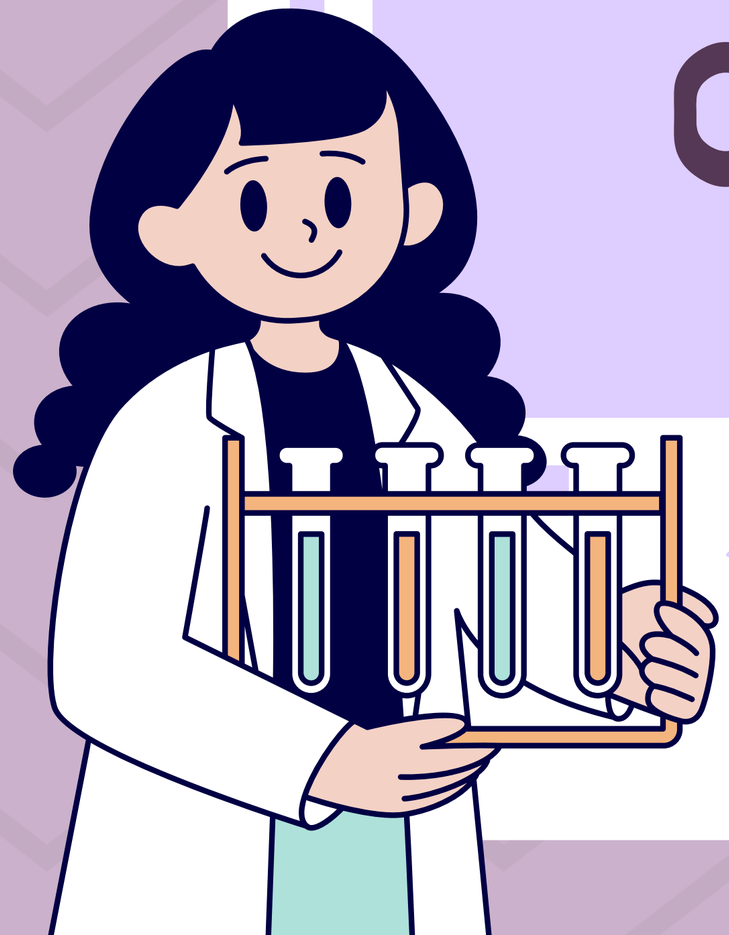
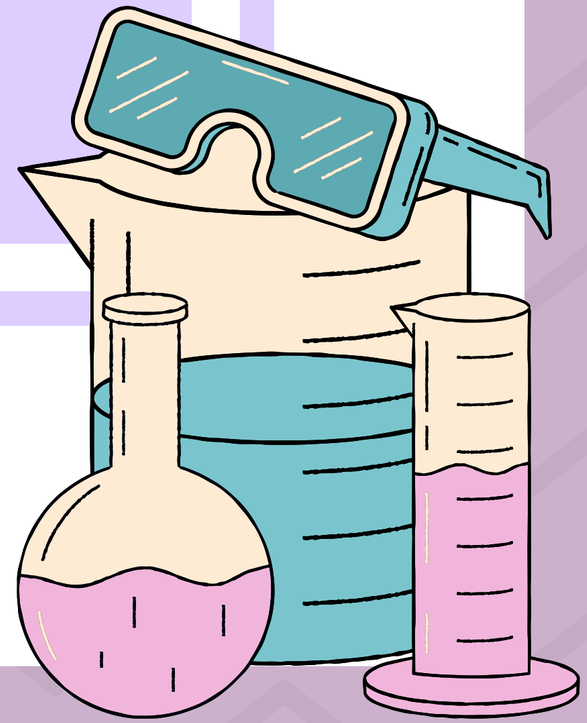
DIPLOMA IN BIOTECHNOLOGY
POLITEKNIK NILAI

