Preparation of Laboratory Solutions And Reagents

By
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The mind is everything. What you think you become.

Science is fun if you want and make it fun!!!

Accuracy versus. Precision ???

- ***Accuracy*** means getting a result that is close to the real answer. **(صحت)**

- ***Precision*** means getting a similar result every time you try. **(دقت)**

Hazard Symbols

Topics

1. *What is the importance of calculations in bioscience?*
2. *What is solution?*
3. *Where do solution recipes come from?*
4. *Concentration of solute: calculations*
5. *Preparing solutions &Making diluted solutions from concentrated ones*
6. *Enzyme units.*

2. What is a solution?

A solution is a homogeneous mixture
The components of a solution are:
solute: substance (or substances) present in lesser amount
being dissolved
solvent: substance present in greater amount doing the dissolving

3. Where Do Solution "Recipes" Come From?

- Original Scientific Literature
- Lab manuals (instructional)
- Manufacturers and suppliers
- Internet

Terminology

Definitions:

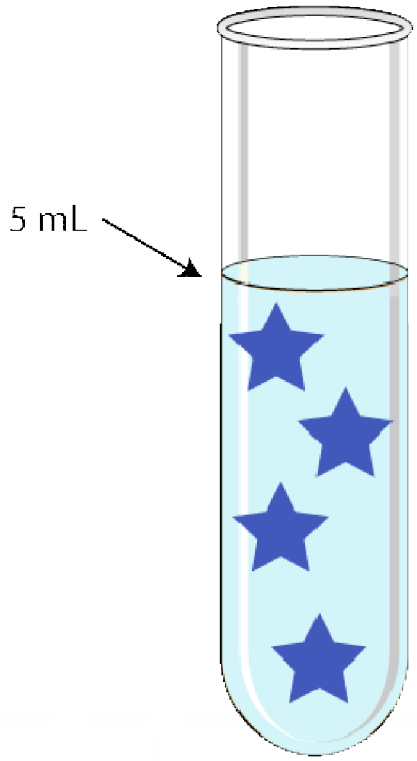
- **Solutes** -- substances that are dissolved
- **Solvents** -- substance in which solutes are dissolved (usually water)
- **Amount** -- how much

Question??

Each star represents 1 mg of NaCl.

What is the total amount of NaCl in the tube? _____

What is the concentration of NaCl in the tube (in mg/mL)? _____



Ways To Express Concentration *of Solute*

1. ***Weight Per Volume*** *(gr/ L)*
2. ***Molarity***
3. ***Normality***
4. ***Percents (Three Kinds)***
5. ***PARTS***

More Concentration Expressions

Amounts of solutes as "parts"

a. Parts per Million (ppm)
b. Parts per Billion (ppb)

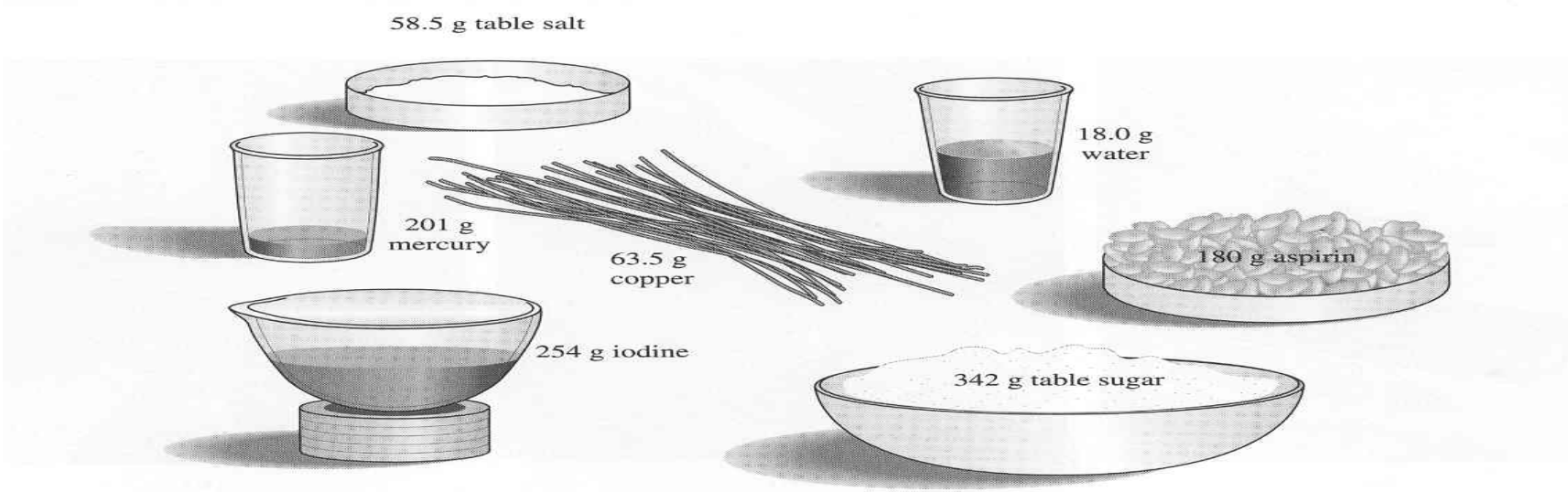
2. Molarity

- ***Molarity*** *is: number of moles of a solute that are dissolved per liter of total solution.*

- ***A 1 M solution contains 1 mole of solute per liter total volume.***

Mole

- How much is a mole?



