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 µl of antibody diluted 1:2000 for your blot:

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

\[
\frac{10,000 \mu l}{2000} = 5 \mu l
\]

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

\[C_1 \times V_1 = C_2 \times V_2\]

The formula \(C_1 \times V_1 = C_2 \times V_2\) 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 \(C_1\) is the concentration of the starting solution and \(V_1\) is the volume of the starting solution, and \(C_2\) is the concentration of the new solution and \(V_2\) 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 \((C_1)\) of the antibody stock provided in the vial and you know both the final concentration \((C_2)\) and final volume \((V_2)\) 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 \(V_1\) which represents how much of the starting solution we need to add to the final volume of diluent \((V_2)\).

Rearranging the formula \(C_1 \times V_1 = C_2 \times V_2\) to solve for \(V_1\):

\[
V_1 = \frac{V_2 \times C_2}{C_1}
\]

\[
V_1 = \frac{0.04 \frac{\mu g}{ml} \times 20 \text{ ml}}{200 \frac{\mu g}{ml}}
\]
\[ V_1 = 0.004 \text{ ml} \]

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 \( \frac{C_1}{C_2} = \frac{V_2}{V_1} \).

\[ \frac{V_2}{V_1} = \text{dilution factor} \]

\( \frac{20,000}{4} = \frac{5,000}{1} = 5,000 \text{ dilution factor or 1:5,000 dilution} \)

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

\[ \frac{C_1}{C_2} = \text{dilution factor} \]

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