PREPARATION OF CHEMICAL BUFFER

POCKETBOOK EDITION





MUNIRAH BINTI MIHAT

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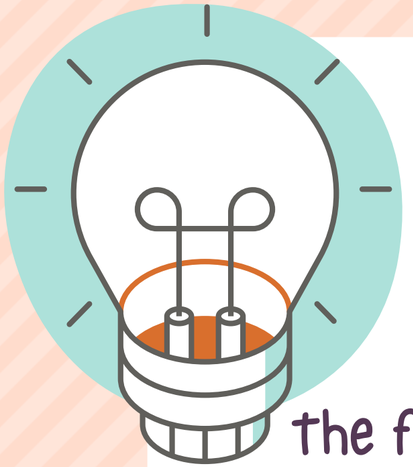
PREFACE

As a science student, you are required to prepare and mix your own chemical solutions and buffers for your experiment. This book provides the general guidelines on buffer preparation as well as the equations needed to calculate the chemicals needed for your buffer preparation. This book specifies on how to calculate the amount of chemicals needed based on molarity, percentage, and stock.

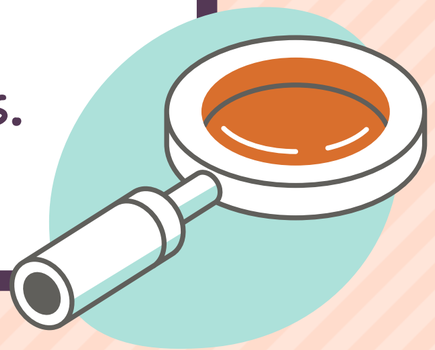
This book also includes practice problems with the answers showing how the calculations are made. The steps for mixing the chemicals are also given with the main points that should be taken when calculating the amount of chemicals for the buffers.

You may refer to **Prep of Solutions** by Fauziah Shaheen as an extra guideline.



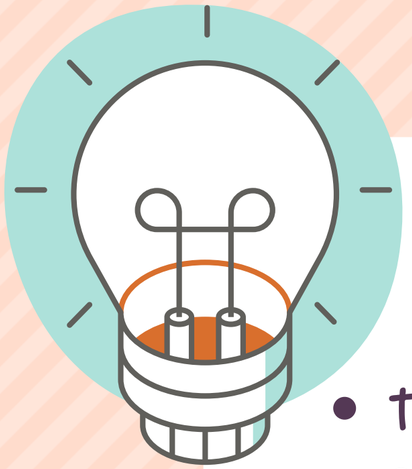
INTRODUCTION

the foundation of any science experiment is reproducible and consistent outcomes, which depend on accurate preparation and appropriate buffer selection. Students must be able to combine chemicals into solutions properly in order to carry out a good laboratory experiment. Thus, this book will serve as a reference on how to properly and successfully mix your solutions so that you may concentrate more on your experiment and less on deciding which chemicals to combine and in what proportions.



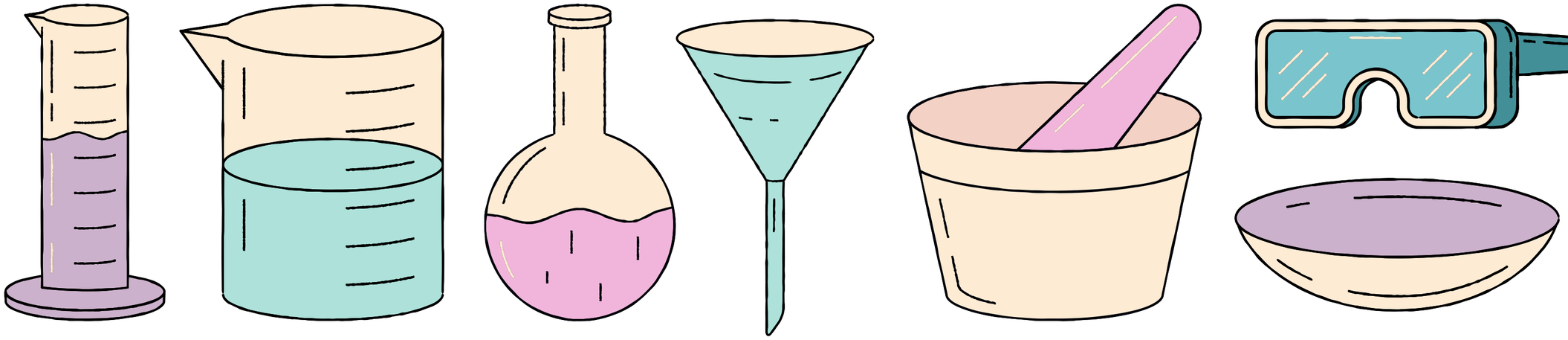
General rules for mixing chemical solutions

