

Materials**Reagents**

Tubes of blue, red, yellow colored water and plain water.

Equipment

8 Falcon tubes (15 mL) and rack

10 mL pipettes with controller

96 well plate

100 and 1000 uL pipettes

100 and 1000 uL pipette tips

Learning to Use Micropipettes: Making Suncatchers

Background

To get more pipetting practice for upcoming labs, we will practice making serial dilutions; pipetting different colored liquids to form a picture in a 96 well plate. Serial dilutions are a step-wise way to decrease the concentration by the same amount each time. For example, the dilutions you will make will be 1 to 2, or 1 part in 2 parts, and each subsequent dilution will be $\frac{1}{2}$ the previous concentration.

Just like Battleship, 96-well plates have letters and numbers to note the location of individual wells. The first well in the upper left corner is A1, below that is B1 and so on. A scientist can use these coordinates to separate 96 different experiments in this small space. You'll be using the tiny wells to pipette different concentrations of different colors of solution into different positions on the plate. This will create a "mystery picture"!

The purpose of this lab is to give you practice using a micropipette, practice making serial dilutions, and to test your pipetting accuracy as well.

Procedure Part 1: Making Serial Dilutions.

1. Collect 8 falcon tubes and mark them 1 – 8 along with the first letter of the color you will be diluting. For example, if you are diluting red for your lab group of 4 people, mark your falcon tubes 1R through 8 R. Be sure that each person in your lab group of 4 has a different color; red, blue, green or yellow. You'll be doing this first part individually.
2. Using the 10 mL pipette and controller, put 5 mL of water into each of the tubes 2-8. Skip tube #1.
3. Using your designated color put 10 mL of the stock solution in your 1st Eppendorf tube.
4. Remove 5 mL ($\frac{1}{2}$ the volume in the tube) from tube #1 and put it into tube #2. Mix gently by pipetting up and down. This is now half as concentrated as tube #1.

5. Remove 5 mL from tube #2 and put into tube # 3. Mix.
6. Remove 5 mL from tube #3 and put into tube #4. Mix.
7. Continue down the line till you have all 8 Falcon tubes with 5 mL of colored solution except the last one which will have 10 mL (because you didn't transfer any). You and your partners should now have a gradually decreasing set of colors. Put them in the center so you all have access to the colors you will need.
8. Obtain a coordinate instruction sheet from your teacher. You and your partner can share one, either A or B, but the other lab partners at your table should take the opposite, so you don't both do the same one!
9. Load each of the wells according to the instruction sheet. Remember: you are now using **micropipettes**, so they need tips, which you should change between colors.
10. When finished with pipetting, determine what picture you created.
11. Using colored pencils or crayons, color in the 96-well diagram, cut it out and paste it into your lab notebook.

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
B	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
F	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
G	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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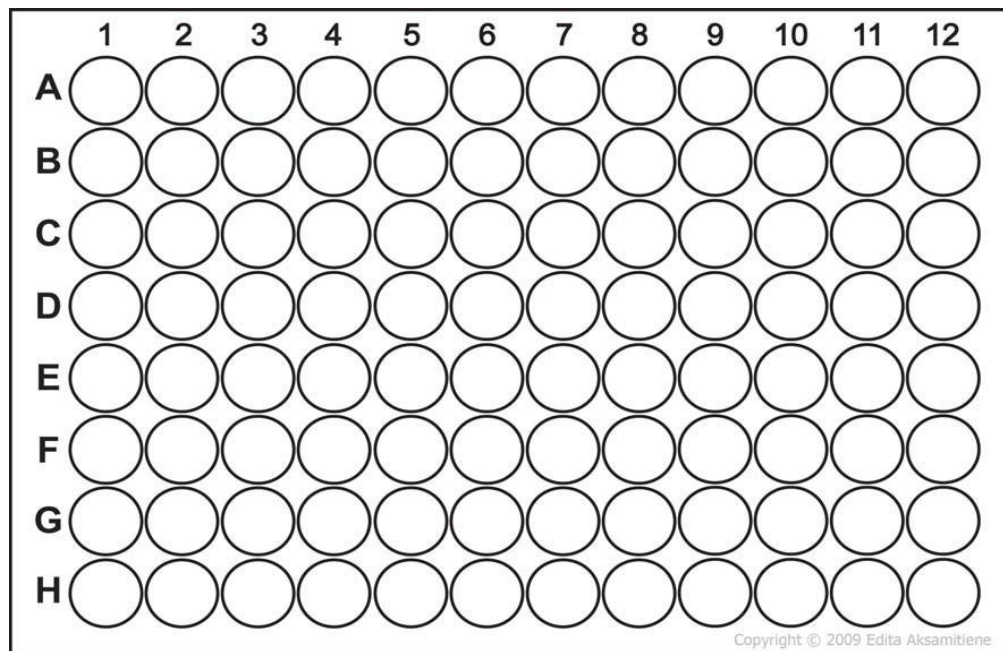
We picked picture (circle one) A B

Our picture was a _____

Procedure Part 2: Accuracy in Pipetting.

1. Obtain the coordinate instructions for the picture that you will create. Note the number in the plate diagram below.
2. Figure out which colors will be used in your picture choice. The primary colors are provided (red, blue, yellow). Others will need to be made by you (green, purple, brown).
3. Weigh your 96 well plate first. You will use the fact that 1 uL of water is approximately 0.001 g of water (or 1.0 mL of water is 1.0 g) to calculate the total mass of colored water that you will be adding. Show your work for this in your lab notebook!
4. If the color has been provided for you, pipette 150 uL that colored solution into the appropriate well(s).
5. If you have to mix colors to create the color (i.e., Blue + Yellow = Green) use 75 uL of two colors for a total volume of 150uL, or 50 uL of three colors for a total of 150 uL. Gently pipette up and down in the well to mix.
6. When finished with your pipetting, determine what picture was created.
7. Using Colored pencils or crayons, color in the 96-well diagram, cut out, and paste into your lab notebook.
8. Mass your plate with the color picture and determine your percent error using the formula: $\frac{\text{Calculated mass} - \text{Actual mass}}{\text{Calculated mass}} \times 100$

Include this calculation in your notebook as well.



We picked picture # _____

Our picture was a _____

Calculations: Show all your work!!!

1. Calculations for predicted mass: water = 0.001 g/uL

Mass empty plate: _____g

Predicted mass of plate with picture: _____g

2. Percent Error:

$$\frac{\text{Calculated mass} - \text{Actual mass}}{\text{Calculated mass}} \times 100$$