



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L020

Reagent and Standard Preparation

Standard Operating Procedure

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1. Purpose

This procedure describes general best practices for preparing, labeling, storing, and documenting laboratory reagents and calibration standards used across EA Program analytical methods. Individual analytical SOPs specify the exact reagents and concentrations required for each method; this SOP provides the overarching framework for solution preparation that ensures traceability, accuracy, and safety.

2. Scope

- **Applies to:** Preparation of aqueous reagent solutions, extraction solutions, calibration standards, buffer solutions, and indicator solutions used in EA Program laboratory analyses
- **Does not apply to:** Preparation of biological growth media (covered in microbiology SOPs); preparation of molecular biology reagents (master mixes, primers, probes; see SOP-L701 and related SOPs); field reagent preparation (covered in field SOPs)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve reagent-grade specifications
Lab Manager	Maintain chemical inventory, order reagents, prepare shared stock solutions
Trained Personnel	Prepare working solutions per this SOP and the relevant analytical SOP
New Students	Complete training before preparing solutions independently

4. Definitions

- **Blank solution:** A solution prepared identically to calibration standards and samples but containing no analyte; used to correct for background signal and verify the absence of contamination in reagents and glassware.
- **Calibration standard:** A solution of known analyte concentration prepared from a primary or secondary standard; used to establish the relationship between instrument signal and concentration.
- **Matrix-matched standard:** A calibration standard prepared in the same solvent or extract matrix as the samples (e.g., 2 M KCl for soil extracts); minimizes matrix effects on instrument response.
- **Primary standard:** A highly pure, chemically stable substance whose mass can be accurately weighed to prepare a solution of precisely known concentration without prior standardization; examples include potassium hydrogen phthalate (KHP) and anhydrous sodium carbonate.

- **Reagent grade:** A chemical purity classification (also called ACS grade) meeting minimum purity specifications set by the American Chemical Society; appropriate for most analytical work; trace-metal-grade or ultrapure-grade reagents are required for trace analysis.
- **Secondary standard (working standard):** A solution prepared from a primary standard or a certified reference material (CRM) and used as the working calibration solution; must be verified against the primary standard periodically.
- **Stock solution:** A concentrated solution of a reagent or standard prepared in bulk and used to prepare working solutions by dilution; labeled with concentration, preparation date, expiration date, preparer's name, and any hazard information.
- **Volumetric dilution:** Dilution performed using Class A volumetric glassware (volumetric flasks, pipettes) to achieve accurate, traceable concentrations; pipette to volume, mix thoroughly, then bring to final volume.

5. Safety and Hazards

Hazards

- **Chemical:** Concentrated acids, bases, and organic solvents used in reagent preparation are corrosive, toxic, and/or flammable. Review the SDS for every chemical before use.
- **Thermal:** Dissolving some solutes (e.g., NaOH, H₂SO₄ dilution) is exothermic. Add solute to solvent slowly, never the reverse for acid dilutions.
- **Glassware:** Volumetric flasks and graduated cylinders can break during mixing. Inspect for cracks before use.

5.1. Required PPE

- ☐ Safety glasses or splash goggles (for concentrated acids/bases)
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ Fume hood (for volatile or fuming reagents)

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes. For spills, follow [SOP-L004 Chemical Spill Response](#).

6. Water Quality

All reagent and standard preparation must use water of appropriate purity:

Water Grade	Source	Use
Type I (18.2 MOhm, ultrapure)	Milli-Q system (see future SOP-L100)	Calibration standards, trace metal work, DOC analysis, molecular biology
Type II (deionized)	Central DI supply	General reagent preparation, rinsing
Tap water	Building supply	Initial glassware rinse only; never for reagent preparation

i Note

Verify Milli-Q resistivity (should read 18.2 MOhm-cm at 25 C) before preparing trace-level standards. If the reading is below 18.0, replace the cartridge or consult the lab manager.

7. Procedure

7.1. Planning

1. Consult the relevant analytical SOP for the exact reagent name, concentration, and grade required
2. Calculate the mass or volume of solute needed using the target concentration and final volume. Document the calculation in the lab notebook.
3. Verify that the chemical in inventory matches the required grade (ACS reagent grade, trace metal grade, molecular biology grade, etc.)

7.2. Weighing Solids

1. Calibrate or verify the analytical balance per [SOP-L200](#)
2. Use a clean, dry weighing vessel (weigh boat or beaker)
3. Tare the vessel on the balance
4. Transfer the required mass of solid reagent using a clean spatula. Record the actual mass to the precision of the balance.
5. Return the reagent bottle cap immediately to prevent contamination and moisture uptake

7.3. Dissolving and Diluting

1. Transfer the weighed solid to a clean beaker and add approximately 75% of the final volume of water. Stir with a magnetic stir bar or glass rod until dissolved.
2. For acid dilutions: always add acid to water (“Do as you oughta, add acid to water”). Stir continuously and allow the solution to cool before adjusting to final volume.
3. Transfer the solution quantitatively to a volumetric flask of the target volume
4. Rinse the beaker and stir bar with small portions of water and add the rinses to the flask
5. Bring to the mark on the volumetric flask at room temperature. Read the meniscus at eye level.
6. Stopper the flask and invert 10 times to mix

7.4. Preparing Calibration Standards by Serial Dilution

1. Prepare or obtain a stock standard of known concentration (certified reference material or gravimetrically prepared stock)
2. Using calibrated volumetric pipettes or micropipettes ([SOP-L103](#)), transfer the calculated aliquot of stock solution into a volumetric flask
3. Dilute to the mark with the appropriate matrix (water, extractant solution, or acid matrix matching the samples)
4. Prepare the full calibration curve (typically 5–7 points plus a blank) in order from lowest to highest concentration to minimize carryover contamination
5. Record all dilution steps, pipette volumes, and flask sizes in the lab notebook

Tip

For trace-level calibration standards (ppb range), prepare fresh daily or per the stability guidance in the analytical SOP. Dilute standards degrade faster than concentrated stocks due to adsorption to container walls.

