Making reagents and buffers

Making buffers and reagents is one of the most essential skills in laboratory work. While not difficult, it requires care and precision. At first glance it may seem that all you have to do is "just add water"—but good preparation involves more: selecting the right concentrations, adjusting pH, sterilizing when needed, and avoiding common mistakes. The quality of your reagents can make or break an experiment.

This protocol provides a step-by-step guide on how to prepare aqueous solutions from powders and stock solutions, with emphasis on building good habits for consistent and reproducible results. While written with novice users in mind, it includes reminders that benefit experienced researchers as well.

Risk assessment

- ▷ Touch chemical containers and shared equipment such as balances or the pH meter only with gloved hands
- ▷ Handle HAZARDOUS MATERIALS, irritating, allergenic, or volatile substances, in a certified chemical fume hood
- ▷ Wear splash goggles when handling corrosive, reactive, or flammable liquids, for example, when diluting strong acids or bases
- ▷ Wear an ancillary full-face shield when handling >1 L of any strong corrosive
- ▷ Wear gloves, lab coat, safety glasses
- ☐ Collect material as HAZARDOUS WASTE unless otherwise instructed
- ☐ Dispose waste according to the CHEMICAL HYGIENE PLAN; only some substances are safe for SINK DISPOSAL; flush with plenty of water

In the event of EXPOSURE:

- ▷ **Wash affected area for 15 min**
- ▷ **Seek medical attention**

Report to:

- **Principal Investigator/Supervisor**

Reviewed: Nov 19, 2023

Procedures

›› *Determine which reagents you need*

(1.) Go through the *entire protocol* for your experiment. Determine how much of each solution you need. Plan for a reasonable excess unless the material is expensive or particularly hazardous.

- Are the ingredients stable once dissolved, or do they need to be prepared freshly?
- How often will you repeat the experiment?

> ***Hint:*** If less than 100 mL are needed, consider composing the solution from concentrated stock solutions rather than weighing out dry powders. Keep one or two liters of autoclaved reagent-grade water in the refrigerator to make cold buffers from stocks.

(2.) Decide on a "straight" or concentrated solution.

> ***Hint:*** Many complex buffers are made at 5×, 10×, 20×, or 50× concentration. When making a new stock solution, choose a concentration that is easy to pipette, compatible with solubility, and practical for routine use. Many stock solutions in this collection fulfill these criteria. Concentrated solutions are often stored at room temperature to avoid precipitation.

(3.) *Optional:* Make a checklist of required reagents. Include any details about concentration, pH adjustment, sterilization, or special handling such as light- or temperature-sensitive components.

(4.) *Critical:* Know the hazards of all materials you work with. No one else will do this for you!

> ***Safety:*** Consult the safety data sheet (SDS) from the supplier and inspect the container for hazard and health warnings. If you see no warnings, do not assume it is safe. Look it up in the *Merck Index* or another authoritative resource.

(5.) Check the inventory for all chemicals you need. If something is missing or outdated, notify the lab manager or order it before proceeding.

> ***Quality assurance:*** Use *ACS grade* or *Reagent grade* chemicals. For sensitive work, select higher-grade reagents of *Molecular Biology, HPLC,* or *Tissue Culture grade.* Avoid Laboratory or Technical grades except for non-critical uses like 70% ethanol. Ultra-pure or pharmacological grades (USP, BP, EP) are unnecessary for most academic work.

> ***Hint:*** If a salt is available in hydrated form, this is the preferred, longer-lived, and usually cheaper option.

›› *Calculate how much you need*

(1.) For each reagent, calculate how much to weigh or dilute to achieve the target concentration in the final volume V_{ij} of the solution to be prepared.

- From solid to molar solution. To prepare a solution of concentration c_i from a solid compound with molecular weight M_i, calculate the required mass m_i as

  $$m_i\,[\mathrm{g}] = \boxed{c_i}\left[\mathrm{M} \equiv \frac{\mathrm{mol}}{\mathrm{L}}\right] \times M_i\left[\frac{\mathrm{g}}{\mathrm{mol}}\right] \times V_{ij}\,[\mathrm{L}]$$

  Critical: Some reagents are sold as hydrates, acid/base forms, or different salts. Always check the molecular weight on the container or safety data sheet (SDS) and adjust your calculations accordingly.