From *Basic Laboratory Methods for Biotechnology: Textbook and Laboratory Reference,* Seidman and Moore, 2000

Example: Sulfuric Acid

For a particular compound, add the atomic weights of the atoms that compose the compound.

H_2SO_4:

2 hydrogen atoms	*2 X 1.00 g =*	*2.00 g*
1 sulfur atom	*1 X 32.06 g =*	*32.06 g*
4 oxygen atoms	*4 X 16.00 g =*	*64.00 g*
		98.06 g

Example Continued

- A 1M solution of sulfuric acid contains 98.06 g of sulfuric acid in 1 liter of total solution.
- *"Mole" is an expression of amount*
- *"Molarity" is an expression of concentration*.

Definitions

- "Millimolar", mM, millimole/L.
  – A millimole is *1/1000 of a mole*.

- "Micromolar", µM, µmole/L.
  – A µmole is *1/1,000,000 of a mole.*

Formula

How much solute is needed for a solution of a particular molarity and volume?

$$\frac{(g\ solute)}{1\ mole} \times \frac{(mole)}{L} \times (L) = g\ solute\ needed$$

or

FW X molarity x volume = g solute needed

Question???

How much solute is required to make 300 mL of 0.8 M $CaCl_2$?

To Make Solution Of Given Molarity And Volume

1. Find the FW of the solute, usually from **label**.

2. Determine the **molarity** desired.

3. Determine the **volume** desired.

4. Determine **how much** solute is necessary by using the formula.

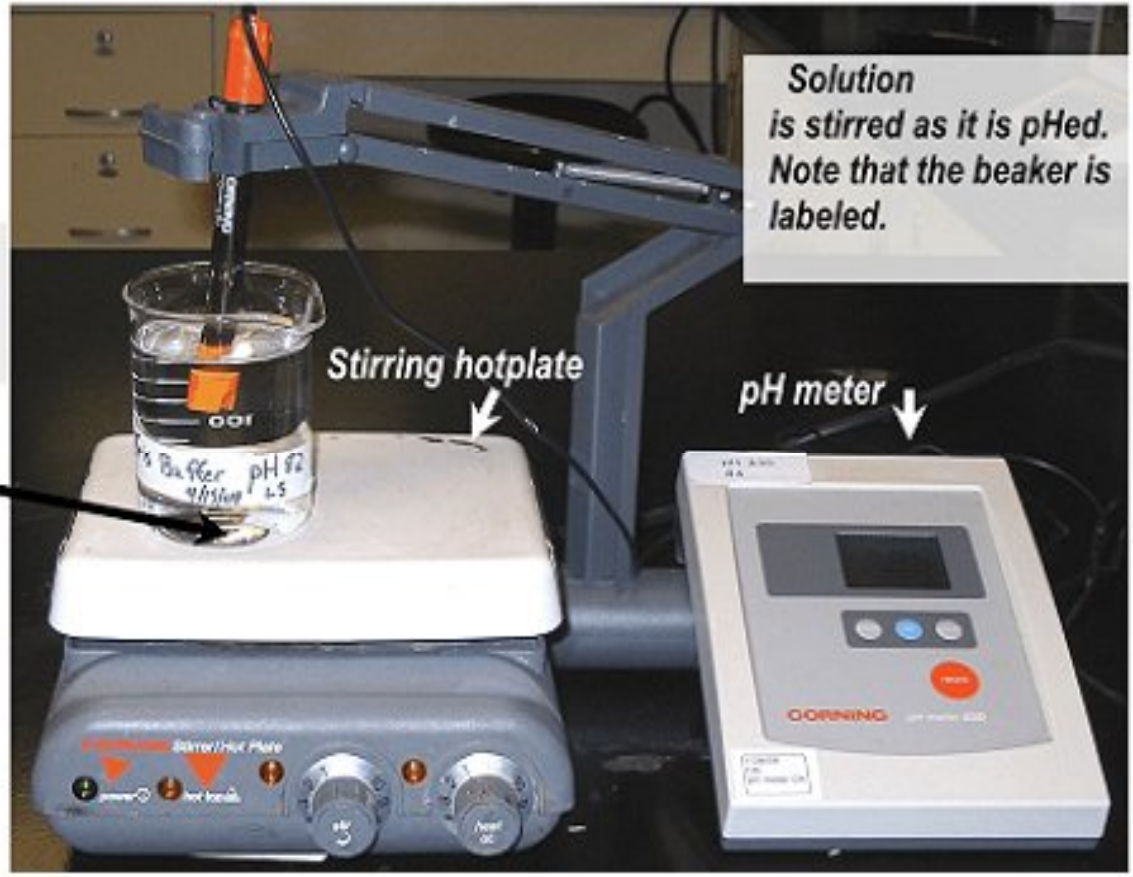
Procedure Cont.

