EXERCISE 3: LABORATORY CALCULATIONS

Introduction

Laboratory calculations and making solutions are some of the most important procedures in a laboratory. If solutions are not prepared accurately, experiments may fail. Biological experiments are particularly sensitive to alterations in chemical composition of solutions. For example, enzymatic reactions are extremely sensitive to alterations in pH, and salt concentrations; incorrectly prepared media may inhibit bacterial growth; DNA migration in agarose gels can be altered by inexact calculation.

Therefore, knowledge of how to solve "dilution problems" is immensely important for students in all biological fields. Often you will encounter these kinds of problems in future science courses as well as in future place of employment. For example, you may be asked to prepare a solution of a particular concentration or you may be asked what the concentration of a solution is if you added a known amount of water to a given solution. There are various solutions used throughout this practical and these have been prepared for you but it is important that you would know how you would go about doing this yourself.

If you are confident about calculations that allow laboratory solutions to be prepared then go straight to the questions throughout this exercise. If you are unsure, or need a reminder then read on for some hints and tips. Below you will find worked examples to show you how to do various calculations. However, if you are still unsure, please ask a demonstrator to help, as different people think about these things differently!

Quantitative Expressions of Concentration

Units of mass and volume:

Mass units:

1 gram (g)	= 10^{-3} kg
1 milligram (mg)	= 10^{-3} g
1 microgram (μg)	= 10^{-6} g
1 nanogram (ng)	= 10^{-9} g
1 picogram (pg)	= 10^{-12} g

Volume units:

1 millilitre (ml)	= 10^{-3} L
1 microliter (μL)	= 10^{-6} L

1. Concentrations based on molarity (mol/L)

Molarity is the number of moles of solute in a litre of solution and this is the most common way of expressing the concentration of solution. To calculate the molarity of a solute in a solution we need to know:

- The moles of solute present in the solution.
- The volume of solution (in litres) containing the solute.

One mole of any substance equals its molecular weight (MW) in grams.

A molar solution = A solution containing one mole of solute / litre (1M/L, or 1 mol/L).

Example: How can you prepare exactly 0.5L (500ml) of a 0.25M NaCl solution in water? The MW of NaCl is 58.5g.

Method - you could remember a general formula for this approach:

g = volume wanted (L) x M (moles) x MW, remembering that you always have to put the volume wanted in litres

= 0.5 x 0.25 x 58.5

= 7.31g

Remember that if you are making 500mls of this solution, you are not adding 7.31g to 500mls of water, but you putting 7.31g **into a final volume of 500mls**

Now try the following questions:

Q1. Solutions of Tris and EDTA are commonly used in buffers in the laboratory. How would you prepare 500mls of each of these stock solutions at 0.5M Tris and 100mM EDTA concentrations? molecular weight of Tris - 121.14g/mol, molecular weight of EDTA – 372.2g/mol.

Q2. You use competent bacteria in the lab for the process of transformation. These cells are typically prepared using a $CaCl_2$ method, which creates pores in bacterial cells by suspending them in a solution containing high concentration of calcium. The concentration of $CaCl_2$ used is 100mM. Given the molecular weight of $CaCl_2$ is 110.98 g/mol, how would you prepare one litre of a 100mM solution?

Q3. Bacterial cells that have been transformed with DNA are plated onto L-agar which contains 50 µg/ml ampicillin, 50 µg/ml IPTG and 50 µg/ml X-gal. The following is the basic recipe to prepare 500ml of this L-agar:

5g Tryptone

2.5 g Yeast extract

2.5 g NaCl

1.5 % Agar (7.5g in 500 ml)

Distilled water to 500 ml.

Given the molecular weight of NaCl is 58.44 g/mol, what is the molar concentration of this in the above recipe?

Q4. In the PCR clean-up protocol, the PCR product is prepared for the clean-up procedure by mixing it with 'Membrane Binding Solution', which consists of 4.5M guanidine isothiocyanate and 0.5M potassium acetate (pH 5.0). Given that the molecular weight of these is 118.16g/mol and 95.15 g/mol, respectively, how would you prepare 100mls of membrane binding solution and ensure that the final pH was correct?

2. Concentrations based on %

Calculation of concentration based on % is more straightforward that molarity. Weight/volume (w/v) or volume/volume (v/v) can determine these percentages.

Example: How would you prepare 100mls of a solution containing 10% NaCl?

Method - 10% (w/v) solution = 10g NaCl/100mls

10g NaCl would be dissolved in water and the volume adjusted in a measuring cylinder to 100mls

Example: How would you prepare 150mls of a solution containing 1% HCl (this is a liquid)?

Method - 1% (v/v) solution = 1.5ml/150mls

1.5mls would be made up to the 150mls with water using a measuring cylinder.