- For percent-based solutions. Calculate the mass m_i of solid to weigh out for a w_i (w/w) or β_i (w/v) percent solution. For σ_i (v/v) percentages, calculate the volume V_i of a liquid to add as

  $$m_i\,[\mathrm{g}] = \boxed{w_i}\left[\frac{\mathrm{g}}{\mathrm{g}} \equiv \frac{\%}{100}\right] \times m_{ij}\,[\mathrm{g}] \qquad \approx \boxed{\beta_i}\left[\frac{\mathrm{g}}{\mathrm{L}} \equiv \frac{\%}{0.1}\right] \times V_{ij}\,[\mathrm{L}]$$

  $$V_i\,[\mathrm{L}] = \boxed{\sigma_i}\left[\frac{\mathrm{L}}{\mathrm{L}} \equiv \frac{\%}{100}\right] \times V_{ij}\,[\mathrm{L}]$$

  This is why: The use of percentage weight per volume β_i (w/v) is convenient but technically imprecise, since percentages are dimensionless. It is acceptable only for dilute aqueous solutions where the density is $\rho_i \approx 1\,\mathrm{g/mL}$.

- Diluting from a stock. When diluting a concentrated stock solution of concentration c_1 to a desired concentration c_2, calculate the volume V_1 to add as

  $$V_1\,[\mathrm{L}] = \boxed{c_2}\left[\mathrm{M} \equiv \frac{\mathrm{mol}}{\mathrm{L}}\right] / c_1\left[\mathrm{M} \equiv \frac{\mathrm{mol}}{\mathrm{L}}\right] \times V_2\,[\mathrm{L}]$$

  Critical: Double-check units and make sure concentrations are in the same format, for example, both in M or mg/mL.

- When you've already measured or pipetted the stock volume V_1 and want to calculate how much diluent to add directly for a target concentration c_2, use

  $$V_2 - V_1\,[\mathrm{L}] = V_1\,[\mathrm{L}] \times \left(1 - \boxed{c_2}\left[\mathrm{M} \equiv \frac{\mathrm{mol}}{\mathrm{L}}\right] / c_1\left[\mathrm{M} \equiv \frac{\mathrm{mol}}{\mathrm{L}}\right]\right)$$

(2.) *Optional:* Use a spreadsheet, calculator, or online dilution tool to minimize arithmetic errors, especially when preparing multiple reagents or complex mixtures.

›› *Weighing out solid reagents*

(1.) Wear a *fully-bottomed* lab coat, a *new pair* of gloves, and safety glasses.

Safety: Take this task seriously to protect you and the chemicals you are dipping into: Balances are shared equipment, and powders can be messy. You don't want to bring any of these chemicals home.

(2.) Gather all materials and choose an appropriate balance for the required accuracy. Double-check if the molecular weight on the container matches the one used in your calculation.

Hint: Use a top-loading balance and weighing boats if weighing more than 1 g and accuracy is less critical. For small amounts or expensive reagents, find an analytical balance with an accuracy of 0.1 mg or less and use weighing paper.

Critical: When weighing amounts below 20 mg, it is often more accurate to weigh a larger amount, dissolve in a defined volume, and then pipette an appropriate aliquot.

(3.) *Critical:* Let containers come to room temperature before you open them.

This is why: If a container is opened while still cold, the moist laboratory air will condense inside the container, eventually altering the chemical composition and weight of the substance even if the substance is not hygroscopic.

(4.) Place a clean, dry weighing container (weigh boat or paper) on the balance. Fold weighing papers once and reopen. Tare to zero before adding any reagent.

Critical: Beakers or large tubes are too heavy for analytical balances to accurately weigh out small amounts!

(5.) Transfer some of the substance to a clean reservoir or secondary container. Do not return excess material to the stock container to avoid contamination.

> ***Hint:*** If possible, do not put spatulas into chemical containers to avoid contamination. Many substances can be tipped with care directly into a wide-mouthed beaker or another container from which you operate. If you overshoot, do not put the excess back into the bottle! Assume it has been contaminated and throw it away.

(6.) With the spatula, remove a small amount of material from the reservoir and place it in the weighing container on the balance. Add gradually until the target weight is reached.

(7.) *Optional:* Record the exact weight. Use this value in your dilution or volume calculation if it's different from the planned amount.

(8.) Transfer the weighed material into a clean beaker or flask appropriate for further preparation steps: Grasp weighing boats on opposite ends and gently bend the ends towards each other to make a funnel-like opening. Pour slowly. Knock off or rinse down any sticky material.

(9.) Clean the balance. Use a brush to sweep any stray bits off the pan. Wipe the weighing area.

> ***Safety:*** Do this immediately! You are the only person who knows the nature of the powder and thus the correct way of disposal.

›› *Mixing*

(1.) Use a beaker or Erlenmeyer flask large enough to allow stirring and pH adjustment. *Do not mix in graduated cylinders*—they are for measuring only. Select the largest stir bar that rotates freely in the beaker. For measuring, choose a graduated cylinder as close as possible to the target volume.

> ***Hint:*** To prepare a final volume of 0.5 L, use a 0.8–1.0 L beaker.

