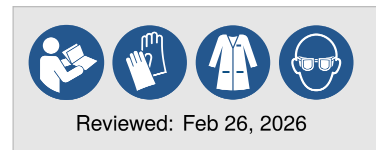
Designing and performing dilutions

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.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This is a bench card. Full protocol available online.

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.

> ***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.

(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.

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

(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*

(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

(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?

›› *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).

> ***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 negligible 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.

Resources (available online)   Troubleshooting (available online)   Notes (available online)

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