Designing and performing dilutions

Whether adjusting concentrations, preparing a standard curve, titrating a reagent, or normalizing samples across experiments, thoughtful dilution design can improve accuracy, reproducibility, and resource use in nearly every quantitative experiment in the life sciences.

This protocol guides users through proper pipetting techniques, breaking down a dilution factor into manageable steps, selecting appropriate diluents and making deliberate choices to dilute samples efficiently across tubes or multiwell plates when preparing standard curves or viability or activity assays.

Risk assessment

- ▷ Take ergonomic posture when pipetting for extended periods; maintain a neutral wrist posture, keep elbows at a right angle and close to your body, avoid over-gripping
- ▷ Wear gloves, lab coat, safety glasses

Reviewed: Feb 26, 2026

Procedures

›› *Accurate volume transfer and pipetting*

(1.) Choose the appropriate pipette and tip for the volume range to minimize error. Aim to operate near the middle of the pipette's range. Set the desired volume.

> ***Hint:*** For example, use a P200 for 20–200 µL, but avoid using it for 5 µL. A P20 would be more accurate.

> ***Quality assurance:*** To achieve maximum accuracy, set the volume by decreasing (rather than increasing) the volume. When increasing the volume setting, pass the required value by one third of a turn and then slowly decrease.

(2.) Pre-wet the pipette tip by aspirating and dispensing the liquid sample one to three times.

> ***This is why:*** This improves accuracy by equilibrating temperature and humidity of the air cushion inside the tip.

(3.) Press the push button down to the first stop. Immerse the pipette tip vertically into the liquid sample. Keep the tip at a constant depth below the surface of the liquid:

Model	**P2**	**P10**	**P20**	**P100**	**P200**	**P1000**	**P5000**
Immersion depth	1 mm	1 mm	2–3 mm	2–4 mm	2–4 mm	3–6 mm	5–7 mm
Wait time	1 s	1 s	1 s	1 s	2–3 s	4–5 s	4–5 s

(4.) Aspirate the liquid by allowing the push button to slide back slowly. Avoid releasing the plunger too quickly, which may draw liquid into the barrel.

> ***Critical:*** Never set a pipette with liquid in the tip on the bench. This can lead to contamination or backflow.

(5.) *Optional:* Use reverse pipetting when working with viscous liquids, volatile solvents, or when full volume accuracy is essential.

> ***This is why:*** In reverse pipetting, you aspirate a small excess and dispense the target volume, leaving a small residual volume in the tip. This prevents under-delivery due to surface tension or viscosity.

(6.) Dispense at an angle, touching the tip to the wall of the receiving container.

> ***Hint:*** When pipetting into plates, touch the side of the well instead of pipetting into the center if volumes are small. If done carefully, a single tip can be used each time to deliver up to four different solutions to different sides of the well.

(7.) Change tips when switching between samples or when diluting across concentrations.

> ***Critical:*** Reuse tips only when transferring from lower to higher concentrations, or from a neutral diluent such as reagent-grade water. Do not reuse tips if contamination would affect downstream results.

›› *Designing a dilution strategy*

- Understand that in a serial dilution, each step transfers material from the previous tube or well to the next. The concentration decreases by a constant factor at each step, producing an exponential (logarithmic) series.
- Errors in any step propagate to all subsequent dilutions.

(1.) Consider the purpose of your dilution, as it will guide the optimal dilution strategy:

Goal	Recommended strategy	Consideration/Outcome
Adjust concentration	Straight (single-step) dilution	Dilution <1:10
	Multi-step dilution	Dilution >1:10, preferably >1:100
Prepare standard curve	1D dilution series, in replicates	Even logarithmic or linear spacing
Dose–response curve	1D dilution series	Broad initial (1:10 to 1:20), then finer spacing (1:2 to 1:5)
Dose–response curve, steep	2D dilution series, full or partial	Very fine spacing (1:1.5 to 1:2)
Titrate two factors	2D dilution series	Independent gradients across rows and columns

(2.) Break down your dilution into manageable steps where pipetting volumes remain between 5–200 µL.

Notation A ("Make up to")	**1 : 2**	**2 : 5**	**1 : 3**	**3 : 10**	**1 : 4**	**1 : 5**	**...**	**1 : 10**	**1 : 1**
Notation B ("Add")	**1 + 1**	**2 + 3**	**1 + 2**	**3 + 7**	**1 + 3**	**1 + 4**	**...**	**1 + 9**	**Undiluted**
Decimal factor	**0.50**	**0.40**	**0.33**	**0.30**	**0.25**	**0.20**	**...**	**0.10**	**1.00**
Volume of stock	1 vol	2 vol	1 vol	3 vol	1 vol	1 vol	...	1 vol	1 vol
Volume of diluent	1 vol	3 vol	2 vol	7 vol	1 vol	4 vol	...	9 vol	0 vol

This is why: The colon notation $1 : D$ means "one part sample made up to D parts total." The plus notation $1 + D$ means "one part sample plus D parts diluent." Both describe the same dilution when D in the plus notation equals $D - 1$ in the colon notation. Some people write "1 : 1" (ambiguous) when they mean 1 + 1. Avoid such confusion!

