M3013 The Dilution Solution MiniLab - Student's Guide
September 11, 2020
Estimated time: 60 minutes for all 3 components

Introduction:
Have you ever made orange juice from concentrate? Added a scoop of liquid soap to a bucket of hot water? Measured a teaspoon of vanilla into cake batter? If so, you already know how to do a dilution! Nearly everyone does dilutions as a part of their everyday lives. Dilutions can range from rough estimates, like adding liquid detergent to a load of laundry, to the extremely precise measurements, like diluting a chemical stock to make a series of calibration standards.

No matter which field of science you are interested in, making accurate dilutions is an essential part of your laboratory skill set. Far from being a routine chore, mastering dilutions requires quantitative thinking and a precise attention to detail. This lab activity gives you an opportunity to experiment with different methods of preparing dilutions and even create your own protocol.

Before we get started, we need to cover some basic terminology. A concentrated solution to be diluted is called a ***stock solution***. A ***working solution*** is used routinely for experiments and is often prepared by diluting a stock solution. Preparing working solutions from stocks saves work and reduces error and since you don't have to measure powdered chemicals each time you want to make a working solution.

Let's say you want to dilute a stock solution with water to make a working solution that is one tenth as concentrated. This could also be called a ***ten-fold*** dilution or a "10X" dilution, because the working solution will be ten times as dilute as the stock. Here the ***dilution factor*** is 10.

How do you know how much stock and how much water to combine? Start by determining the final volume you want then divide by the dilution factor to find the volume of stock solution you will need to use:

Final volume = 1000 mL (1 L)
Dilution factor: 10X
Stock volume: 1000 mL/10 = 100 mL

Now you need to calculate the amount of water you will add the 100 mL stock solution to. Do this by subtracting 100 mL from the final volume:

Final volume: 1000 mL
Volume of water to add: 1000 mL – 100 mL = 900 mL

Following this calculation, you will add 100 mL stock to 900 mL water. In fact we can calculate any dilution using this equation:

$$\text{Volume of stock} = \frac{\text{Final volume}}{\text{Dilution factor}}$$

The equation also allows you to calculate the dilution factor if you know the volumes of stock and water that was used. Let's say your lab partner mixed 200 mL of a stock with 800 mL of water. What is the dilution factor of the new solution?

$$\text{Volume of stock} = \frac{\text{Final volume}}{\text{Dilution factor}}$$

$$\text{Dilution Factor} = \frac{\text{Final volume}}{\text{Volume of Stock}}$$

$$\text{Dilution Factor} = \frac{800\ \text{mL} + 200\ \text{mL}}{200\ \text{mL}}$$

$$\text{Dilution Factor} = 5$$

→ Your lab partner made a 5X dilution

A ten-fold dilution is sometimes also written as a 1:10 dilution. Here 1:10 shows the ratio between the stock and the final volume of the working solution (100 mL : 1000 mL -> 1:10). 1:10 is the same as the fraction 1/10 or 0.1, indicating that the final solution is one tenth as concentrated as the stock. Sometimes you will simply be told "add one part stock to nine parts water". The discussion above should make clear why this would be a ten-fold dilution.

Confusingly, you will sometimes see stocks labeled by how concentrated they are relative to the working solutions that are prepared from them. Using this terminology, a "10X" stock might be diluted by adding 100 mL of the stock to 900 mL to produce a "1X" working solution.

As you are diluting a stock, you are making the dissolved solutes less concentrated. For example, if your stock is 100 mM NaCl, a ten-fold dilution in water would give a 10 mM NaCl solution. You can calculate the concentration of a diluted solution by dividing the concentration of the stock by the dilution factor:

$$\frac{\text{Stock concentration}}{\text{Dilution factor}} = \text{Final concentration}$$

$$\text{Example: } \frac{100\text{M}}{10} = 10\text{ mM}$$

In this lab we will be working with a stock solution of a fluorescent compound called fluorescein. Fluorescent chemicals are different from the colored chemicals in food dye. Colored dye molecules in food dye, paint, and magic markers absorb some colors of light and reflect others, but they don't emit any light of their own, so you would not say that they "glow".

Fluorescent materials on the other hand are able to absorb light of one color and emit light of another color. If you have ever seen something glowing under a black light, then you have seen fluorescence. Highlighters, scorpions, tonic water, and fabric treated with bleach all absorb the UV light from a black light and emit light of different colors. Tonic water is an interesting example because it does not appear to have any color until it is illuminated with UV light. You will notice that the sample you will work with in this lab also has only a faint color under regular room light, but changes dramatically when exposed to a blue light.

Learning objectives:
Students will be able to

- Dilute a solution using multiple methods.
- Calculate the volumes needed to prepare a specified dilution.
- Calculate the concentration of a diluted solution.
- Describe the characteristics of fluorescence.
- Reflect on sources of error in an experiment.

Pre lab Questions:

1. Can you think of an example from your everyday life when you have made a dilution?

2. A recipe for soup says to add 1 cup of vegetable broth to 4 cups of boiling water. What is the dilution factor for the vegetable broth?

