1	SGM 133	Camera-equipped for image capture

6.2. Compound Microscopes

Item	Qty	Location	Notes
Compound light microscope	*TBD*	SGM 133	Objectives: 4x, 10x, 40x, 100x (oil)

6.3. Consumables

Item	Notes
Microscope slides (25 x 75 mm)	Standard glass
Cover slips (No. 1.5, 0.17 mm)	For compound microscope use
Immersion oil (Type A or NF)	For 100x oil immersion objective only
Lens cleaning solution	Optical-grade, for objectives and oculars
Lens cleaning tissue (lint-free)	Use optical-grade tissue, not Kimwipes on coated optics
Compressed gas duster	For removing dust; use short bursts only
Depression slides	For wet mounts of live specimens

7. Procedure

7.1. Choosing the Right Microscope

Task	Microscope Type	Typical Magnification
Sorting macroinvertebrates from debris	Dissecting	7–20x
Gross specimen identification	Dissecting	10–45x
Examining head capsules, chaetae, gill structures	Compound	100–400x
Counting algae or diatoms	Compound	200–400x
Identifying microplastics on filters	Dissecting	10–45x
Viewing stained tissue sections	Compound	40–1000x

7.2. Dissecting Microscope: Setup and Use

1. Plug in the microscope and turn on the illumination using the switch on the base. Most units have both **incident** (top) and **transmitted** (bottom) light sources. Select the mode appropriate for the specimen:
   - Incident light: opaque specimens (insects, soil, rocks)
   - Transmitted light: translucent specimens (small organisms in water, thin sections)
   - Both: general sorting when mixed materials are present
2. Adjust the zoom to the lowest magnification (widest field of view) to locate the area of interest
3. Place the specimen on the stage plate. Use a white plate for dark specimens and a black plate for light or translucent specimens to improve contrast.
4. Adjust the interpupillary distance by sliding the eyepiece tubes until you see a single merged circle of light
5. Focus using the focus knob (single knob on most stereo scopes). With both eyes open, adjust the diopter ring on one eyepiece until the image is sharp in both eyes simultaneously.
6. Increase magnification by turning the zoom dial. Refine focus as needed.
7. When finished, return the zoom to the lowest setting, remove the specimen, turn off the illumination, and unplug the unit if lab policy requires it

7.3. Compound Microscope: Setup and Use

1. Plug in the microscope and turn on the light source using the power switch. Adjust the intensity to a comfortable level.
2. Rotate the nosepiece to the lowest-magnification objective (4x)
3. Place the prepared slide on the stage, securing it with the stage clips. Center the specimen over the condenser aperture.
4. Using the **coarse focus knob**, raise the stage until the specimen comes into approximate focus. Watch from the side to avoid driving the slide into the objective.
5. Sharpen the image with the **fine focus knob**
6. Adjust the condenser height and the iris diaphragm to optimize contrast and resolution. For most work, the condenser should be nearly fully raised (close to the slide) and the diaphragm partially closed to improve contrast without sacrificing too much resolution (Murphy & Davidson, 2012).
7. Increase magnification by rotating the nosepiece to the next objective (10x, then 40x). Refocus with the fine focus knob only.

> **!** Avoid Slide Damage
>
> When switching from the 10x to the 40x objective, watch from the side to confirm that the longer 40x lens does not contact the cover slip. If the working distance is too short, lower the stage slightly before rotating the objective into position.