Hint: For large dilution factors, it is often more accurate to perform multiple smaller dilutions rather than a single extreme one. For example, a $1 : 1,000$ dilution can be achieved by three consecutive 1 : 10 steps, or by a $1 : 20 \times 1 : 50$ series. For multi-step dilutions with unequal factors, start with the highest dilution first.

(3.) For straight dilutions, plan about 10–20% excess volume to account for pipetting losses.

(4.) For serial dilutions, work backward from the assay volume. How much volume must remain for the assay or downstream use? How much volume will you transfer to the next dilution?

For example, where 100 µL must remain after each transfer, the following starting volumes may be practical:

Dilution factor	**1 : 2**	**1 : 5**	**1 : 10**
Starting volume	200 µL	125 µL	110 µL
Diluent volume	100 µL	100 µL	99 µL
Volume transferred	100 µL	25 µL	11 µL
Remaining volume after dilution	100 µL	100 µL	99 µL

(5.) Sketch out source, destination, and any intermediate tubes or wells in your strategy.

(6.) Double-check: Are the volumes large enough? Is there enough margin?

Note: Minor corrections made during planning are much easier than troubleshooting after pipetting begins. If the well or tube cannot support both the transfer and the assay, adjust the dilution plan before proceeding. Plate readers typically require at least 50–100 µL per well for reliable measurements.

›› *Pipetting a dilution series*

(1.) Confirm the diluent to be used. It should match the assay conditions unless otherwise specified.

> ***Critical:*** For biological assays, use assay buffer or medium rather than water. If you prepare a dilution series from which you will take equal volumes to assay, consider using the solvent that was used to prepare the stock. Using the wrong diluent can alter sample behavior and assay outcome significantly.

(2.) Label tubes or plate wells clearly. Arrange them in the order of pipetting.

(3.) Add the diluent to all tubes or wells except the starting wells (those receiving the stock solution).

> ***Hint:*** For uniform serial dilutions, this is typically the assay volume. Distribute the diluent using the same pipette tip if contamination risk is minimal, or use a multi-stepper pipette for speed.

> ***Quality assurance:*** When pipetting very small volumes, add the smaller volume first, followed by the larger volume, to minimize surface tension errors. Pre-dilution is preferred when possible. Alternatively, wipe the outer surface of the tip carefully with a lint-free tissue if needed. Whenever possible, use larger transfer volumes: precision improves dramatically above 10 µL.

(4.) Add the calculated volume of stock solution to the first tube or well only.

(5.) Mix the first dilution thoroughly by aspirating and dispensing at least three times.

> ***Critical:*** Insufficient mixing leads to inaccurate concentrations that will propagate across the dilution series.

(6.) Transfer the calculated volume from the first dilution to the next tube or well. Mix thoroughly after transfer. Discard the pipette tip between dilution steps.

> ***Critical:*** The carryover from high to low concentration tubes can erratically make the actual concentration higher than calculated. This is not the time to conserve pipette tips. When sampling from the completed series for the assay, you can work from low to high concentration with a single tip, since the carryover effect is usually neglible in that direction.

(7.) Repeat the transfer and mixing step until the end of the series is reached. Discard excess volume from the final tube/well if necessary.

(8.) *Optional:* To extend into a 2D dilution series, use the 1D series as a source and repeat the same pipetting across a second axis, for example, columns if the first dilution was along rows.

> ***Critical:*** Plan starting volumes with extra margin! In 2D dilutions, each transfer draws from an earlier dilution step, so running out of volume can disrupt the entire layout. Always mix source wells/tubes again just before transferring into the second axis if the plate has been sitting for more than a few minutes.

(9.) Seal the plate (or cap tubes) after pipetting is complete to avoid evaporation. Proceed immediately to the assay if possible.

> ***Hint:*** If the plate must be stored temporarily, keep it covered and at the correct temperature to maintain sample integrity.

Troubleshooting

Accurate volume transfer and pipetting

In Step 6:

- Liquid remains in the tip after dispensing
  - Check that you pressed to the second stop and held it long enough to allow full outflow.
  - For viscous samples, use reverse pipetting or a larger tip diameter.
- Inconsistent volumes across replicates
  - Verify that tips are seated properly and that the plunger is fully released before aspirating.
- Plunger sticks or feels rough
  - Clean or service the pipette according to manufacturer instructions. Lubrication or replacement of internal parts may be needed.

