Making reagents and buffers

Objectives

- To learn how to calculate concentrations and prepare stock solutions and buffers.

Preparation of solutions is a skill that is critical to research, since experimental results will depend on the quality of the reagents used (concentration, chemical purity, pH).

Glossary of basic terms

Solute: The substance (reagent) which dissolves in a solution.

Solvent: The substance which dissolves another to form a solution. For example, in a sugar and water solution, water is the solvent; sugar is the solute.

Buffer: A buffer is a chemical solution containing a specific mixture of salts, buffering agents, and eventually reducing agents, detergents or cofactors, etc., in which each of the components has a purpose and is included to optimize the reaction (e.g restriction enzyme digestion buffer, PCR buffer).

Mole (symbol mol): The SI unit for a large number of elementary particles (atoms, molecules, ions, electrons, etc) of any substance. 1 mole (symbol mol) is 6.02×10^{23} molecules of that substance (Avogadro's number).

Molecular weight (symbol MW) is the mean molecular weight of 1 mol of the substance. One can find the molecular weight of the solute from the bottle of the chemical, from the MSDS, or from a chemical company's website.

Molarity (symbol M): The concentration of a solution is commonly expressed in molarity, defined as the number of moles of solute in one liter of solution

M = mol/ L

Molarity = moles of solute / liter of solution

1 M (one molar) solution contains one mole of solute per liter of solution.
1 mM (one millimolar) solution contains one millimole of solute per liter of solution.
1 µM (one micromolar) solution contains one micromole of solute per liter of solution etc.

Example: Preparing stock solution
Prepare a **1 M** (pronounce 1 molar) solution of NaCl.
The molecular weight of a sodium chloride (NaCl) is 58.44 g (by definition containing 1 mol NaCl molecules).
Dissolve **58.44 g** of NaCl in a final volume of **1 liter** water = **1 M NaCl** solution.
To make molar NaCl solutions of other concentrations dilute the mass of salt to 1000 ml of solution as follows:
0.1 M NaCl solution requires 0.1 mol/l x 58.44 g/mol NaCl = 5.844 g/l
0.5 M NaCl solution requires 0.5 mol/l x 58.44 g/mol NaCl = 29.22 g/l
2 M NaCl solution requires 2.0 mol/l x 58.44 g/mol NaCl = 116.88 g/l

Percent solutions
Percent by volume (v/v) are defined as milliliters of solute per 100 ml of solution.

Examples: Prepare a 70% Ethanol solution: mix 70 ml ethanol with 30 ml of water.
Prepare a 10% stock solution of SDS: mix 10 ml of SDS with 90 ml of water.

Percent weight by volume (w/v) solutions are defined based on the grams of solute per 100 ml of solvent.

Example:
Prepare a 20% solution of NaCl.
Dissolve 20 g of sodium chloride in 70 ml of water, then bring the volume up to 100 ml.
This technique accounts for the extra volume contributed by the solute.

Concentrated stock solutions – using "X" factor

In a molecular biology lab, you will often deal with solutions that are labelled "10X," "5X," "100X," etc. The "X" factor indicates that the solution is in a concentrated form that must usually be diluted to a "1X" concentration for use.

Example: You have a stock of 10X TAE buffer for agarose gel electrophoresis. Dilute 100 ml 10X TAE buffer with 900 ml of water giving a final volume of 1 liter.

Example: You are using a concentrated solution of Bovine Serum Albumin that is labelled BSA (100X). For a total reaction volume of 150 µl use 1.5 µl BSA (100X).

Dilutions: The **dilution factor** is the total number of unit volumes in which the reagent (for example stock solution or biological material) will be dissolved. For example, a 1:5 dilution (pronounced as "one to five" dilution) entails combining 1 unit volume of reagent to be diluted + 4 unit volumes of the solvent (hence, 1 + 4 = 5 = dilution factor). A **serial dilution** is a series of dilutions, which amplifies the dilution factor quickly beginning with a small initial quantity of material (e.g. a bacterial culture, a chemical, etc.).

Example: **Preparing a serial dilution**
Make a serial dilution of a 1 mg/ml stock solution of DNA to achieve final concentrations of 100, 10 and 1 ng/ml:
Prepare 1:10 serial dilutions corresponding to dilutions of 10^{-1}, 10^{-2} and 10^{-3}.
Label 3 tubes and add 900 µl of water into each tube.
Pipette 100 µl of DNA solution into tube 1 and mix by vortexing.
Transfer 100 µl of tube 1 into tube 2, etc.

Example: Preparing Buffers from one stock solution

Prepare 1 liter of 1X TBE buffer from a 10X TBE stock solution. Use the dilution formula: $\mathbf{C_1V_1 = C_2V_2}$. The formula relates the concentration (molarity) and volume of a starting, concentrated solution to a new, diluted solution.

V_1= volume of stock buffer = ?	$C_1V_1 = C_2V_2$
C_2= concentration of stock buffer = 10X	? (10X) = (1 liter) (1X)
V_2= volume of diluted buffer = 1 liter	? = (1 liter) (1X) / (10X)
C_2= concentration of diluted buffer = 1X	? = 0.1 liter

To prepare 1 liter of 1X TBE from a 10X concentrated TBE stock, you add 0.1 L (100 ml) of 10X TBE to 0.9 L (900 ml) of water.

Example: Preparing Buffers from multiple stock solutions

It is common in biochemistry and molecular biology to prepare complex buffers by diluting stock solutions. This technique is quicker and more accurate than weighing out the different chemicals and dissolving them. For example, to prepare 100 ml of TE buffer (0.1 mM EDTA, 10 mM Tris), it is best to simply dilute stocks of concentrated EDTA and Tris solutions. To calculate the volume of the stock solution needed to prepare the buffer use the dilution formula: $\mathbf{C_1V_1 = C_2V_2}$ Calculate the volume needed for **each** component independently.