- When chemicals measuring is required, avoid returning chemicals to their original packaging to avoid cross contamination.
- Keep your chemical containers closed while mixing your buffer. Dust and vapour may escape from an open container, while gases and suspended materials may penetrate, changing the chemical's nature.
- Never place a spatula, a stirrer, or any other items inside a chemical storage container. Spatulas can be used in laboratory reagent containers, but must be cleaned before using, and avoid using the same spatula for measuring different chemicals.



General rules for mixing chemical solutions

- To avoid label destruction from spills, the label should always be facing upward when pouring from bottles.
- When handling concentrated acids, extra care must be exercised. Acids should be diluted by adding them to water while swirling continually. Add acid into water and not water to acid.
- Always use correct protective equipment when handling any chemicals such as gloves, goggles, etc.
- Label your container properly; include the buffer's name, pH and concentration, and the date of production (to ensure buffer expiry date).



SOLUTION - homogeneous mixture, which may be liquid, gas or solid, formed by dissolving one or more substances

BUFFER - a solution that can resist pH change upon the addition of an acidic or basic component. It is able to neutralize small amount of added acid or base, thus maintaining the relatively stable pH of the solution.

BUFFER VS SOLUTION

BASIC CONCEPTS

MOLARITY

Molar concentration is a unit of measurement for the concentration of a chemical species, specifically a solute, in a solution, expressed as the amount of substance per unit volume of solution.

PERCENTAGE WEIGHT/VOLUME (W/V)

Solutions with w/v measurements often consist of a solid chemical dissolved in a liquid solvent, such as water.

PERCENTAGE VOLUME/VOLUME (V/V)

Based on v/v measurements, solutions are liquid diluted into a liquid.

STOCK

A stock solution is made by weighing a suitable portion of a pure solid or measuring a suitable volume of a pure liquid, placing it in a suitable flask, and diluting to a known volume.

THE EQUATIONS

MOLARITY

No of moles = $\frac{\text{mass (g)}}{\text{molar mass (g/mol)}}$ ------Equation 1

No of moles = concentration(M) x volume (L)
------Equation 2

Combine equation 1 and 2;

$\frac{\text{mass (g)}}{\text{molar mass}}$ = concentration (M) x volume (L)

Only use if chemicals are in **solid form**

THE EQUATIONS

PERCENTAGE

VOLUME/VOLUME (V/V)

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

Only use if chemicals are in **liquid form**

THE EQUATIONS

PERCENTAGE

WEIGHT/VOLUME (W/V)

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

Only use if chemicals are in **solid form**

THE EQUATIONS

STOCK

use this when you have a stock with higher Molarity, and you want to make another buffer with smaller Molarity

$$M1\ V1 = M2\ V2$$

M1= initial Molarity (M)
V1= initial Volume (mL)
M2= final Molarity (M)
V2 = final volume (mL)

BUFFER WITH VARIOUS MOLARITY

Let's make general lysis Buffer, a buffer with all solid chemicals

Use the Molarity equation ;

Let's say we are making 500mL buffer

Chemical	Concentration	Molar mass
Tris-HCl	50mM	157.60
NaCl	100mM	58.44
DTT	5mM	154.25
EDTA	1mM	292.24

