Lab Calculations

Scientific Notation

Number	Scientific	Prefix	Symbol
1,000,000,000	$1x10^9$	Giga	G
1,000,000	$1x10^6$	Mega	M
1,000	$1x10^3$	Kilo	k
1	1	-	
0.001	$1x10^{-3}$	Milli	m
0.0000001	$1x10^{-6}$	Micro	u
0.0000000001	$1x10^{-9}$	Nano	n

1g = 1000mg
1mg = 1000ug
1ug = 1000ng

Understanding and converting between these units is essential to molecular biology

This tutorial sheet gives examples of common calculations you will need to be able to do in the lab

After the examples, there are a set of questions to test your understanding of these important lab calculations which all research scientists must understand. You will get a copy of these as part of the lab induction, please return to Sam, and ask if there are any areas you need help with 😊

Section A: Concentration Calculations

C1xV1 = C2xV2

$$\mathbf{V1 = \frac{C2}{C1} \times V2}$$

$$\text{Volume needed} = \frac{\text{Concentration wanted}}{\text{Stock concentration}} \times \text{Final volume}$$

- V1 = Volume you want to calculate
- V2 = Final volume
- C1 = Stock concentration
- C2 = Final desired concentration

Example Calculation 1:

You have a DNA stock which is at **925ng/ul**. This concentration is too high to use directly, so you want to make **50ul** of a diluted stock at **200ng/ul**.

$$V1 = \frac{200ng/ul}{925ng/ul} \times 50ul = 10.8ul$$

∴ To make 50ul of a 200ng/ul stock, add **10.8ul** of the original stock to **39.2ul** of NF H_2O

Example Calculation 2:

You want to set up a PCR with **50ng** of DNA. You have a stock of DNA which you have measured by nanodrop spectrophotometry to be **122ng/ul**. What volume of stock do you need to add for 50ng in your PCR reaction?

$$\frac{50ng}{122ng/ul} = 0.4ul$$

∴ To get 50ng of template DNA in your PCR, add **0.4ul** of the 122ng/ul stock.

Section B: Molarity Calculations

Molar mass is the mass of a substance containing 1 mol of molecules: units = g/mol. It is often used as an approximation of molecular weight.

Molar concentration (M) is the amount of the solute in moles/volume of a solution. 1M = 1mol/L

Example Calculation 1:

You need to make **100ml** of a **500mM stock** of NaCl from powdered stock.

A 1M solution = 1mole in 1L
Molecular Weight (Mol.wt) of NaCl = 58.5g/mol

$\therefore$ To make a 1L of a 1M solution of NaCl, dissolve 58.5g NaCl in 1L NF H_2O

1M = 58g/1L

First work out the amount needed for 1L of a 0.5M stock:

$$\frac{0.5M}{1M} \times 58.5g/L = 29.25g/L$$

Then work out how much would be needed for 100ml:

$$\frac{0.1ml}{1ml} \times 29.25g = 2.925g / 100ml$$

So to make 100ml of a 500mM stock, dissolve **2.925g** of NaCl in **100ml** NF H_2O

Example Calculation 2:

You have a 3M stock solution of $MgSO_4$, you need to dilute this to make **200ml** of a **250mM stock** of $MgSO_4$ for an experiment.

$$\frac{0.25M}{3M} \times 200ml = 50ml$$

$\therefore$ To make 200ml of a 250mM stock, add **50ml** of the original stock to **150ml** of NF H_2O

Example Calculation 3:

You have a **1M** stock of Tris Base. How much of this stock do you need to add to have **50mM** in a **10ml** solution?

$$\frac{0.05mM}{1mM} \times 10ml = 0.5ml$$

$\therefore$ To make a 10ml solution containing 50mM Tris Base, add **500ul** of 1M stock to 9.5ml

Section C: Dilution Calculations

Many lab solutions are made up as concentrated stocks (e.g. 5X, 10X, 20X etc…)
Final concentrations used are always a 1X solution.

- A 5X stock needs to be 1/5th of the final volume
- A 10X stock needs to be1/10th of the final volume
- A 20X stock needs to be 1/20th of the final volume

Example Calculation 1:

You have a **20x stock** of TBST buffer, you need to make **100ml of 1X** TBST for your experiment.

$$\frac{1X}{20X} \times 100ml = 5ul$$

∴ To make a 100ml of 1X TBST, add **5ml** 20X TBST to **95ml** NF H_2O

Example Calculation 2:

You have a **5X stock** of Transfer buffer, you need to make **500ml of 1X** Transfer buffer with **10% Methanol** for your experiment.

