Solutions

- Concentration & Dilution
- Micropipetting & Assays

A Solution

- Substances can react when they encounter each other
  - Must be fluid or dissolved in a fluid
- **Solution**: **solutes** dissolved in **solvent**
  - **Aqueous solution**: solvent is **water**
- **Concentration**: amount of a solute in the total volume of solution

Concentration

= Number of solutes in a given volume

- Measurable units
  - Milliliters per 100ml (v%)
  - Grams per 100ml (g%)
  - Micrograms per milliliter (µg/ml)
  - Parts per thousand (ppt)
- But a gram of sucrose has fewer molecules than a gram of glucose,
  - so 1% sucrose ≠ 1% glucose concentration

Concentration

= Number of solutes in a given volume

- "Real" units:
  number of solute molecules per volume
  - **Molar** (*M*)
    = **Moles per liter** = millimoles per milliliter

  1 Mole = 6.023×10^{23}
- 1M glucose = 6.023×10^{23} glucose molecules per 1 liter of total solution
- 1 M glucose = 1M sucrose in concentration
  = $1M = 10^3$ mM = 10^6 µM

Concentration

But how can you make a "real" concentration?

You cannot count the molecules!

Conversion factor
= **molecular mass** [molecular weight (MW)]
= daltons per molecule
= grams per mole of molecules

e.g., $MW_{glucose} = 180$.

I.e., 1 mole of glucose weighs 180 grams

Concentration

So, say you want 10 ml of 2M glucose solution:

Remember, $MW_{glucose} = 180$ g/mole.

10ml x 2 moles glucose/1000 ml x 180g glucose/mole glucose
= 3.6 g glucose needed.

What is the "real" concentration of a 1% glucose solution?

1 g glucose/100 ml x 1 mole/180 g x 1000ml/L
= 0.056 M = 56 mM glucose solution.

Changing Concentration: Dilution

$$C_1V_1=C_2V_2$$

C_1: concentration of the initial solution

V_1: volume of the initial solution

C_2: concentration of the final solution

V_2: volume of the final solution

Changing Concentration: Dilution

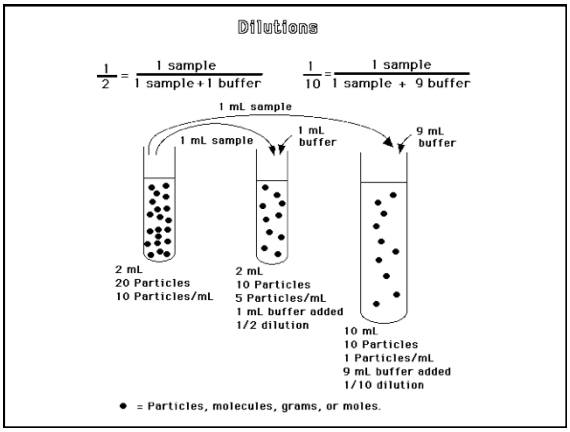
$$C_1V_1=C_2V_2$$

$$\therefore C_1= C_2 V_2/ V_1$$

$$\therefore C_2= C_1 V_1/ V_2$$

$$\therefore V_1= V_2 C_2/ C_1$$

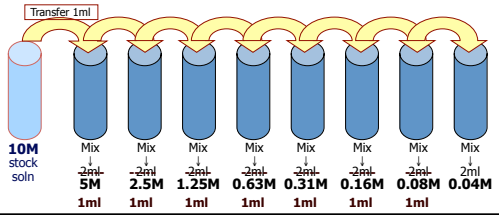
$$\therefore V_2= V_1 C_1/ C_2$$



Changing Concentration: Serial Dilutions

To create a precise series of dilutions, or achieve a low concentration in a small volume.

