Dilutions Worksheet

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Knowing how to obtain the correct concentration of your reagents is extremely important! Your reaction will work best with the proper relative amounts of reagents.

The Basics

1. Concentration formulas generally follow this format:

$$\frac{\text{amount of substance}}{\text{volume of total mixture}}$$

2. Specifically, molarity is given by $\frac{\text{moles solute}}{\text{liters of solution}}$. The mole is a standard scientific unit of amount, abbreviated mol. Molarity is often denoted by M.
3. A quick way to convert between concentrations is equating their amounts, giving the formula $M_1V_1 = M_2V_2$.
4. And, one last thing: 1 nanoliter (nL) = 10^{-9} liters, 1 microliter (μL) = 10^{-6} liters, 1 milliliter (mL) = 10^{-3} liters. Keep all of your units in terms of microliters for this worksheet.

Examples

- Say you want to do a titration to verify an unknown concentration of base, but 5 M hydrochloric acid is too concentrated! What volume of 5 M acid do you need to obtain a 100 mL solution of 1 M HCl?

- **Solution**: In 100 mL of the desired solution, you need 0.1 L * 1 M or 0.1 mol HCl. To get the volume of 5 M acid needed, divide this by the concentration, and you'll find you need 0.02 L or 20 mL.
- Let's change it up a little. You have a 4X enzyme mix, and need 1X in your final solution. How much do you need to add to 30 mL of your reaction solution

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- **Solution**: 1 part of 4X enzyme mix in 4 parts total solution will give you a final concentration of 1X—it's a division by 4. From this, the ratio of existing solution to enzyme mix is 3:1, thus you need to add 10 mL of the 4X mix into 30 mL of existing solution.

Now you try!

Remember that individual components of a complicated solution can be considered separately; only the ratio of their amounts to the whole mixture are relevant.

For today's experiment, we want a final reaction volume of 50 microliters/50 µL. Work in groups to calculate the volumes of reagent needed for the PCR.

1. You need to dilute the 10X Taq Buffer to 1X. It should comprise a tenth of the final volume. What volume Taq Buffer will you add?

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2. From a stock of 2 mM dNTP mix, you'll want a final concentration of 200 µM.
   How much of the mix is needed?

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3. You'll need 0.2 µM of each of the forward and reverse primers, which conveniently have the same initial concentration of 1 µM. How much of each primer will you need?

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4. You have .1 ng per µL DNA, and you need 0.3 ng total in your 50 µL solution. How much should you add? Don't overthink this one!

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5. Almost there! The last component is Taq polymerase. You have .25 U/µL, and need 1.25 U total in your reaction mixture. How much Taq polymerase should you add?

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6. Okay, now you've got everything you need! However, the total amount of liquid doesn't add up to 50. How much water do you need to top it off?

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Congrats! You did it!