



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L115

Sonicator – Ultrasonic Bath and Probe

Standard Operating Procedure

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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