5. Weigh out the amount of solute.

6. Dissolve the solute in ***less than*** the desired final volume of solvent.

7. Place the solution in a volumetric flask or graduated cylinder. Add solvent until exactly the required volume is reached, *Bring To Volume, BTV*.

ADJUSTING THE pH OF A SOLUTION.



BE CAREFUL NOT TO CRASH THE ELECTRODE WITH THE STIR BAR.

Relationship between Molarity and normality???

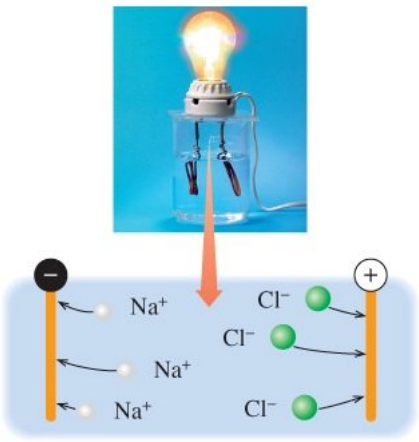
$$N = n\,M$$

(What is Equivalent and mEq/l)???

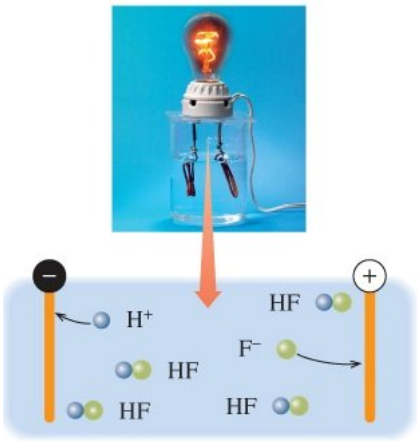
Solutes and Ionic Charge

In water,

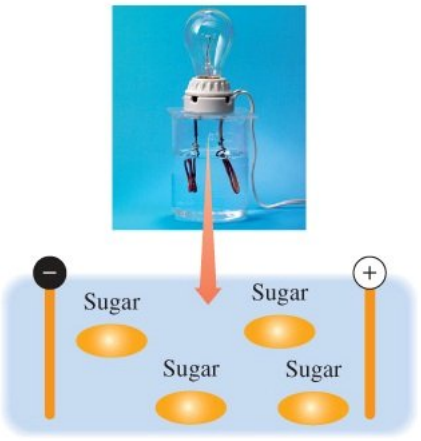
- **strong electrolytes** produce ions and conduct an electric current.
- **weak electrolytes** produce a few ions.
- **nonelectrolytes** do not produce ions.



Strong electrolyte



Weak electrolyte



Nonelectrolyte

Pre al solutions

By: Dr. Mehdi Imani

Equivalents

An equivalent (Eq) is the amount of an electrolyte or an ion that provides 1 mole of electrical charge (+ or -).

1 mole of Na^+ = 1 equivalent

1 mole of Cl^- = 1 equivalent

1 mole of Ca^{2+} = 2 equivalents

1 mole of Fe^{3+} = 3 equivalents

Electrolytes in Body Fluids

TABLE 7.5 Some Typical Concentrations of Electrolytes in Blood Plasma

Electrolyte	Concentration (mEq/L)
Cations	
Na^+	138
K^+	5
Mg^{2+}	3
Ca^{2+}	4
Total	150
Anions	
Cl^-	110
HCO_3^-	30
HPO_4^{2-}	4
Proteins	6
Total	150

Ringer's Solution

In replacement solutions for body fluids, the electrolytes are given in milliequivalents per liter (mEq/L).

Na^+	147 mEq/L	Cl^-	155 mEq/L
K^+	4 mEq/L		
Ca^{2+}	4 mEq/L		

The milliequivalents per liter of cations must equal the milliequivalents per liter of anions.

Learning Check

A. In 1 mole of Fe^{3+}, there are

1) 1 Eq. 2) 2 Eq. 3) 3 Eq.

B. In 2.5 moles of SO_4^{2-}, there are

1) 2.5 Eq. 2) 5.0 Eq. 3) 1.0 Eq.

C. An IV bottle contains NaCl. If the Na^+ is 34 mEq/L, the Cl^- is

1) 34 mEq/L. 2) 0 mEq/L. 3) 68 mEq/L.

What about protein concentration in cells or plasma?!!!

The reference range for *albumin concentrations in serum* is approximately 35 - 50 g/L (3.5 - 5.0 g/dL).

Mw: 67 kDa
Calculate its molarity ???!!!

4. Percents

4.1 Weight/volume %
4.2 Volume / Volume %
4.3 Weight / Weight %

Questions?

1. **How many grams of dextrose are required to prepare 4000 mL of a 5% solution?**

2. **What is the percentage strength (w/v) of a solution of urea, if 80 mL contain 12 g?**

✓**2 mg/mL proteinase K**
Means 2 mg of proteinase K in each mL of solution.

