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.

This is a bench card. Full protocol available online.

In the event of EXPOSURE:

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

Report to:

- **Principal Investigator/Supervisor**

Reviewed: Feb 25, 2026

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?

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

(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 If something is missing or outdated, notify the lab manager or order it before proceeding.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.

›› *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. Three conventions are common: weight per weight w_i (w/w), weight per volume β_i (w/v), and volume per volume σ_i (v/v). To find how much solid m_i to weigh out for a desired percent concentration, or how much liquid V_i to measure for a volume percentage, use the relationships below.

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

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

- 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.

- Alternatively, if you have already dispensed a known volume V_1 of stock and need to know how much solvent to add to reach a target concentration c_2, rearrange the dilution equation to solve for the volume of diluent directly:

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

(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-buttoned* 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.

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.

(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.

(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.

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›› *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.

(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).

(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. Position the electrode at one side of the beaker, half-way between the center and the wall, ensuring the stirring bar clears it.

(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.

> ***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.

> ***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.

> ***Quality assurance:*** Use autoclave indicator tape on each container to confirm exposure to steam. Tape alone does not guarantee sterility. For critical applications, include a chemical integrator strip inside the load or run periodic biological indicator tests with *Geobacillus stearothermophilus* spore ampules.

(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.

(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)
- 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.

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

(5.) Maintain a digital (or paper) inventory log of stored solutions and aliquoted materials SOP 0036. Record any deviations from the standard recipe, the date of preparation, when the container was opened, and when the batch was discarded.

Troubleshooting (available online) Notes (available online)