Pipetting a dilution series

In Step 4:

- Calculated dilution volumes are impractical for available pipettes
  - Introduce an intermediate stock at a more convenient concentration to allow larger transfer volumes.
  - Round volumes slightly to the nearest practical value and adjust downstream calculations if accuracy allows.

In Step 7:

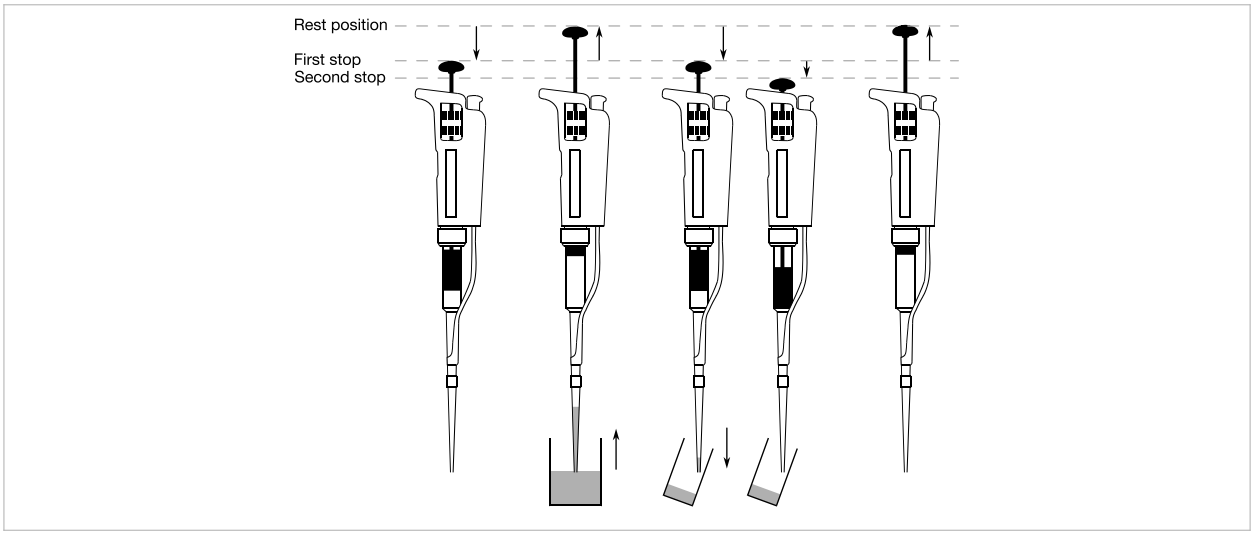
- Missed a well or tube during dilution
  - Partial dilution errors are difficult to detect afterward. Pause immediately. Mark the affected tube or well, restart the series from the last confirmed correct step.
  - When in doubt, repeat the entire dilution series.

In Step 9:

- Dilution error discovered after completing part of the series
  - For minor deviations (within 10%), document the deviation in your records and adjust analysis accordingly.
  - Redo the series if the error critically affects assay interpretation.