1. **How much proteinase K is required to make 50 mL of solution at a concentration of 2 mg/mL?**

5. Concentration Expressions (Most Common)

- **PARTS** (Common in environmental sciences, for example)

Amounts of solutes expressed as "parts"

a. Parts per Million (ppm)
b. Parts per Billion (ppb)
c. Might see parts per Thousand (ppt)
d. Percents are same category (pph %)

ppm and ppb

- **ppm:** The number of parts of solute per 1 million parts of total solution.
- **ppb:** The number of parts of solute per billion parts of solution.

5 ppm chlorine = 5 g of chlorine in 1 million g of solution,

Conversions

To convert ppm or ppb to simple weight per volume expressions:

5 ppm chlorine = $\frac{\text{5 g chlorine}}{10^6 \text{ g water}}$ = $\frac{\text{5 g chlorine}}{10^6 \text{ mL water}}$

= **5 mg/1 L water**

= 5 X 10^{-6} g chlorine/ 1 mL water

= **5 micrograms/mL**

1 ppm in water = $\frac{\text{1 microgram}}{\text{ml}}$

Preparing Dilute Solutions From Concentrated Ones

- Concentrated solution = ***stock*** solution
- Use this equation to decide how much stock solution you will need: $C_1V_1=C_2V_2$
  - C_1 = concentration of stock solution
  - C_2 = concentration you want your dilute solution to be
  - V_1 = how much stock solution you will need
  - V_2 = how much of the dilute solution you want to make

How would you prepare 1000 mL of a 1 M solution of Tris buffer from a 3 M stock of Tris buffer?

Bacterial Growth Media (LB)

نام ماده	٪	مقدار (گرم)
تریپتون	1%	10
عصاره مخمر	0.5%	5
NaCl	1%	10

اجزای محیط کشت LB

Antibiotics Calculation For Growth Media

نام آنتی بیوتیک	غلظت محلول استوک	غلظت نهایی مورد استفاده در محیط کشتLB
آمپی-سیلین	100 mg/ml DDW	100 µg/ml
کاناماپسین	100 mg/ml DDW	25 µg/ml
شرایط نگهداری : $-۲۰C^{o}$		

Osmosis???

1. Normal human reference range of osmolality in plasma is **about 285-295** milli-osmoles per kilogram

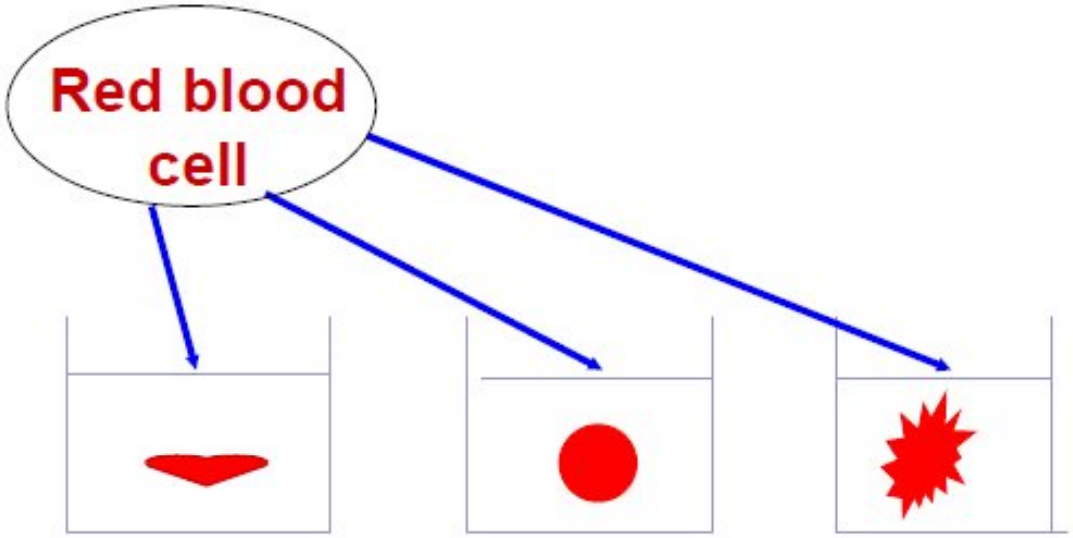
2. Plasma osmolarity of some reptiles, especial those from a freshwater aquatic environment may be lower than that of mammals (e.g. < 260 mOsm/L) during favourable conditions. Consequently, solutions osmotically balanced for mammals (e.g., 0.9% normal saline) are likely to be mildly hypertonic for such animals

3. **Calculated osmolarity = 2 Na + Glucose + Urea (all in mmol/L).**

- The term **Isotonic** should be restricted to solutions having equal osmotic pressures which respect to a particular membrane (Husa)

- **Isotonicity value**…the concentration of an aqueous NaCl soln. having the same colligative properties as soln. (Goyan & Reck)