GET THIS FROM YOUR CHEMICAL BOTTLE

BUFFER WITH VARIOUS MOLARITY

Volume= 500mL

TRIS-HCL

mass (g) = concentration (M) x volume (L)
molar mass

mass (g) = 0.05M x 0.5 L
157.60

mass (g) = 3.94g

Volume= 500mL

NaCl

mass (g) = concentration (M) x volume (L)
molar mass

mass (g) = 0.1M x 0.5 L
58.44

mass (g) = 2.922

BUFFER WITH VARIOUS MOLARITY

Volume= 500mL

DTT

mass (g) = concentration (M) x volume (L)
molar mass

mass (g) = 0.005M x 0.5 L
154.25

mass (g) = 0.385g

Volume= 500mL

EDTA

mass (g) = concentration (M) x volume (L)
molar mass

mass (g) = 0.001M x 0.5 L
292.24

mass (g) = 0.146g

Mixing up the buffer (lysis buffer)

step 1

Measure 3.94g of Tris-HCl

step 2

Measure 2.922g of NaCL

step 3

Measure 0.385g of DTT

step 4

Measure 0.146g of EDTA

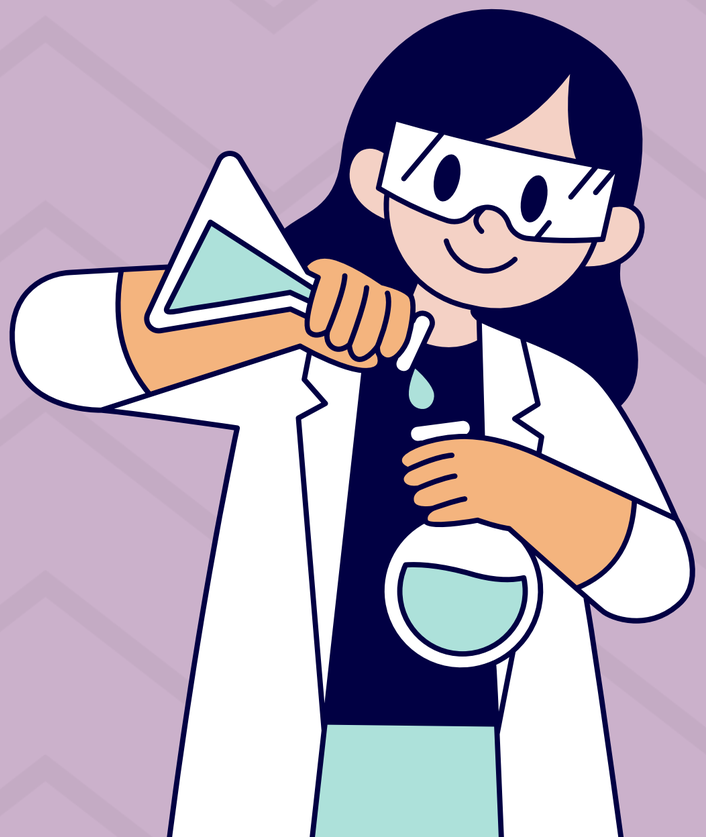
step 5

Put all chemicals in a beaker and dissolve them in a 300mL ddH20

step 6

After all chemical have dissolved, top up the buffer with ddH20 until 500 mL

MIXING UP THE BUFFER



TAKE NOTE;

- measure out all the chemicals needed
- make sure you clean your spatula in between the different chemicals, or use different spatula
- use separate weighing boat, and do not put the extra chemicals back to the container
- take moderate amount of chemicals. DO NOT WASTE.
- dissolved all chemicals before making up the correct volume needed for the buffer

DO YOU GET IT?

TEST YOUR UNDERSTANDING

PRACTICE PROBLEM

Calculate the corresponding mass of chemicals for this PBS buffer (2.5L)

Chemicals	Concentration	molar mass
NaCl	137mM	58.44
KCl	2.7mM	74.55
Na2HPO4	10mM	141.96
KH2PO4	1.8mM	136.09

DO YOU GET IT?