(2.) Add 80% of the final volume of solvent. Measure the solvent with the graduated cylinder and pour it into the beaker or flask.

(3.) Gently drop the magnetic stir bar into the beaker, place on the stir plate, and turn on the stirrer.

(4.) Pour the weighed reagents slowly into the beaker while stirring. Let solids dissolve almost completely before you add more. Rinse the weighing container or transfer funnel with solvent.

(5.) *Optional:* Bring to 90% of the final volume and adjust the pH (see below).

> ***Note:*** For some buffers, pH should be adjusted only after dilution to working concentration. Consult a the preparation guide.

(6.) Pour the solution back into the graduated cylinder and make up with water to the final volume.

(7.) *Optional:* Sterilize the solution if required (see below).

(8.) Transfer to a storage bottle or container.

⊞ **Optional:** *Adjusting the pH by titration*

(1.) Transfer the solution to a clean glass beaker and add a magnetic stir bar.

> ***Critical:*** For applications where the pH and ionic strength of the buffer are of paramount importance, it is preferable to mix the acidic and basic components in the correct proportions. For most laboratory procedures, and provided the pH meter is well cared for and properly calibrated, buffers can be adjusted by titration.

(2.) Rinse the electrode with laboratory-grade (deionized) water from the wash bottle. Blot gently with a soft paper tissue.

(3.) *Critical:* Calibrate the pH meter using at least two pH standard solutions bracketing your target pH. Typically, standard solutions are pH 4.0, pH 7.0, or pH 10.0.

> ***Critical:*** Always calibrate the pH meter before adjusting pH, especially if it's the first use of the day or every 20 to 30 samples. Don't scrimp by standardizing only to one buffer. It does make a difference and it is ridiculous to go through the trouble of pH-ing if it isn't done correctly.

(4.) Rinse the electrode again, then immerse only the lower quarter of the probe into your buffer.

> ***Note:*** Make sure that the stirring bar clears the electrode. Dip the electrode in at one side of the beaker, half-way between the center and the border, away from the stir bar.

(5.) Stir moderately to lessen the chance of electrode damage and avoid splatters.

(6.) Measure the initial pH. Wait for the readings to stabilize.

(7.) If adjustment is needed, add small amounts of acid or base dropwise with a transfer pipette while stirring. Wait for the pH to stabilize before continuing.

> ***Hint:*** If the pH is off by more than a unit, use concentrated monoprotic acids such as 10 M titrant. Near the target pH, use diluted acid or base such as 0.1–1.0 M HCl, acetic acid, NaOH, or KOH.

> ***Hint:*** Use appropriate acids or bases as available. A Tris-Cl buffer means that after dissolving the base form of Tris, the pH was adjusted with HCl. A Tris-acetate buffer means that the pH was adjusted with acetic acid.

> ***Critical:*** Carelessness or the use of titrant that is too strong relative to the buffer, will inevitably lead to overshooting with the net effect of adding more salt to the buffer. You must start over again. The pH should only be adjusted once.

(8.) Check the solution for clarity. If any material remains undissolved, consult the SDS or supplier's documentation for solubility behavior.

> ***Critical:*** Never adjust pH of a turbid or undissolved solution unless instructed otherwise.

(9.) Once pH is adjusted, remove the stir bar and rinse the beaker walls into the solution with a small amount of reagent-grade water.

Optional: *Sterilizing buffers and solutions*

(1.) Decide whether each solution is compatible with heat sterilization or requires filter sterilization.

> ***Hint:*** Most aqueous buffers, salts, and growth media without heat-labile supplements can be autoclaved. Antibiotics, serum, and reducing agents should be filter-sterilized.

> ***Safety:*** DO NOT AUTOCLAVE flammable liquids or solids such as ethanol, methanol, or chloroform; radioactive materials; chemicals that emit toxic or carcinogenic fumes when heated such as aldehydes or thiols; strong corrosives, including phenol, or bleach. Solutions that contain detergents can easily boil over.

(2.) For autoclaving, loosely cap containers (one full turn) and wrap in autoclave paper or place in an autoclave tray. Run a liquid cycle for 20–60 min depending on container volume.

> ***Critical:*** Steam-sterilize at 121 °C (250 °F) and 1.03–1.38 bar (15–20 psi). Allow slow exhaust to prevent boiling over.

(3.) After cycle completion, wait 10–15 min for vapor removal before opening. Ensure that liquid remains clear and capped bottles are intact. Make up volume differences with autoclaved water.

(4.) *Optional:* Add supplements or adjust the pH of growth media or agar with sterile solutions when the autoclaved goods have cooled down.