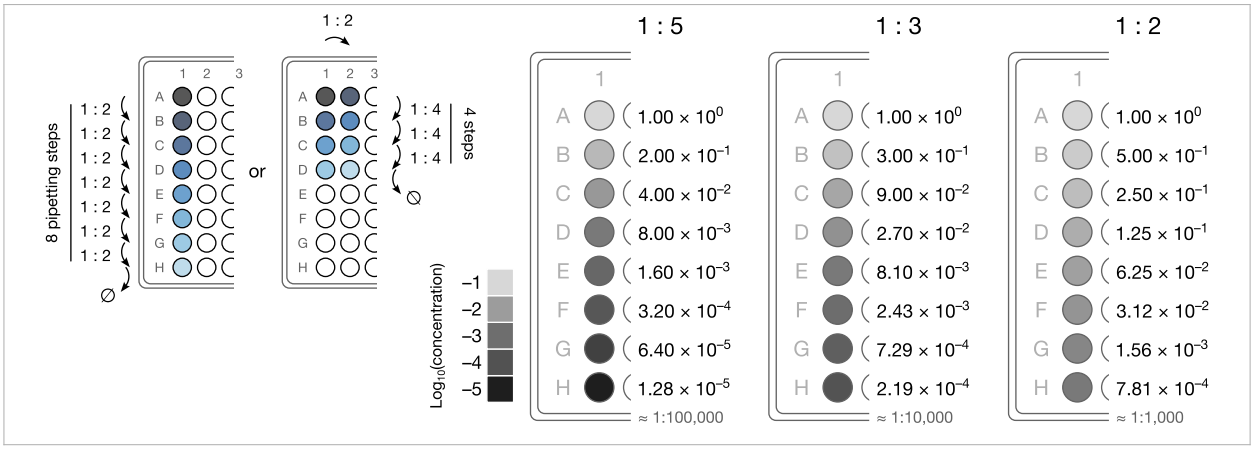
Resources

Accurate volume transfer and pipetting

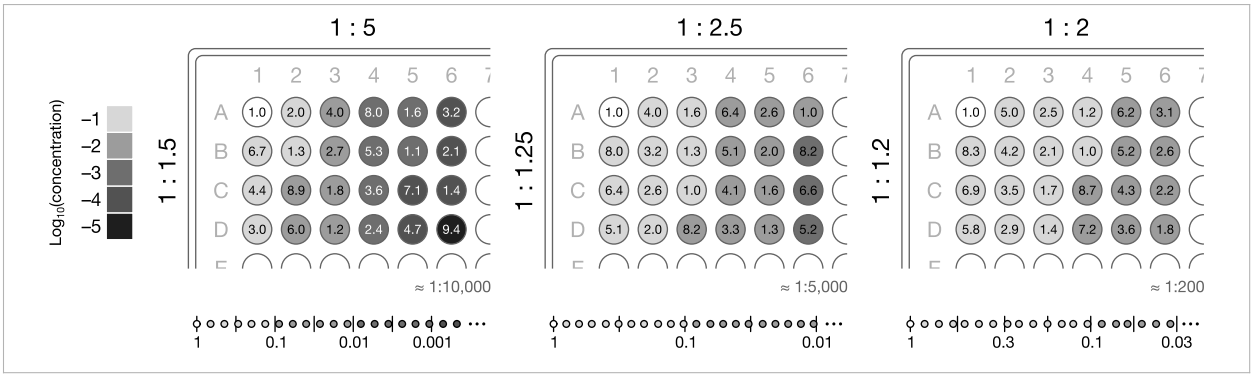


In Step 3: Pipetting motion during aspirating and dispensing. Modified from Gilson PIPETMAN® User's Guide Pub. No. LT801122/E.

Designing a dilution strategy

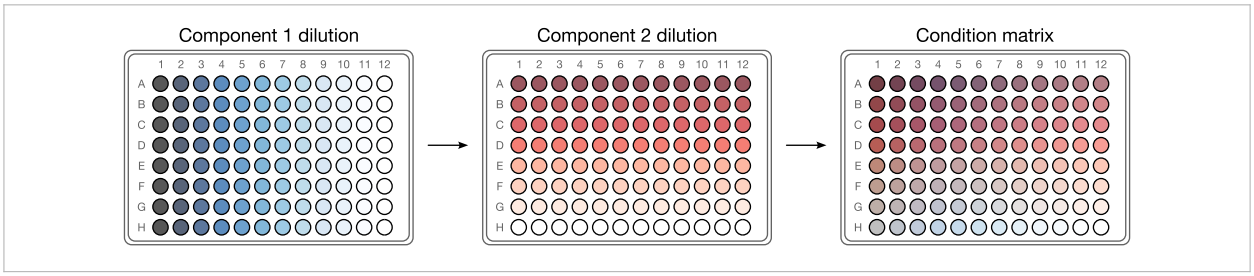


In Step 2: Even logarithmic serial dilutions with constant dilution factors of 1 : 5, 1 : 3, and 1 : 2 in a 1D layout. Each step reduces the concentration by the stated factor. Larger dilution factors cover mor orders of magnitude in fewer steps but with coarser spacing between concentrations. If a multichannel pipette is available, pipetting steps can be reduced.



In Step 2: Combing two dilution factors across rows and columns produces finer logarithmic, almost linear spacing than either factor alone.

Pipetting a dilution series



In Step 8: Pipetting workflow for a two-component 2D dilution. Compoent 1 is serially diluted across columns; component 2 is independently diluted across rows. If enough excess is prepared, the second dilution can be applied within the same plate. The resulting matrix covers a wide combinatorial range of conditions, enabling the analysis of synergy, antagonism, or dose-response effects between drugs, ligands, enzymes, or cells.

Change log

2021-12-20	Benjamin C. Buchmuller	Adaptation as SOP.
2023-11-22	Benjamin C. Buchmuller	Reformatting.
2025-04-24	Benjamin C. Buchmuller	Added guidance on pipetting and two-dimensional serial dilutions.
2026-02-26	Benjamin C. Buchmuller	Integrate resource table into procedure.