**Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**SERIAL DILUTIONS**

Dilutions are a standard part of many laboratory procedures and are commonly used to create working solutions or in the preparation of samples. You will be making many serial dilutions in the Phage Discovery lab this fall, so it is important you know this material well.

First, a few terms to be covered.

1. Dilution: expressed as a ratio of the quantity of a solute (serum, phage stock, chemical solution) contained in a solvent (phage buffer, PBS, diluents). For example, a 1:10 dilution of serum is prepared by adding 1 part serum to 9 parts diluents to make a total of 10 parts. Thus, in the lab if you wanted to make a 1:10 dilution of serum in PBS (phosphate buffered saline solution), you would add 1 mL serum to 9 mL PBS and mix well.
2. “Diluted to”: This is essentially the same term as dilution. If 1 mL of serum is diluted to 10 mL then 9 mL of diluent was added to the 1mL of serum to generate 10 mL of total solution.
3. “Added to”: This IS NOT the same as “dilution” or “diluted to.” “Added to” refers to the volume of solute added to a specified volume of solvent. For example, if 1 mL of serum is added to 10 mL of PBS, the total volume would be 11 mL. This is not a 1:10 dilution, but rather a 1:11 dilution.
4. Serial dilution: This term refers to multiple dilutions made in succession using the same dilution factor each time. For example, to prepare 1:5 serial dilutions 1 mL of serum (Dilution Zero or D0) is added to 4 mL PBS and the solution is mixed well. This is now Dilution 1 (D1). Then 1 mL of D1 is added to 4 mL of PBS and mixed well. This is now Dilution 2 (D2). This same process is continued as needed.

Dilutions must be calculated carefully to avoid compounding errors. Be sure to double check your calculations and label tubes well to avoid confusion while working in the lab.

**Preparation of Diluted Solutions**

Many solutions in the laboratory will be purchased as a concentration solution that needs to be diluted to generate working stock solutions. To determine the dilution factor needed to prepare the working stock, look at the bottle to determine the –fold concentration of the stock solution. For example, TAE and TBE buffers (used for gel electrophoresis) often come in 10X, 25X or 50X stock solutions. If we purchase a 10X TAE buffer, we will need to dilute the stock TAE 1:10 using dH20 prior to use.

Question: How would you prepare 500 mL of working TBE buffer if the stock solution was purchased as 25X TBE?

Answer: You would need to make a 1:25 dilution of the TBE. But we are not making just 25 mL of the 1X solution- we want to make 500 mL! So we would need to divide 500 mL by 25 (the dilution factor), which gives us 20. This is the total amount of the concentrated solution (25X) that will be used to make the 500 mL of 1X solution. In other words, we would measure out 20 mL of the 25X stock, and add it to 480 mL of water to make a total of 500 mL of solution (now at 1X concentration). We can double check this by looking at the ratio created: 20 mL/500 mL is the same as 1:25 or 1/25 (or 0.04).

**Preparation of Serial Dilutions**

In the Phage Discovery lab, we will make several serial dilutions. To calculate a dilution at a particular point in the series, multiply each dilution factor involved in preparation of the tube. 

Example: A student prepared five 4-fold serial dilutions of their original phage stock. What was the dilution in each tube? Note: 4-fold is another way of stating 1:4.

Solution:



**Example**: A student was making serial dilutions to be able to count phage plaques on the plate. They started by adding 20 µL of phage stock to 80 µL of phage buffer in the first tube, then proceeded to make 5-fold serial dilutions for a total of 6 tubes. What was the dilution in the last microfuge tube?

Solution:

$\left(\frac{20 uL phage stock}{100 uL total solution}\right)\left(\frac{1}{5}\right)\left(\frac{1}{5}\right)\left(\frac{1}{5}\right)\left(\frac{1}{5}\right)\left(\frac{1}{5}\right)= \left(\frac{1}{15,625}\right)$

**Practice Problems:**

1. A student labeled 5 tubes 0, 1, 2, 3, 4, 5. Tube 0 contained undiluted phage lysate. The student used the undiluted lysate to prepare 10-fold serial dilutions in the tubes labeled 1-5. What is the final dilution of each of the tubes?
2. A student needed to prepare 500 mL of 1X TAE buffer to run a QC gel. The stock solution in the lab is 5X TAE. What volumes of stock TAE and water are needed to prepare the 1X working TAE buffer?

**SERIAL DILUTION PRACTICE PROBLEMS ANSWER KEY**

**Practice Problems:**

1. A student labeled 5 tubes 0, 1, 2, 3, 4, 5. Tube 0 contained undiluted phage lysate. The student used the undiluted lysate to prepare 10-fold serial dilutions in the tubes labeled 1-5. What is the final dilution of each of the tubes?

Answer:

| Tube # | 0 (undiluted) | 1 | 2 | 3 | 4 | 5 |
| --- | --- | --- | --- | --- | --- | --- |
| Dilution factor | no dilution | 1/10 | 1/10 | 1/10 | 1/10 | 1/10 |
| Final dilution | no dilution | 1/10 | 1/100 | 1/1,000 | 1/10,000 | 1/100,000 |

1. A student needed to prepare 500 mL of 1X TAE buffer to run a QC gel. The stock solution in the lab is 5X TAE. What volumes of stock TAE and water are needed to prepare the 1X working TAE buffer?

Answer: $\frac{1}{5}=\frac{x}{500 mL}$

Solve for x; (1 x 500 mL) ÷ 5 = 100 mL 5xTAE

To make a 500 mL total solution, add 400 mL of water