$$\frac{1X}{5X} \times 500ml = 100ml$$

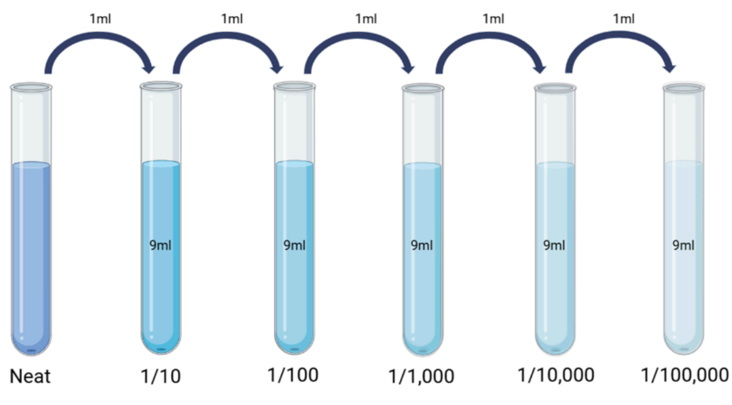
$$\frac{10\%}{100\%} \times 500ml = 50ml$$

∴ To make a 500ml of 1X Transfer buffer with 10% Methanol, add **100ml** 5X Transfer buffer + **50ml** 100% methanol to **350ml** NF H_2O.

Section D: Serial Dilutions

Serial dilutions involve making a stepwise dilution of a substance with a consistent dilution factor (i.e the concentration decreases by the same quantity between each tube)

Example:



Section E: Standard Curve & Plotting Protein Concentration

For quantification of protein samples, we measure absorbance using a BSA or Bradford assay.

First, we need to generate a set of standards of known protein concentration (e.g. for BCA assay we use 2000ug/ml, 1500ug/ml, 1000ug/ml, 750ug/ml, 500ug/ml, 250ug/ml, 125ug/ml, 25ug/ml, 0ug/ml)

Absorbance readings are taken for the standards and 1/10 dilutions of your unknown protein samples (in triplicate).

1. The standards are plotted as a scatter plot (typically on Excel)
2. The equation of the line is obtained from the graph
3. Concentration of your unknown samples is calculated from the equation:

$$\text{Concentration (ug/ml)} = \frac{\text{Average OD} - \text{Line Intercept Value}}{\text{Line Slope Value}}$$

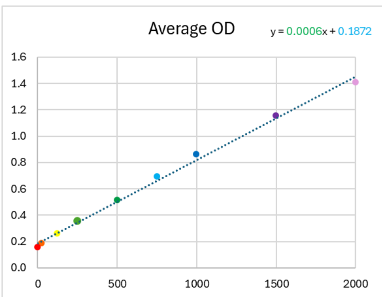
Example:

Measure Absorbance (OD) for your BSA protein standards
Work out the Average OD values from your three readings

Standard	ug/ml	OD1	OD2	OD3	Average OD
St01	2000	1.419	1.410	1.390	1.406
St02	1500	1.154	1.160	1.149	1.154
St03	1000	0.864	0.859	0.861	0.861
St04	750	0.680	0.700	0.690	0.690
St05	500	0.514	0.502	0.523	0.513
St06	250	0.353	0.358	0.348	0.353
St07	125	0.262	0.259	0.260	0.260
St08	25	0.183	0.176	0.186	0.182
St09	0	0.158	0.151	0.160	0.156

Plot The Average OD values against your standard concentrations on Scatter Plot 

Standard	ug/ml	Average OD
St01	2000.000	1.406
St02	1500.000	1.154
St03	1000.000	0.861
St04	750.000	0.690
St05	500.000	0.513
St06	250.000	0.353
St07	125.000	0.260
St08	25.000	0.183
St09	0.000	0.156



Equation of the line: y = ax +b
Where:
(a) Slope
(b) Intercept
(x) sample concentration
(y) Sample OD

So to work out sample concentration:
(x = y - b / a)

To add a line and display the equation of the line on the chart, go to:

Add Chart Element > Trendline > More trendline options > Trendline Options > ☐Linear, ☐ Display Equation on chart

You will now have the Slope value (0.0006) and Intercept value (0.1872) for your graph

Next, work out the average ODs for your (1/10 diluted) protein samples

	OD1	OD2	OD3	Average OD
Sample 1	0.86	0.81	0.85	0.840
Sample 2	1.02	1.1	1.08	1.067
Sample 3	0.98	0.97	1.01	0.987

Use the equation of the line (from the scatter plot) to calculate the concentration e.g. For sample 1:

$$\frac{0.840 - 0.1872}{0.0006} = 1088\text{ug/ml}$$

Since these samples are 1/10 dilutions, the actual concentration is **10880ug/ml (10.88ug/ul)**