RBC in solutions with different tonicity



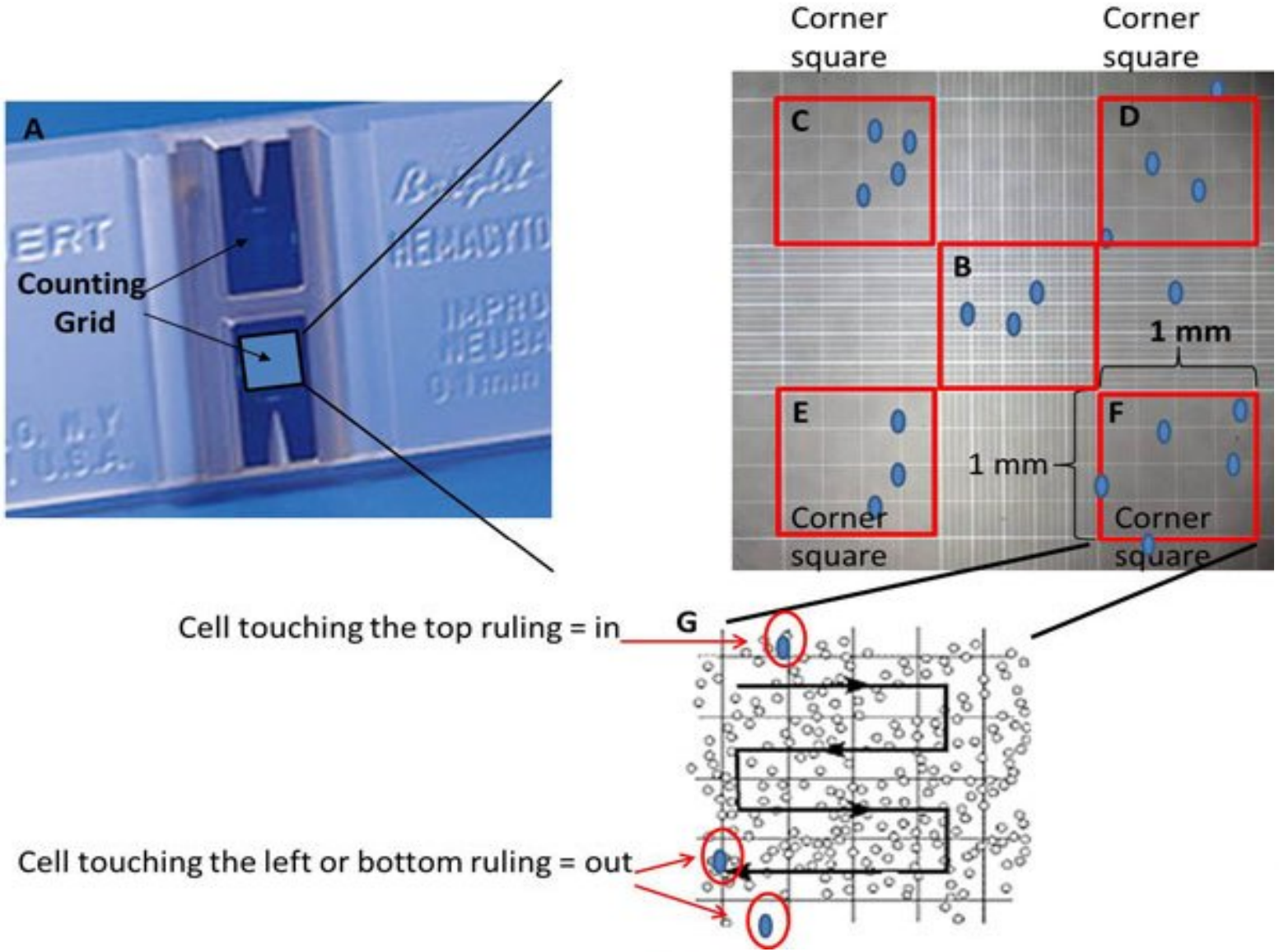
NaCl solution

2.0 % Hypertonic, Shrink

0.9 % Isotonic

0.2 % Hypotonic, Hemolysis

Cell culture calculations


A
Counting
Grid
Corner
square
Corner
square
C
D
B
1 mm
E
F
1 mm
Corner
square
Corner
square
Cell touching the top ruling = in
G
Cell touching the left or bottom ruling = out

Transfer 200 µl of the cell suspension into a 1.5 ml microfuge tube.
• Add 300 µl of PBS and 500 µl of 0.4% trypan blue solution to the cell suspension (creating a dilution factor of 5) in the centrifuge tube

1. Each square of the hemacytometer (with cover slip in place) represents a total volume **of 0.1 mm3 or 10-4 cm3**. Since 1 cm3 is equivalent to 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations.
2. Cells per ml = the average count per square x the dilution factor x 104 (count 10 squares)
3. Example: If the average count per square is **45 cells** x **5** x104 = 2,250,000 or **2.25 x 106 cells/ml.**
4. Total cell number = cells per ml x the original volume of fluid from which cell sample was removed.
5. Example: 2.25 x 106 (cell per ml) x 10 ml (original volume) = 2.25 x 107 total cells

6. **Question**? What would you do If you want to prepare 10 ml cell with the concentration of 1x104?