NaCl

Volume= 2.5L

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{58.44} = 0.137\text{M} \times 2.5\ \text{L}$$

mass (g) = 20.015g

DO YOU GET IT?

KCl

Volume= 2.5L

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{74.55} = 0.0027\text{M} \times 2.5\ \text{L}$$

$$\text{mass (g)} = 0.503\text{g}$$

DO YOU GET IT?

Na_2HPO_4

Volume= 2.5L

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{141.96} = 0.01M \times 2.5\ L$$

mass (g) = 3.549g

DO YOU GET IT?

KH_2PO_4

Volume= 2.5L

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{136.09} = 0.0018\text{M} \times 2.5\text{ L}$$

$$\text{mass (g)} = 0.612\text{g}$$

BUFFER WITH VARIOUS PERCENTAGE

MONOMER STOCK SOLUTION

Identify the state of your chemicals.

In this situation, all chemicals used will be in solid form; hence use the equation percentage w/v

Let's make a 500mL buffer

CHEMICALS	CONCENTRATION
Acrylamide	30%
N,N'-methylenebisacrylamide	0.8%

BUFFER WITH VARIOUS PERCENTAGE

Volume= 500mL

Acrylamide

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$30\ \% = \frac{\text{weight of solute (g)}}{500\text{mL}} \times 100\%$$

weight (g) = 150g

BUFFER WITH VARIOUS PERCENTAGE

Volume= 500mL

N,N'-methylenebisacrylamide

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$0.8\ \% = \frac{\text{weight of solute (g)}}{500\text{mL}} \times 100\%$$

weight (g) = 4g

BUFFER WITH VARIOUS PERCENTAGE

FIXING SOLUTION

Identify the state of your chemicals.

In this situation, all chemicals used will be in liquid form; hence, use the equation percentage v/v

Let's make a 500mL buffer

Chemical	Concentration
Methanol	50%
Glacial Acetic acid	10%

BUFFER WITH VARIOUS PERCENTAGE

Volume=
500mL

Methanol

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

$$50\% = \frac{\text{volume of solute (mL)}}{500\text{mL}} \times 100\%$$

volume (mL) = 250mL

BUFFER WITH VARIOUS PERCENTAGE

Volume=
500mL

Glacial Acetic Acid

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

$$10\% = \frac{\text{volume of solute (mL)}}{500\text{mL}} \times 100\%$$

volume (mL) = 50mL

DO YOU GET IT?

TEST YOUR UNDERSTANDING

PRACTICE PROBLEM

Calculate the corresponding mass of chemicals for this DNA Loading buffer(100mL)

Chemicals	Concentration
Glycerol (l)	30%
Bromophenol blue (s)	0.25%
Xylene Cyanol FF(s)	0.25%

DO YOU GET IT?

Glycerol

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

$$30\% = \frac{\text{volume of solute (mL)}}{100\text{mL}} \times 100\%$$

volume (mL) = 30mL

Volume= 100mL

DO YOU GET IT?

Bromophenol blue (w/v)

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$0.25\% = \frac{\text{weight of solute (g)}}{100\text{mL}} \times 100\%$$

mass (g) = 0.25

Volume= 100mL

Xylene Cyanol FF (w/v)

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$0.25\% = \frac{\text{weight of solute (g)}}{100\text{mL}} \times 100\%$$

mass (g) = 0.25

BUFFER WITH VARIOUS PERCENTAGE AND MOLARITY

1. Identify the state of your chemicals- solid or liquid
2. Identify the concentration- Molarity or percentage
3. Identify which formula to be used- Molarity; w/v; v/v
4. Calculate the amount of chemicals needed (in g or mL) respectively

BUFFER WITH VARIOUS PERCENTAGE AND MOLARITY

Let's make LAEMLI BUFFER (1L)