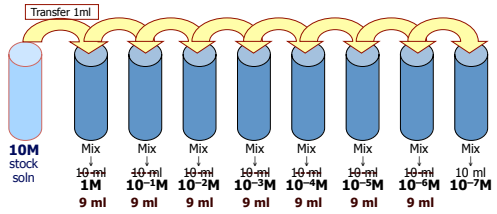
E.g., a **1:2 dilution series**: Starting stock solution = 10M.
Place 1 ml solvent in each dilution tube



Changing Concentration: Serial Dilutions

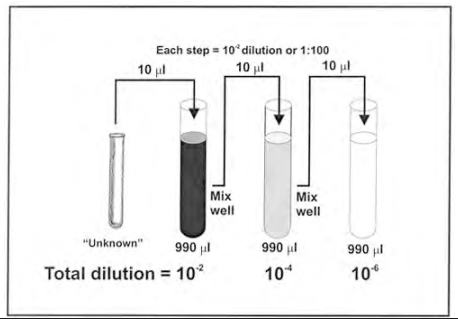
To create a precise series of dilutions, or achieve a low concentration in a small volume.

E.g., a **1:10 dilution series**: Starting stock solution = 10M.
Place **9** ml solvent in each dilution tube



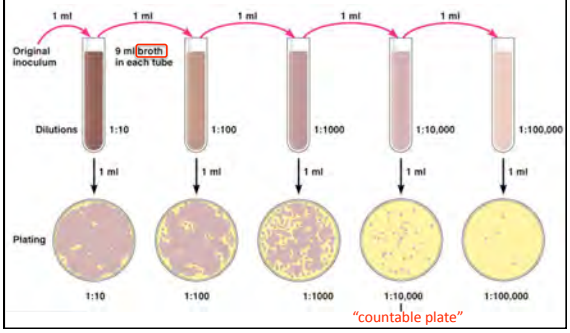
Changing Concentration: Serial Dilutions

- **1:100 dilution series**:



Serial Dilution of Particle Suspension

— e.g., a bacterial culture



Measuring very small volumes

Micropipettes

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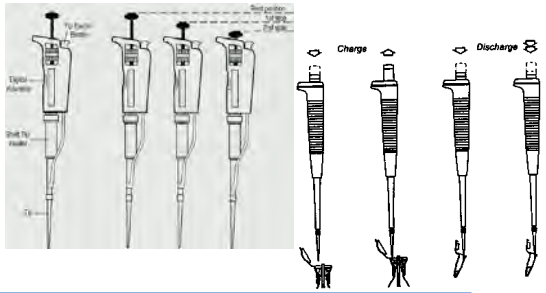
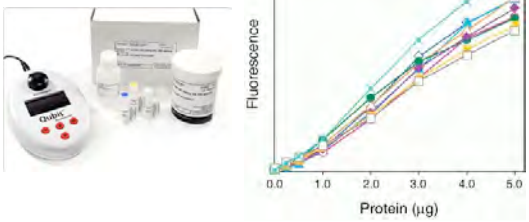


- http://www.teachersdomain.org/special/biot/biot.tools/
- http://www.teachersdomain.org/special/biot/biot.tools/

Quant-iT™ Protein Assay

- http://www.youtube.com/watch?v=6HtnVUHMX_8



Protein Concentration of Fish Tissue Homogenates
by Qubit Fluorescence Assay

Sample[1]	Volume extract in assay[2]	[protein] in assay tube[3]	[protein] in homogenate sample[4]	[protein] in "gel-ready" tube[5]	µl "gel-ready" solution to load onto gel well[6]	µg fish protein loaded in well[7]

1. "Sample" is the name of the fish and/or tissue.
2. Volume of centrifuged fish tissue homogenate added to the protein assay tube. Should be 10µl according to the procedure, but may be less if re-assay because of high concentration.
3. The protein concentration displayed on the Qubit fluorometer. **Be sure to write down the units!**
4. The protein concentration in the centrifuged fish tissue homogenate sample tube calculated from the assay concentration.
5. The protein concentration in the sample tube prepared for electrophoreses according to the "Sample preparation protocol".
6. Volume of protein sample prepared for electrophoreses to be loaded in the gel well. You should attempt to load the same protein amount in each sample well, ideally **60µg** protein, but **cannot exceed 25µl per well**.
7. Actual amount (µg) of fish protein loaded in that well. Ideally should be 60µg, but may be less if concentration is <60µg/25µl.