How to do dilutions

Problem: In biology, we often work with specific inhibitors, or additives, such as hormones, or enzymes that are applied in very small concentrations. To weigh or pipette such small quantities is difficult and the experimental noise by small mistakes in handling is huge. One crystal falling off, when you transfer your compound into a reaction tube, one tiny little droplet that sticks to the wall and does not go into your reaction mix, makes a huge difference and can cause your experiment to fail.

Solution: Try to avoid very small quantities by doing dilutions. Rather than trying to pipet 0.5 µL into your tube, it is better to use a 10-fold dilution and pipet 5 µL, rather than trying to weigh 1.5 mg into the ml in your reaction tube, it is better to weigh 15 mg into 10 mL and then use 1 mL of this solution.

Solution series: If you have to work with very small quantities, it is sometimes necessary to get to the final concentration using a dilution series as shown below.

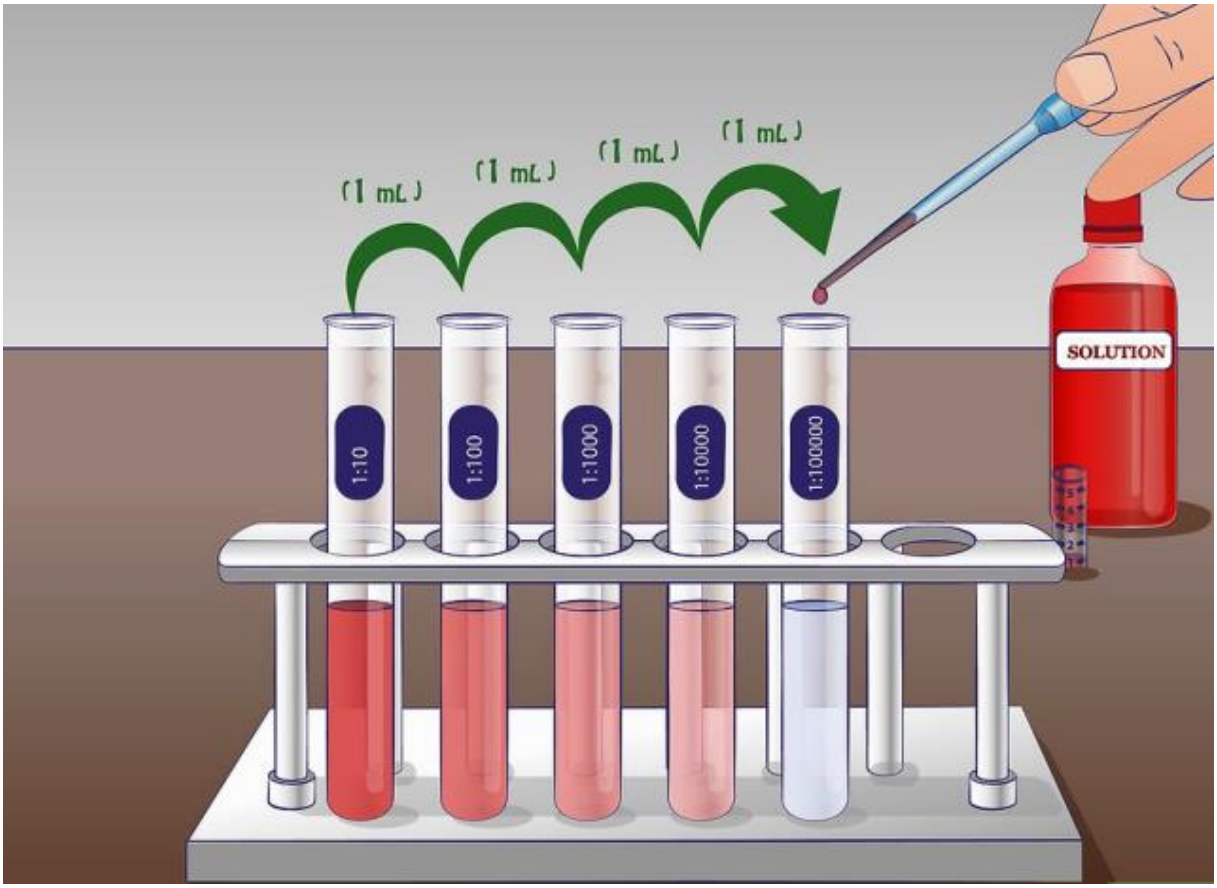
Stock solution: For hormones and inhibitors, it is often useful to have a stock solution which is 100-fold (rarely 1000-fold) concentrated. The volume to pipet should be preferably not smaller than 1 µL

Example: You want to cultivate your cells in the presence of the natural auxin Indole-Acetic Acid (IAA). The working concentration should be 1 µM, your culture flask has 30 mL cell suspension. The molecular weight of IAA is 175, so 1 µM would be 0.000175 g of IAA per liter or 0.00000525 g which you should weigh into your flask – no chance to do this correctly…

What do you do? 1 M of IAA would be 175 g/L, 1 mM of IAA would be 175 mg/L or 1.75 mg/10 mL. This is something, which you just can weigh correctly, if you are careful. When you dilute this stock solution of 1 mM IAA by a factor of 1000, you end up with the desired concentration. When you add into your flask of 30 ml, 30 µL (1/1000), you would get 1 µM IAA. This can be pipetted correctly.

If you have to go to the nM range, which happens with certain inhibitors, you would not be able to do it in one step. Here, you would dilute your stock solution into an intermediate working solution and from there go to your final concentration.

Important: when you do a dilution series, you have to mix very well each time, otherwise your concentrations will not be correct.


(1 mL)
(1 mL)
(1 mL)
(1 mL)
1:10
1:100
1:1000
1:10000
1:100000
SOLUTION