CHEMICALS	CONCENTRATION	MOLAR MASS
trisbase	250mM	121.14
Glycine	1.92M	75.06
SDS	1%	

BUFFER WITH VARIOUS PERCENTAGE AND MOLARITY

trisbase

Volume=
1L

mass (g) = concentration (M) x volume (L)
molar mass

mass (g) = 0.25M x 1L
121.14

mass (g) = 30.285g

BUFFER WITH VARIOUS PERCENTAGE AND MOLARITY

Glycine

Volume= 1L

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{75.06} = 1.92\text{M} \times 1\text{L}$$

mass (g) = 144.115g

BUFFER WITH VARIOUS PERCENTAGE AND MOLARITY

Volume=
1L

SDS

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$1\% = \frac{\text{weight of solute (g)}}{1000\text{mL}} \times 100\%$$

weight (g) = 10g

Mixing up the buffer (Leamli buffer)

step 1

Measure 30.285g of Trisbase

step 2

Measure 144.115g of Glycine

step 3

Measure 10g of DTT

step 4

Put all chemicals in a beaker and dissolve them in a 800mL ddH20

step 5

After all chemicals have dissolved, top up the buffer with ddH20 until 1L

BUFFER WITH VARIOUS PERCENTAGE, MOLARITY AND STOCK

SDS LOADING BUFFER

- Identify the state of your chemicals- solid, liquid, or stock
- Identify the concentration- Molarity or percentage
- Identify which formula to be used- Molarity; w/v; v/v, or m1v1
- Calculate the amount of chemicals needed (in g or mL) respectively

CHEMICALS	CONCENTRATION	MOLAR MASS/STOCK
SDS	10%	
Glycerol	20%	
Tris-HCL	0.2M	stock (1M)
DTT	10mM	154.253

Volume= 50mL

BUFFER WITH VARIOUS PERCENTAGE, MOLARITY AND STOCK

SDS

$$\text{Percentage \%} = \frac{\text{weight of solute (g)}}{\text{volume of solution (mL)}} \times 100\%$$

$$10\% = \frac{\text{weight of solute (g)}}{50\text{mL}} \times 100\%$$

weight (g) = 5g

Volume= 50mL

BUFFER WITH VARIOUS PERCENTAGE, MOLARITY AND STOCK

GLYCEROL

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

$$20\% = \frac{\text{volume of solute (mL)}}{50\text{mL}} \times 100\%$$

volume (mL) = 10mL

Volume= 50mL

BUFFER WITH VARIOUS PERCENTAGE, MOLARITY AND STOCK

TRIS-HCL

M1 V1= M2 V2

M1= initial Molarity (M)
V1= initial Volume (mL)
M2= final Molarity (M)
V2 = final volume (mL)

1M x (V1) = 0.2M x 50mL
V1= 10mL

Volume= 50mL

BUFFER WITH VARIOUS PERCENTAGE, MOLARITY AND STOCK

DTT

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{154.253} = 0.01\text{M} \times 0.05\ \text{L}$$

$$\text{mass (g)} = 0.077\text{g}$$

Volume=
50mL

Mixing up the buffer (Leamli buffer)

step 1

Measure out all solids (DTT and SDS

step 2

Measure out all liquids (Tris-HCl and Glycerol

step 3

Put all chemicals in a beaker and dissolve them in a 40mL ddH20

step 4

After all chemicals have dissolved, top up the buffer with ddH20 until 50mL

DO YOU GET IT?

TEST YOUR UNDERSTANDING

Calculate the corresponding mass of chemicals for this Extraction buffer (50mL)

Chemicals	Concentration	Molar mass/stock
Tris-HCl	200mM	Stock (2M)
EDTA	25mM	292.24
NaCl	250mM	58.44
Tween 80	0.5%	

DO YOU GET IT?

TRIS-HCL

M1 V1= M2 V2

M1= initial Molarity (M)
V1= initial Volume (mL)
M2= final Molarity (M)
V2 = final volume (mL)

2M x (V1) = 0.2M x 50mL
V1= 5mL

Volume= 50mL

DO YOU GET IT?