7.5. pH Adjustment (When Required)

1. After dissolving, measure the pH with a calibrated pH meter per [SOP-L300](#)
2. Adjust to the target pH using the appropriate acid or base (as specified in the analytical SOP), added dropwise with stirring
3. Record the final pH and the volume of acid/base added

7.6. Labeling

Every prepared solution must be labeled with the following information before it leaves the preparation bench:

- Solution name and concentration (e.g., “0.5 M K₂SO₄”)
- Solvent or matrix (e.g., “in DI water”)
- Date prepared
- Expiration date or shelf life (per the analytical SOP or manufacturer recommendation)
- Preparer’s initials
- Hazard warnings if applicable (e.g., “Corrosive,” “Flammable”)

Important

Never use an unlabeled solution. If you find an unlabeled container, do not use it. Dispose per [SOP-L006 Waste Disposal](#).

7.7. Storage

Solution Type	Storage Conditions
Aqueous inorganic standards	Room temperature, dark; amber bottles for light-sensitive analytes
Organic solvent solutions	Flammable cabinet; fume hood if volatile
Buffer solutions	Room temperature or 4 C per the analytical SOP
Concentrated acids and bases	Acid cabinet (acids) or base shelf (bases); never store together
Molecular biology reagents	-20 C (primers, probes, enzymes) or 4 C (buffers) per manufacturer

7.8. Documentation

Record the following in the lab notebook for every prepared solution:

- Reagent name, lot number, manufacturer, and grade
- Calculation showing target concentration and volumes/masses
- Actual mass weighed (to balance precision)
- Final volume and flask size
- Date prepared, preparer's initials
- Expiration date
- Any deviations from the standard procedure

8. Quality Control

- Verify the concentration of each new stock standard by analyzing it against the previous batch or against a certified reference material (CRM). Agreement should be within 5%.
- For critical calibration standards, prepare from a second independent source (different manufacturer or lot) and compare
- Discard solutions past their expiration date; do not extend shelf life without re-verification
- Check volumetric glassware calibration (Class A tolerance) annually by gravimetric verification
- Verify micropipette accuracy quarterly per [SOP-L103](#)

9. Common Reagent Recipes

The table below lists frequently used reagent solutions across EA Program methods. Refer to the source SOP for complete preparation details.

Solution	Concentration	Source SOP	Shelf Life
KCl extraction solution	2 M	L403	6 months
K ₂ SO ₄ extraction solution	0.5 M	L409	6 months
Sodium hexametaphosphate (HMP)	50 g/L	L401	1 month
HNO ₃ (for sample acidification)	2%	L410	Prepare fresh
KHP stock standard (DOC)	1,000 mg C/L	L407	1 month (refrigerated)
KNO ₃ stock standard (TN)	1,000 mg N/L	L407	6 months
NaOH (for pH adjustment)	1 M	Various	6 months
HCl (for pH adjustment)	1 M	Various	6 months
Longmire's buffer (eDNA preservation)	As formulated	F700	6 months (refrigerated)

10. Waste Disposal

Dispose of expired or unwanted reagent solutions per [SOP-L006 Waste Disposal](#). Key points:

- Hazardous reagents (acids, bases, solvents, metal solutions) go to the appropriate waste stream in the SAA
- Dilute, non-hazardous salt solutions at neutral pH may be drained with copious water if approved by EH&S
- Never pour an unknown or unlabeled solution down the drain

11. References

- National Research Council. (2011). *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards* (updated ed.). National Academies Press.
- Harris, D.C. (2016). *Quantitative Chemical Analysis*. 9th ed. W.H. Freeman.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. Section 1080: Reagent Water.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L003	Hazardous Materials Handling	Chemical safety
L005	Chemical Inventory	Reagent sourcing and tracking
L006	Waste Disposal	Disposing of expired reagents
L102	Using Balances	Weighing technique
L103	Micropipettes	Volumetric transfers
L200	Balance Calibration	Ensuring mass accuracy

SOP	Title	Relationship
L300	pH and Conductivity	pH verification of prepared solutions

13. Training Record

Before preparing reagents or standards independently, personnel must:

1. Read this SOP and [SOP-L003](#) in full
2. Demonstrate proper use of the analytical balance (SOP-L102/L200) and micropipettes (SOP-L103)
3. Prepare one reagent solution and one set of calibration standards under supervision, demonstrating correct calculation, weighing, dissolving, volumetric dilution, labeling, and documentation
4. Demonstrate correct acid dilution technique (acid to water)
5. Be signed off by the lab manager or PI