Volume-to-Volume Dilutions

Volume-to-volume dilutions describe the ratio of a solute to the final volume of the diluted solution. A majority of the time, antibody manufacturers suggest a certain starting dilution of antibody to use for a specific application. So if the manufacturer suggests a 1:2000 dilution of antibody for a western blot, this would mean 1 part of the stock antibody to 1999 parts of diluent (blocking buffer). The dilution factor is equal to the final volume divided by the initial volume. So for a 1:2000 dilution:

$$\frac{2000}{1} = 2000 = dilution\ factor$$

If you need a final volume of 10 ml or 10,000 µl of antibody diluted 1:2000 for your blot:

$$\frac{final\ volume\ you\ want}{dilution\ factor} = volume\ of\ stock\ antibody\ to\ add\ to\ diluent$$

$$\frac{10{,}000\ \mu l}{2000} = 5\ \mu\text{l}$$

Then you would need to add $5\mu l$ of antibody to $9{,}995\ \mu l$ of diluent for a final volume of $10{,}000$ µl or $10\ ml$ of diluted antibody.

$\mathbf{C1 \times V1 = C2 \times V2}$

The formula $C1 \times V1 = C2 \times V2$ is useful for determining how to dilute an antibody or stock solution of a known concentration to a desired final concentration and desired volume.

In this formula $C1$ is the concentration of the starting solution and $V1$ is the volume of the starting solution, and $C2$ is the concentration of the new solution and $V2$ is the volume of the new solution.

So let's say you have an antibody stock at a concentration of $\frac{0.2\ \mu g}{ml}$ OR $\frac{200\ \mu g*}{ml}$ and you need $20\ ml$ of antibody diluted to a concentration of $\frac{0.04\ \mu g*}{ml}$.

**When performing these calculations it is important to keep the units the same throughout the equation.*

You know the starting concentration ($C1$) of the antibody stock provided in the vial and you know both the final concentration ($C2$) and final volume ($V2$) of solution that you want (in the case of diluting antibodies, the final solution would be in a diluent of blocking or staining buffer). We need to find $V1$ which represents how much of the starting solution we need to add to the final volume of diluent($V2$).

Rearranging the formula $C1 \times V1 = C2 \times V2$ to solve for $V1$:

$$V1 = \frac{V2 \times C2}{C1}$$

$$V1 = \frac{0.04 \frac{ug}{ml} \times 20ml}{200 \frac{\mu g}{ml}}$$

$V1 = \ 0.004\, ml$

Converting $0.004\, ml$ to $\mu l = 0.004\, ml \times \frac{1000\, \mu l}{mL} \ = 4.0\, \mu l$

So you need to take $4.0\, \mu l$ of the original $\frac{200\, \mu g}{ml}$ antibody solution and add it to $19{,}996\, ul\ (19.996\, ml)$ of diluent. The final $20\, ml$ solution will represent a solution of $\frac{0.04\, \mu g}{ml}$ of antibody.

Now that we have diluted the antibody we can calculate what volume-to-volume dilution we actually performed (the dilution factor) because of the relationship $\frac{C1}{C2} = \frac{V2}{V1}$:

$$\frac{V2}{V1} = dilution\ factor$$

$$\frac{20{,}000}{4} = \frac{5{,}000}{1} = 5{,}000\ dilution\ factor\ or\ 1{:}\,5{,}000\ dilution$$

The dilution factor can also be calculated by dividing the concentration of the starting stock solution by the concentration of the new solution:

$$\frac{C1}{C2} = dilution\ factor$$

$$\frac{200\ \ \mu g/ml}{0.04\, ug/ml} = 5000\ dilution\ factor\ or\ 1{:}\,5{,}000\ dilution$$