3. If the concentration of salt in your vegetable broth is 75 mM, what is the concentration of salt in the final concentration of salt in the soup?

4. Can you think of an example of something from your everyday life that is fluorescent?

5. If you dilute a fluorescent solution, how will the diluted solution look different from the original solution?

6. Fluorescein is a common fluorescent compound. Can you identify some uses and why fluorescence is helpful in these applications?

Purpose: Correctly preparing dilutions is essential for getting accurate and reproducible results from your experiments. Whether you are a plant scientist, geneticist, or chemist, ensuring that your materials are prepared at the right concentrations is imperative. However, calculating and preparing dilutions takes practice and patience. In this activity you will use a fluorescent solution and practice this important technique by creating your own protocol for diluting it.

Materials for each student group:
12 x 1.5 mL microcentrifuge tubes
3 microcentrifuge tubes with stock fluorescein solution
15 mL water
1 x MiniOne Winston Fluorescence Reader with photo hood (charged ahead of time)
1 x 100-1000 µL adjustable volume micropipette with tips OR 1 mL transfer pipettes with gradations
1 x 2-20 µL adjustable volume micropipette and tips
1 x Microcentrifuge tube rack (optional)
1 x marking pen to label tubes

Part 1: Discover Dilution
Estimated Time: 20 min

Your job is to prepare a series of dilutions to make increasingly lower concentrations of the solution. You will experiment by choosing random volumes of stock solution and water in order to see the differences in dilutions.

1. View the stock fluorescein solution in the MiniOne Winston Fluorescence Reader. What do you think the diluted solutions will look like in the viewer?

2. Calculate the volumes of stock solution and water needed to dilute the stock fluorescein solution. Each diluted solution should have a total volume of 1 mL or 1000 µL. In the left column in the table below, the example solution has a dilution factor of 2X meaning you will add 500 µL of stock solution to 500 µL of water.

3. Fill in the table below with the quantity of stock fluorescein solution and the quantity of water you will add for each dilution. You may choose your volumes but be sure that they vary and that each dilution is more dilute than the one before it. **Leave the Dilution Factor blank for now:**

Example	**Tube 1A**	**Tube 1B**	**Tube 1C**	**Tube 1D**
Dilution Factor: 2X	Dilution Factor: __X	Dilution Factor: __X	Dilution Factor: __X	Dilution Factor: __X
Vol of Stock: 500 µL	Vol of Stock: ____ µL	Vol of Stock: ____ µL	Vol of Stock: ____ µL	Vol of Stock: ____ µL
Vol of Water: 500 µL	Vol of Water: ____ µL	Vol of Water: ____ µL	Vol of Water: ____ µL	Vol of Water: ____ µL
Total Vol: 1000 µL	**Total Vol: 1000 µL**	**Total Vol: 1000 µL**	**Total Vol: 1000 µL**	**Total Vol: 1000 µL**
Concentration: 0.5 µg/mL	**Concentration:**	**Concentration:**	**Concentration:**	**Concentration:**

4. Label your four microcentrifuge tubes, 1A, 1B, 1C, and 1D to correspond to the dilution factors you will create.

5. Use your adjustable volume micropipette or transfer pipette to add the volume of water you have calculated to each of four labeled microcentrifuge tubes.

6. Use your adjustable volume micropipette to add the volume of stock fluorescein solution you calculated above to each of the tubes.

7. Close the lids and invert each tube repeatedly until the dye and water are completely mixed.

8. Place the tubes in the MiniOne Winston Fluorescence Reader and put the photo hood on top.

9. Document your dilution series by taking a picture with your cell phone. Place your phone's camera directly on the orange photo hood over the viewing portal. Do not zoom in!

10. Dispose of your tubes in the proper waste disposal container when done.

11. Calculate the dilution factors you created. To calculate the dilution factor you would use the equation:

$$\frac{\text{Total volume}}{\text{Volume of stock solution added}} = \text{Dilution Factor}$$

In the table in Step 3 above, the example dilution factor would be 1000/500 = 2, so a 2X dilution was done. Calculate the dilution factors for the dilutions you created and record in the table above.

12. The concentration of the stock solution is 1 µg/mL. Use the dilution factor for each of your tubes to calculate the concentration and record in the table above.

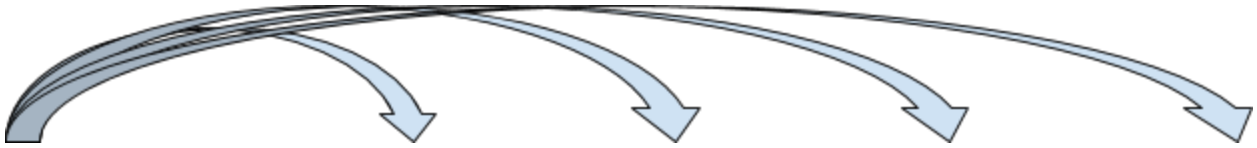
Part 2. Change the Dilution Factor

Estimated time: 20 minutes

In the first experiment you made your own dilutions based on how much stock solution and water you wanted to use, then calculated the dilution factors. In a lab you need to use very specific dilution factors. What if you wanted a 2X dilution series where each solution is 2X as dilute as the previous one?