EDTA	Tris
V_1 = volume of stock buffer = ?	V1 = volume of stock buffer = ?
C_1= concentration of stock buffer = 0.5 M	C1= concentration of stock buffer = 1 M
V_2= volume of dilute buffer = 100 ml	V2= volume of dilute buffer = 100 ml
C_2= concentration of dilute buffer = 0.1 mM	C2= concentration of dilute buffer = 10 mM
(?) = (100 ml) (0.1 mM) / (500 mM)	(?) = (100 ml) (10 mM) / (1000 mM)
(?) = 0.02 ml	(?) = 1 ml

To prepare 100 ml of TE Buffer, mix:

- 0.02 ml of 0.5 M EDTA
- 1 ml of 1 M Tris
- 98.98 ml H_2O.

Guidelines for preparing solutions

- **Determine what you need.** How much? Which concentration? If possible, make concentrated stock solutions (e.g. SDS, PBS, NaCl)
  Safety: Check Material Safety Data Sheet (MSDS) for safety and handling of solutions.

- **Plastic and Glassware**
  To make solutions you will need graduated cylinders, beakers and Erlenmeyer flasks. Glass bottles are used for buffer storage.

- **Water** is available in many different qualities that differ in price, environmental impact and use. The most commonly used grades are:
  - Standard tap water: for washing
  - Laboratory grade: to make buffers
  - Ultrapure (filtration): for cell culture and some buffers
  - Nuclease-free (commercial): for work with nucleic acids.

- **Sterilizing solutions**

  When sterile conditions are required buffers are autoclaved or filter sterilized.
  CAUTION: Do not autoclave corrosives (acids, bases, phenol), volatiles (ethanol, methanol, chloroform), radioactive material or liquids containing bleach, formalin or glutaraldehyde, detergents, heat labile substances (vitamins, serum, antibiotics, proteins), dithiothreitol (DTT) or beta-mercaptoethanol (BME) containing solutions. Solutions containing heat-labile ingredients can be filter-sterilized (0.2µm).

- **Make aliquots** if necessary and store at the correct temperature. Avoid freeze/thawing cycles. Some buffers will precipitate in the cold. Light-sensitive reagents should be stored in a brown bottle or covered with aluminium foil. Before use, always verify whether the solution has changed colour, shows precipitates or bacterial/fungal contamination.

Calculating Concentrations

First write down what is provided (with units) and what is unkown. Be methodical about this to avoid mistakes. What is the dilution factor? What total volume of solution is desired? What is the stock solution concentration? What volume of stock is needed? What volume of solvent (water) is needed? It is good practice to record such calculations in your lab notebook (

Exercise 1

Prepare 250 ml of 200 mM NaCl and 150 mM Tris-HCl pH 7.2. You have a stock solution of 5 M NaCl and 1 M Tris-HCl.

Exercise 2

You need to prepare 1 L of Tris-EDTA buffer from a 20X stock. What volumes of stock and distilled water should be mixed?

Exercise 3

You are preparing a 1.2 % agarose gel in a total volume of 50 ml TAE. How much agarose do you need?

Exercise 4

You make 0.8 % agarose gels for 8 groups in a total volume of 400 ml TAE. How much agarose do you need?

Exercise 5

You need to prepare 5 ml of a 2% sucrose solution. You already have a stock solution of 10% sucrose. How many µl of the 10% sucrose solution do you need?

Exercise 6

For PCR you need to prepare 25 mM dNTPs (deoxynucleotide triphosphates) from 100 mM each of dATP, dTTP, dCTP and dGTP stocks. What volume of each stock and water is mixed to obtain 1 ml dNTP working solution?

Exercise 7

You prepare 250 ml of sterile filtered 1 M NaCl stock solution (Figure 1). Using the image below, write down materials and procedures, as well as the amounts of solute and solvent needed.

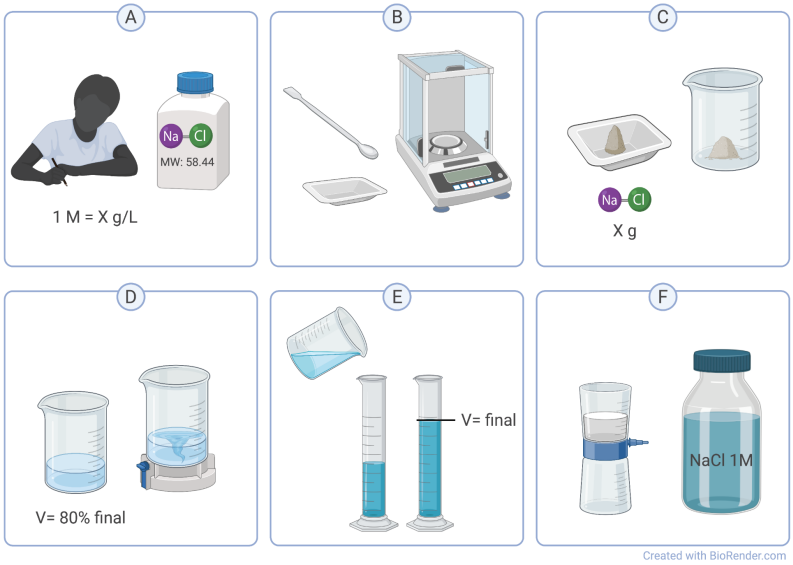

Figure 1: Preparation of 1M NaCl solution.