Now try the following questions:

Q5. How can the following solutions be prepared?:

(a) 200mls of a 40% (w/v) solution of polyethylene glycol (PEG)

(b) 80mls of a 7% (w/v) solution of NaCl

(c) 200mls of a 95% (v/v) solution of ethanol.

Q6. If you have a stock solution of 10% (w/v) SDS and you wish to prepare 50mls of a solution containing 0.02% SDS, how would you use your stock solution to prepare this?

3. Dilutions from Stocks

In a laboratory, you often do not weigh out chemicals every time you want to prepare a solution, for two reasons; (i) inconvenience, and (ii) sometimes it is impossible to weigh out small enough amounts to make a particular solution. Certain commonly used reagents are prepared as a stock solution at a high concentration, and then this would be diluted to the desired concentration. The Master equation for doing this type of calculation would be as follows:

$$\frac{\text{What you want}}{\text{What you have}} \text{ X final volume} = \text{volume to add to solution}$$

Note: **what you want** = the final concentration you want – this can be expressed as %, M, or mg/ml

what you have = the concentration of the stock solution you have – this can be expressed as %, M, or mg/ml

'what you have' and 'what you want' must be expressed in the same units!!

Final volume = means the amount you want to prepare: if you use litres in your answer will be in litres, if you use mls in your units then your final answer will be in mls.

Example: If you want to make 100mls of 1M salt from a 5M stock, you can solve this as follows:

$$\frac{\text{1M (What you want)}}{\text{5M (What you have)}} \text{ X 100mls} = \text{20mls of 5M stock and add 80mls } H_20 \text{ to give a final volume of 100mls}$$

Example: If you want to make 1L of 100mM Tris from a 1M stock, you can solve this as follows:

$$\frac{\text{100mM (What you want)}}{\text{1000mM (What you have)}} \text{ X 1L} = \text{0.1L of 1M Tris and add 0.9L } H_20 \text{ to give a final volume of 1L}$$

Example: If you want to make 40mls of a 0.5% glucose solution from a 20% stock:

$$\frac{0.5\% \text{ (What you want)}}{20\% \text{ (What you have)}} \times 40\text{mls} = 1\text{ml of 20\% glucose and add 39mls } H_20 \text{ to give a final volume of 40mls}$$

Now try the following questions:

Q7. If the stock concentration of the forward and reverse primer in a PCR is 10nM and the final concentration you want of these is 0.4nM. What volume of each primer would be added to the PCR tubes (final volume 50μl)?

Q8. TAE buffer is a buffer solution containing a mixture of Tris base, acetic acid and EDTA. This was used in the preparation of your agarose gel mix (see Appendix 3). In molecular biology it is used in agarose electrophoresis typically for the separation of nucleic acids such as DNA. Assume in the lab you are provided with a 50X stock solution of TAE. How would you prepare 150ml of a 1X TAE gel mix containing 1% agarose?

Q9. To prepare the ligation reaction mix (DAY 2), you are provided with pBluescript plasmid precut with the restriction enzymes BamHI and KpnI. The final concentration of the pBluescript you require for this is 200ng/μl. A stock of plasmid has already been made for you at a concentration of 1mg/ml. How much of this stock would be required to prepare 2mls of plasmid vector ready for use in the reaction?

Q10. Assume that you have stock concentrations of ampicillin prepared at 60mg/ml. How much would you need to add to 500ml of L-agar to have the correct final concentration of 50μg/ml ?

4. Mixed Solutions

In a laboratory, you often have to prepare a solution that is a mixture of many components. Some of these components may have stock solutions, others may have to be weighed out, etc. You may therefore need to use a mix of the above calculations to work out how much of each component is required to prepare the final solution.

Q11. In this lab, you have to carry out a restriction digest a couple of times and this is a common procedure for further DNA analysis. Imagine that you want to digest 2μg of DNA with BamHI; your DNA stock is 1.2mg/ml. In this digest, use 1μl of BamHI and get your final volume to be 30μl. You need to have a final concentration of buffer to be 1X but its' stock is at 10X. How would you prepare this restriction digest mix?

Q12. During the plasmid miniprep two of the solutions you use are called the 'Cell Lysis Solution' (consists of 0.2M NaOH and 1% SDS) and 'Cell Resuspension Solution' (consists of 50mM Tris-HCl pH7.5, 10mM EDTA and 100μg/ml RNase A). Given the following information, how would you prepare 50ml of these two solutions for use in the miniprep?

molecular weight of NaOH is 40 g/mol; Stock solutions of 0.5M Tris-HCl, 10% SDS, 0.1M EDTA, 100mg/ml RNase A