EDTA

$$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$$

$$\frac{\text{mass (g)}}{292.24} = 0.025\text{M} \times 0.05\ \text{L}$$

$$\text{mass (g)} = 0.365$$

Volume= 50mL

DO YOU GET IT?

NACL

$\frac{\text{mass (g)}}{\text{molar mass}} = \text{concentration (M)} \times \text{volume (L)}$

$\frac{\text{mass (g)}}{58.44} = 0.25\text{M} \times 0.05\ \text{L}$

mass (g) = 0.731g

Volume= 50mL

DO YOU GET IT?

TWEEN 80

$$\text{Percentage \%} = \frac{\text{volume of solute (mL)}}{\text{volume of solution (mL)}} \times 100\%$$

$$0.5\% = \frac{\text{volume of solute (mL)}}{50\text{mL}} \times 100\%$$

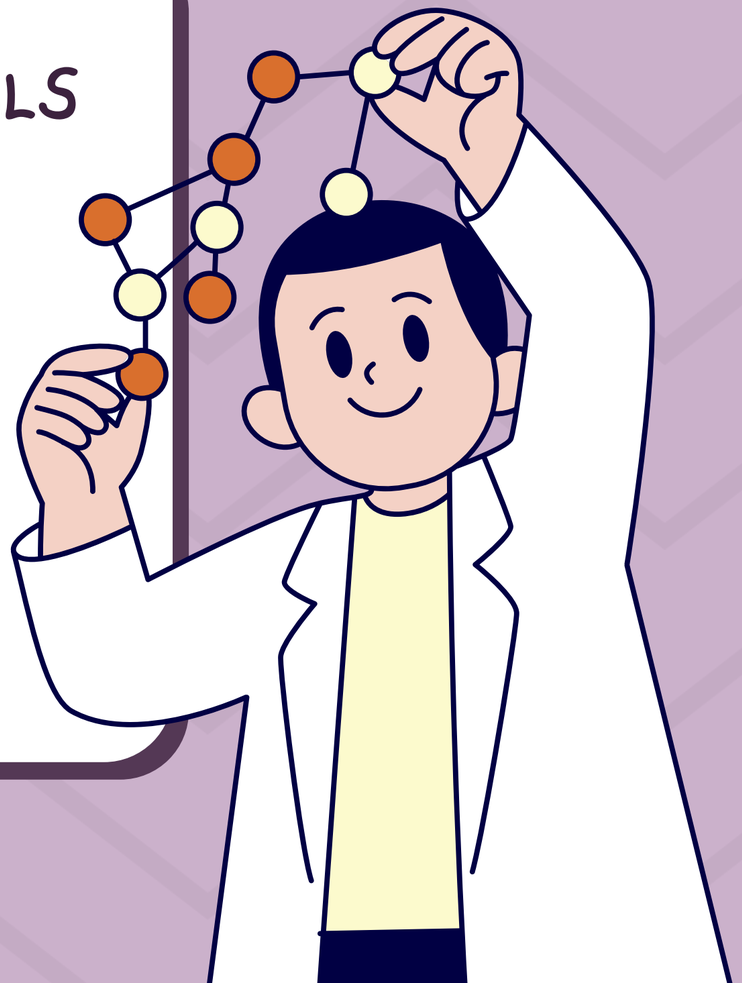
volume (mL) = 0.25mL

Volume= 50mL

CONCLUSION

STEPS FOR CALCULATING YOUR CHEMICALS

- Identify the origin of your chemicals- solid, liquid, or stock
- Identify the state of chemicals- solid, or liquid
- Identify the concentration- Molarity or percentage
- Identify which formula to be used- Molarity; w/v; v/v, or m1v1
- Calculate the amount of chemicals needed (in g or mL) respectively



This e-book is designed as a supplementary material for Diploma in Biotechnology.
This e-book may be useful for any science-based subject.

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