Working with Proteins

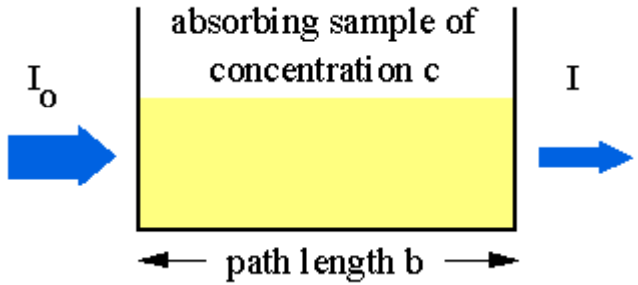
1. Cell lysates for immunobloting

2. Absorbance at 280 nm
   OD280 = 1 equals 1mg/ml

3. Colorimeteric assays

Absorbance at 280 nm

Beer-Lambert law (or Beer's law)



$$- \log(I / I_o) = A = \varepsilon * b * c$$

$$C = A/\varepsilon b \text{ (Molar)}$$

سوال

ضریب خاموشی مولی استیل کولین استراز 5.27x 10^5 می باشد جذب A280 محلولی از این پروتین در کوتی به طول 1 cm معادل 0.22 می باشد غلظت مولی پروتئین چقدر می باشد؟؟

Working with DNA

1. Measure DNA concentration using the **NanoDrop** program. Record the absorbance values at 260 nm and 280 nm.
2. Using the relationship OD260 nm **1 = 50 ng/ul DNA**, **or ug/ml** calculate the concentration and total yield.
3. For **RNA OD 1 = 40 ug/ml**

4. Evaluate the ratio A260/A280. A value of greater than 1.7 indicates essentially pure DNA, while lower values indicate protein contamination.

Estimation DNA concentration by spectrophotometer

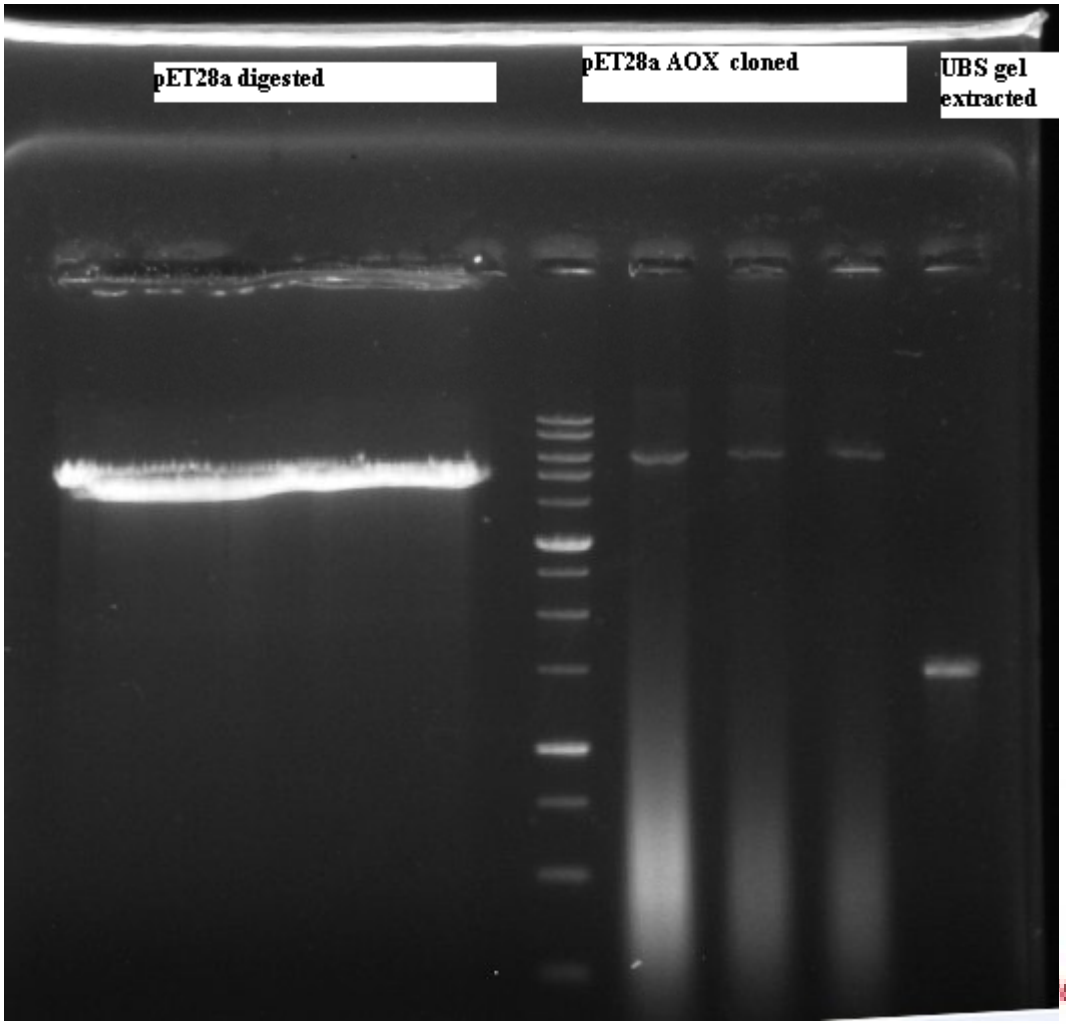
1. Using the relationship OD260 nm **1 = 50 ng/µl DNA**, **or µg/ml** calculate the concentration and total yield.