1. Calculate and record in the table below the volume of stock solution and water needed to create the listed dilution factors. Keep the total volume 1000 µL. *Hint refer to the equation you used to calculate the dilution factors in Part 1*.

2. The concentration of the stock solution is 1 µg/mL. Use the dilution factor for each of your tubes to calculate the concentration and record in the table below.



Stock Solution	**Tube 2A** Dilution Factor: 2X Vol of Stock: ____ Vol of Water: ____ **Total Vol: 1000 µL** **Concentration:**	**Tube 2B** Dilution Factor: 4X Vol of Stock: ____ Vol of Water: ____ **Total Vol: 1000 µL** **Concentration:**	**Tube 2C** Dilution Factor: 8X Vol of Stock: ____ Vol of Water: ____ **Total Vol: 1000 µL** **Concentration:**	**Tube 2D** Dilution Factor: 16X Vol of Stock: ____ Vol of Water: ____ **Total Vol: 1000 µL** **Concentration:**

3. Label 4 new microcentrifuge tubes 2A, 2B, 2C, and 2D.

4. With a new tube of stock solution, use your adjustable volume pipette or transfer pipette to dilute your stock solution so that each tube has the corresponding dilution factor.

5. Place the tubes in the MiniOne Winston Fluorescence Reader and put the photo hood on top.

6. Document your dilution series by taking a picture with your cell phone. Place your phone's camera directly on the orange photo hood over the viewing portal. Do not zoom in!

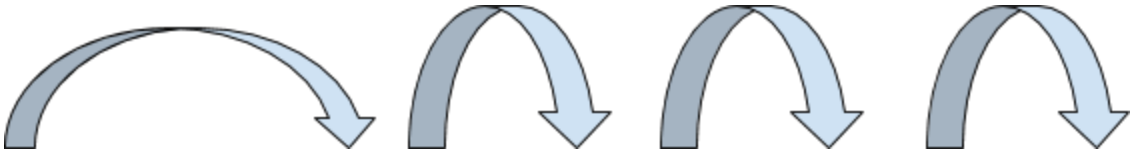
7. Discard your tubes in the appropriate waste container when done.

Part 3. Direct vs. Serial Dilution

Estimated time: 20 minutes

In Part 2, you created specific dilutions directly from the stock solution. Often it's more convenient to prepare serial dilutions. In this example this means each diluted solution is used as the stock to prepare the next diluted solution in a chain of dilutions. *In a serial dilution you will use the same volume of water for each dilution.*

1. You will start with the same concentrated stock solution you used for Parts 1 & 2. Fill in the table below with how much water and previously diluted solution needs to be added to each tube. For example, calculate the volume of 2X solution and the amount of water you will need to make 1000 µL of 4X solution.



Stock Solution	**Tube 3A** Dilution Factor: 2X Vol of Stock: ____ Vol of Water: ____ **Total Vol: 1000 µL**	**Tube 3B** Dilution Factor: 4X Vol of 2X: _____ Vol of Water: ____ **Total Vol: 1000 µL**	**Tube 3C** Dilution Factor: 8X Vol of 4X: _____ Vol of Water: ____ **Total Vol: 1000 µL**	**Tube 3D** Dilution Factor: 16X Vol of 8X: _____ Vol of Water: ____ **Total Vol: 1000 µL**

2. Label four new microcentrifuge tubes 3A, 3B, 3C, and 3D.

3. Use your adjustable volume micropipette or transfer pipette to add the volume of water you have calculated to each of four labeled microcentrifuge tubes.

4. Prepare the 2X dilution first by combining the indicated volume of stock solution and water. Invert each tube repeatedly until the dye and water are completely mixed.

5. Next prepare the 4X solution by combining the calculated volumes of water and the 2X dilution. Note that you will take 500 µL of solution out of the 2X tube, so it will only have 500 µL remaining.

6. Use the calculated volumes of 4X solution and water to create the 8X dilution, then use the 8X dilution and water to create the 16X dilution. Note that at this point you will have three tubes with 500 µL and one tube with 1000 µL.

7. Place the tubes in the MiniOne Winston Fluorescence Reader and put the photo hood on top.

8. Document your dilution series by taking a picture with your cell phone. Place your phone's camera directly on the orange photo hood over the viewing portal. Do not zoom in!

9. Compare this image with your first 2X dilution series. Do they look the same? If they don't, what could account for the differences?

Post Lab Questions:

1. Which type of dilution was easier, direct or serial? Why?

2. Do you think you are more likely to make a mistake with the direct or the serial dilution procedure. Why?

3. You were using a fluorescent solution. What did it look like in the Fluorescence Reader? How did it change as the dilution factor increased?

4. Why can you estimate the concentration of the solution better in the Fluorescence Reader than room light?