

Pipetting



Why do I have a page-long list of pipetting concepts? Pipetting is the one thing you will do EVERY DAY here in the lab. Most of the time, a tiny bit of error is okay, but your science will always be better if you are precise and accurate!

What is a pipet?

A pipet is a tool for moving liquid in accurate volumes. We will focus on micropipettes here, those that move between 0.1uL and 1000uL. These pipets can be thought of as giant syringes. When you set the pipet, imagine deciding where the plunger of a syringe will slide to when sucking up liquid. Inside, springs move a piston to the correct location.

We work with air-displacement pipets, meaning that there is a cushion of air that keeps our liquids from ever actually touching the pipet. This helps keep our pipets safe, but has some disadvantages. Firstly, it makes pipetting some chemicals (like volatiles) a bit tricky. Secondly, the air inside a pipet can get contaminated with chemicals, even with DNA. Careful pipetting will avoid this.

Pipets are fragile little instruments. They are very sensitive to being bumped around, and any major taps (or drops!) can throw off the calibration of the pipet, meaning that it will no longer give the right volume. Please treat them like the sophisticated pieces of lab equipment they are!

Pipetting terms:

Calibration: The pipet has been verified as dispensing the correct amount of liquid (aka if you set it to 100uL, you get between 95-104 uL of liquid. Calibrations are tested annually with water.

Aspirate: pull liquid up into the pipet tip.

Dispense: push liquid out of the pipet tip

First stop: The “syringe” setting that corresponds to the volume set. Feel for it!

Second stop: Your emergency reserve of air to help squeeze that last drop out of the tip.

pXX: How we refer to different sizes of pipets. A p10 dispenses a maximum of 10uL of water, while a p1000 dispenses a maximum of 1000uL of water

Range: The volumes a given pipet can dispense. This is always 10% of the maximum range to the maximum range (aka a p1000 can dispense between 100-1000uL, a p10 can dispense between 1-10uL, etc.) ***Note that on some pipets the last 0 is left off the range, especially common on p1000s. Get to know your pipet to avoid setting it to 1000uL when you wanted 100uL!

Tips: The disposable plastic ends that will actually come in contact with our liquids

Reverse pipetting: (ONLY IF NECESSARY!) A technique in which we break all the rules of pipetting on purpose for greater accuracy. To reverse pipet, go down to the second stop before entering the liquid. Pipet up (we now have too much volume in the tip, but on purpose!). Pipet down to the first stop, and back up. Now move to your receiving container, and pipet out down ONLY TO THE FIRST STOP. The extra liquid in the tip should REMAIN in the tip, but the volume you dispense will be more accurate if the substance is viscous or volatile.



Pipet Tips

1. **Know your stops!** Down to first stop, immerse tip, pipet up gently, move slowly, down to first stop, down to second stop.
2. **NEVER set a pipet to a number outside of its range.** A p1000 will NEVER be set to 1001uL. This also means you need to be gentle and slow when setting pipets to their maximum and minimum values. Cranking that dial too far will damage the pipettor.
3. **ALWAYS use the smallest pipet possible for a given volume.** If you have to pipet 100uL, you could in theory use both a p200 and a p1000, but the p200 will be much more accurate.
4. **Set down to volume.** A pipet set to 9uL from 10uL is more accurate than set to 9uL from 8uL.
5. **Keep that pipet vertical!** That means storage, use, cleaning, etc. You may lay them sideways on the counter briefly if necessary, but never with a tip on and NEVER with liquid in the tip.
6. **Take tips gently and aseptically.** This means you take tips without any banging of the pipet (not needed!), from the edges of a tip box. This keeps your hand from passing over and potentially contaminating other tips.
7. **When in doubt, change tips.** Tips are cheap and disposable; samples are not. If you think you touched the outside of the tube, change tips. If you sneezed, change tips. If you forgot to open your source vial before grabbing a tip, change tips. We will never tell you you've used too many tips.
8. **Wet your tips!** Always, every time. Dry tips are not accurate, ever.
9. **Look at the tip every time.** You need to notice if you accidentally pipetted up a bubble (now your volume is wrong!), or if the amount in the tip makes sense. Common sense rules the day here; if you think the amount in the tip looks wrong, grab another pipettor and check.
10. **Pipet gently!** You should never hear the pipettor click during pipetting; if it's making noise, you need to slow down.
11. **Remember your chemical handling!** This means:
 - **Ethanol:** Saturate your tips! (Reverse pipet if necessary)
 - **Buffered chemicals:** Embrace the bubble (just this once!)
 - **Viscous chemicals:** Go twice as slow, and pause for five seconds before withdrawing. (Reverse pipet if necessary). Remember not to dunk your tip!
 - **Small-necked bottles:** Wipe that pipet down as soon as you're done with that chemical!
 - **Hot or cold samples:** Never use the first take (always put it back, and use the second pull).
12. **Clean pipets before and after use!** A three second wipe-down with 70% ethanol will keep them clean and prevent contamination.
13. **Reset our pipets before you leave!** A pipet should be set to at least 90% of its maximum value for storage. That means the p1000 should be left at $\geq 900\text{uL}$, the p200 left at $\geq 180\text{uL}$, and the p10 left at $\geq 9\text{uL}$. This relieves the tension on the spring and will help our pipets to last longer.
14. **Tell us IMMEDIATELY if a pipet gets dropped,** even just from your hand to the counter. The pipet's calibration will need to be checked before it can be used. This also goes for any pipets that you think may be dispensing the wrong volume.