(5.) For filter sterilization, assemble a 0.2 µm pore size filter unit on a sterile funnel or manifold. Pre-wet the filter with sterile water if recommended by the manufacturer.

> ***Hint:*** A pore size of 0.2 µm is sufficient for buffer sterilization. Some tissue culture media requires 0.1 µm pore filtration.

(6.) Slowly pour or draw the solution through the filter into a sterile bottle. Rinse the filter with 10 mL sterile buffer to maximize yield.

(7.) Label the sterilized solution with the word "sterile-filtered" or "autoclaved".

›› *Storage of prepared buffers and solutions*

(1.) Label each container clearly and persistently. Labels must be understood by everyone. Include:

- Name and concentration of the solution
- *Optional:* Recipe identifier
- Hazard labels (prominently, as applicable)

  Note: This applies to solutions exceeding 100 mL that contain more than 1–10% flammable liquids or strong corrosives, as well as any solution exceeding 10 mL that contains a particularly hazardous substance such as toxic materials, carcinogens, reproductive toxins, or mixtures that pose serious health hazards or hazards to the environment.

- Date (MM/YYYY) of preparation or expiry (as applicable)
- Your initials

(2.) Protect light-sensitive solutions such as solutions containing certain antioxidants by wrapping containers in foil or storing in a dark cabinet, fridge, or freezer.

(3.) *Optional:* For long-term storage (weeks to months) of frozen goods, aliquot into smaller volumes to reduce freeze-thaw cycles. Write the volume aliquoted on the secondary container.

Quality assurance: Aliquot buffers containing labile components such as EDTA-free protease inhibitors into single-use volumes. Aliquots stored refrigerated or frozen should occupy at least half of the container volume.

(4.) Store at the recommended temperature.

Hint: Most common buffers can be stored at room temperature. Buffers containing serum, antibiotics, or unstable reagents such as DTT should be kept at 4 °C. Avoid repeated freeze-thawing of frozen stocks.

Critical: Do not store stocks in a frost-free freezer!

(5.) Maintain a digital (or paper) inventory log of stored solutions, and aliquoted materials. Record any deviations from the standard recipe. Note the date of preparation, when the container was opened and when the batch was discarded.

Hint: Periodically inspect and discard any solutions that appear cloudy, have precipitates, or are past their shelf life.

Troubleshooting

Weighing out solid reagents

In Step 4:

– The balance does not zero in
  - If you are using an analytical balance, close all doors when weighing. These balances really are sensitive and will be affected by ventilation or someone passing by.

In Step 6:

– Hygroscopic substances
  - Tare the container first, weigh quickly, close the container.

– Static substances
  - Use an antistatic brush or ionizing blower if available.
  - Avoid plastic weigh boats.
  - Ground yourself by touching a metal surface before weighing.

Mixing

In Step 3:

– Stir bar loses rhythm
  - Turn off the stirrer, wait until the stir bar settles, and turn the stirrer on very slowly.

In Step 4:

– The reagent does not dissolve readily
  - Some reagents dissolve slowly or incompletely without heat or pH adjustment. Consult the protocol or solubility table. Find a stir plate with a heater and warm the solution to 37–50 °C while stirring. However, all solutions must be cooled to room temperature before you adjust the pH.
  - Use a finer powder, crush lumps with a spatula, or use mortar and pestle to grind.
  - Use a different salt form with higher solubility such as Na^+ or K^+ salt if permitted.
– Hydrophobic or amphipathic compounds clump or float
  - Dissolve in a small volume of ethanol, DMSO, or another compatible organic solvent before adding water.
  - Sonicate if appropriate.
  - Use a mild detergent such as Tween™ 20 if necessary and permitted.

Adjusting the pH by titration

In Step 6:

– Readings are erratic
  - Check for temperature fluctuations; allow the solution to equilibrate.
  - Check whether the electrode buffer may need a refill.
  - If the electrode is cracked or broken, the electrode must be replaced.
  - If a silver/silver chloride reference electrode is used, the silver ions can react with proteins and related substances such as Tris and produce an insoluble precipitate in the porous liquid junction of the electrode. Use an electrode with special junction or a calomel reference element.

Sterilizing buffers and solutions

In Step 3:

– Liquid boiled over or caps popped off
  - Reduce fill volume to 80% of container capacity.
  - Use slow exhaust or shorter sterilization time.

In Step 6:

– Filter clogs or flow is very slow
  - Pre-clarify turbid solutions by low-speed centrifugation and decant supernatant.
  - Use a larger-pore pre-filter (0.45 µm) before sterile filtration with 0.2 µm pore size.

Change log

2021-12-20	Benjamin C. Buchmuller	Adaptation as SOP.
2023-11-19	Benjamin C. Buchmuller	Reformatting.