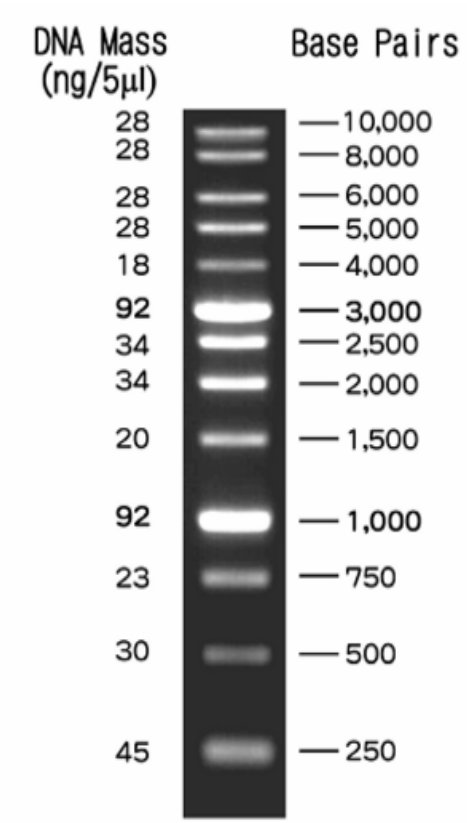
2. For **RNA OD 1 = 40 µg/ml**


Thermo
SCIENTIFIC
NANODROP 2000c
Thermo
SCIENTIFIC
NANODROP 3300


NanoDrop ND-1000 UV/Vis Spectrophotometer
NanoDrop®
ND-1000 Spectrophotometer

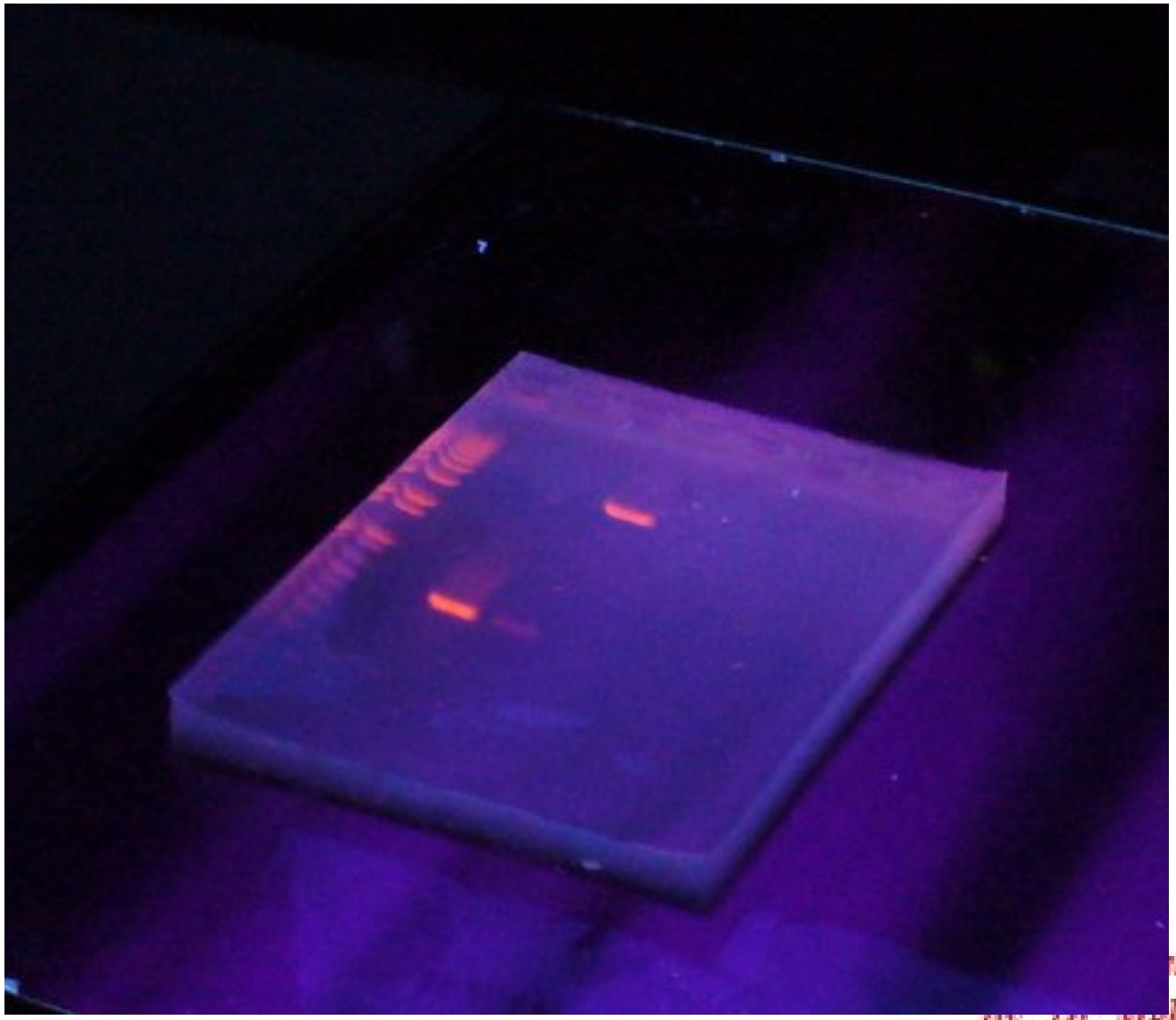
Estimation DNA concentration using gel electrophoresis





