



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L101

Glassware Cleaning and Preparation

Standard Operating Procedure

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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 HNO₃ 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 MΩ·cm) 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 HNO₃ (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, HNO₃) 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 <i>SOP-L11</i>	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

i 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% HNO₃ 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 $\text{NH}_4^+/\text{NO}_3^-$ (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% HNO₃ Acid Bath (Trace Metal Grade)

1. Follow the same steps as above, substituting Trace Metal grade HNO₃ (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