7.4. Using Oil Immersion (100x Objective)

1. Focus the specimen at 40x first
2. Rotate the nosepiece to an empty position (no objective in the light path)
3. Place a single small drop of immersion oil directly on the cover slip at the point of illumination. Do not use excess oil.
4. Rotate the 100x objective into position. The front element of the lens should contact the oil without trapping an air bubble.
5. Focus carefully using the **fine focus knob only**
6. After use, immediately clean the 100x objective with lens cleaning solution and lint-free tissue. Wipe gently from center to edge. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.5. Capturing Images (Camera-Equipped Units)

1. Focus the specimen and center the region of interest in the field of view
2. Open the camera software on the connected computer (software varies by camera model; consult the lab manager for the current setup)
3. Adjust exposure and white balance in the software to match the illumination
4. Capture the image. Record the magnification, scale bar, and sample ID in the file name or metadata.
5. Use a consistent file-naming convention: `YYYY-MM-DD_SampleID_Magnification_Mode.jpg`
6. Export images to the project data folder

7.6. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x on compound; lowest zoom on dissecting)
3. If oil immersion was used, clean the 100x objective immediately (see Section 6.4, step 6)
4. Wipe any residue from the stage with a lint-free tissue
5. Turn off the light source and unplug if required by lab policy
6. Cover the microscope with a dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in the field of view that do not move when the specimen is translated, the optics need cleaning:

1. Start with a few short bursts of compressed gas duster to remove loose dust from the objectives and oculars. Do not invert the can.
2. For objective lens surfaces, apply a small amount of lens cleaning solution to a piece of lint-free optical tissue and gently wipe in a circular motion from center to edge. Do not use dry tissue on coated optics.
3. Clean the oculars (eyepieces) the same way. Fingerprints on the eye lens are the most common source of image degradation.
4. For persistent contamination on internal optics, contact the lab manager. Do not disassemble the microscope.

8.2. Illumination Check

- Replace burned-out bulbs promptly. LED-equipped units should not require bulb replacement during normal use.
- If transmitted light appears uneven, check that the condenser is properly centered and the iris diaphragm is functioning.

8.3. Image Quality Checklist

Before accepting a micrograph for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- A scale bar or magnification is recorded
- The specimen is oriented consistently with project conventions

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal per SOP-L112
Preserved specimen waste (ethanol)	Flammable waste container
Preserved specimen waste (formalin)	Hazardous waste; dispose through EH&S
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. https://doi.org/10.1002/9781118382905

11. Training Record

Before using dissecting or compound microscopes independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper setup, focusing, magnification changes, and shutdown of both dissecting and compound microscopes under supervision
4. If oil immersion work is required, demonstrate proper oil application, focusing, and cleanup
5. If image capture is required, demonstrate camera operation and correct file naming
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

12.1. Dissecting (Stereo) Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Zoom dial	Continuously adjustable magnification (e.g., 0.7–4.5x objective)
Focus knob	Single knob for coarse/fine focus
Stage plate	Removable; white and black plates for contrast
Incident light	Top illumination for opaque specimens
Transmitted light	Bottom illumination for translucent specimens
Diopter adjustment	On one or both eyepieces; corrects for vision differences between eyes

12.2. Compound Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Stage	Holds slide; translatable via X/Y controls
Condenser	Focuses transmitted light onto the specimen
Iris diaphragm	Controls cone angle of illumination; affects contrast and resolution

Component	Function
Coarse focus knob	Rapid focus (large movements)
Fine focus knob	Precise focus (small movements)
Light intensity control	Adjusts brightness of the transmitted light source

13. Appendix B: Total Magnification Reference

Total magnification = eyepiece magnification × objective magnification. For standard 10x eyepieces:

Objective	Total Magnification	Typical Use
4x	40x	Scanning; locating regions of interest
10x	100x	General observation
40x	400x	Detailed cellular structure
100x (oil)	1000x	Fine detail; bacteria, diatom striae, chromosomes

14. Appendix C: Relationship to Other SOPs

SOP	Relationship
SOP-L211	Echo Labs Revolve hybrid microscope (upright/inverted, iPad display, fluorescence)
SOP-L602	Macroinvertebrate sorting and identification (uses dissecting and compound scopes)
SOP-L308	Microplastics enumeration (uses dissecting scope and Revolve)
SOP-L112	Autoclave / sterilization for biohazard waste