

## 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:

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

If you need a final volume of 10 ml or 10,000  $\mu\text{l}$  of antibody diluted 1:2000 for your blot:

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

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

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

## $C1 \times V1 = C2 \times V2$

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  $\frac{0.2 \text{ mg}}{\text{ml}}$  OR  $\frac{200 \mu\text{g}^*}{\text{ml}}$  and you need 20 ml of antibody diluted to a concentration of  $\frac{0.04 \mu\text{g}^*}{\text{ml}}$ .

*\*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  $V1$ :

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

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

$$V1 = 0.004 \text{ ml}$$

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

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  $\mu l$  (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  $\frac{C1}{C2} = \frac{V2}{V1}$ :

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

$$\frac{20,000}{2} = \frac{5,000}{1} = 5,000 \text{ dilution factor or } 1:5,000 \text{ dilution}$$

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

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

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