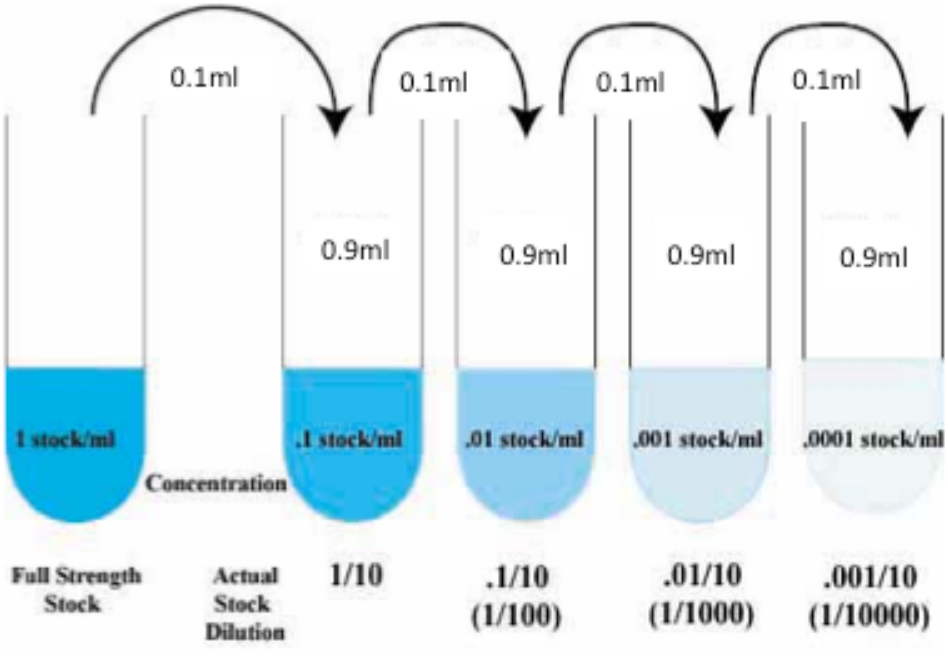
Protocol for Dilution Exercise

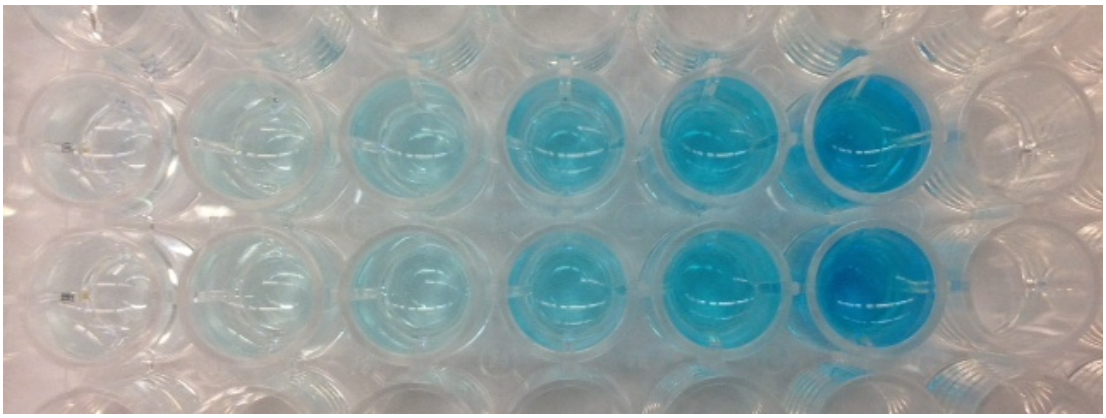
- You will be provided with food dye that has a stock concentration of 10X. In each of 5 tubes you will add 0.9 ml of DI water. Using a pipette carefully pipette 0.5 ml of food dye into a clean centrifuge tube. This is your stock concentration or starting amount.

- Label tubes 1 to 5. Tube 1 is your stock and tubes 2-5 contain 0.9 ml of DI-Water.

- Carefully take 0.1 ml of dye from your stock tube 1 and add it to tube 2. Mix and take 0.1 ml from tube 2 and add it to tube 3…. Etc. Continue the serial dilution until you reach tube 5. This tube should end up having 1 ml of total solution and is very faint. See example below.



- When you are done preparing your serial dilution, transfer an aliquot of 0.2 ml in duplicate using a clean pipette tip to a 96-well plate starting from lowest to highest concentration see figure below.



- Include a blank which is just plain DI-water in one of the empty wells

- Include your unknown sample in another pair of empty wells.

- We will then use a spectrophotometer to determine the absorbance at 595 nm of each standard for the class. With the data for your group fill in the table below

Table showing Conc. vs. Absorbance for standards and unknown sample

Sample	Concentration	Absorbance 595nm
Std1	10X	
Std2		
Std3		
Std4		
Std5		
Unknown Sample		

- Using the data for your group, plot a graph of concentration (X-axis) vs. Absorbance (Y-axis). Use this standard curve to determine the concentration of your unknown sample