1 % TAE agarose gel

aration of biochemical solutions
By: Dr. Mehdi Imani



mical solutions

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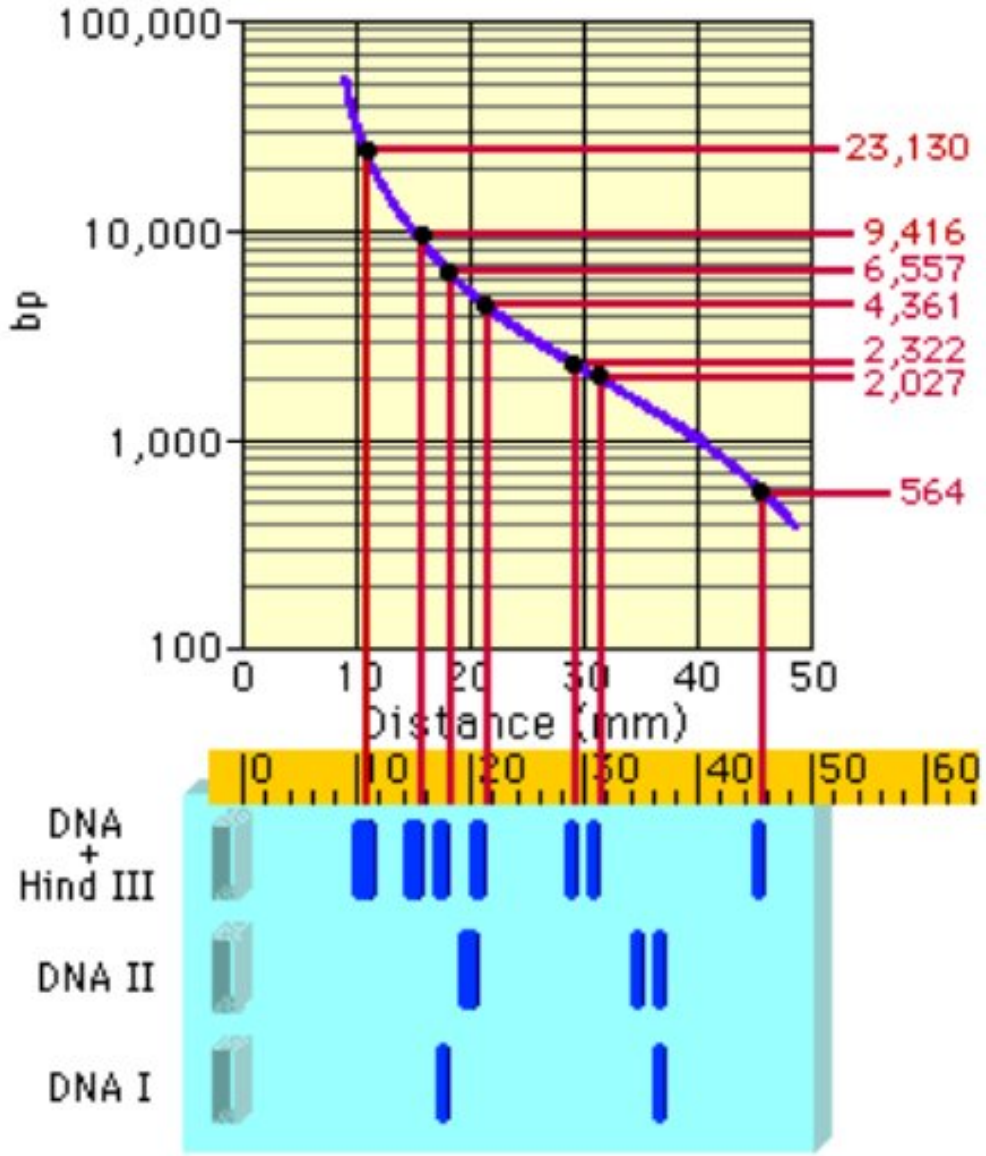
Standard curve for DNA Size determination



Enzyme units in PCR Workstations!!!

Component	25 µl reaction	50 µl reaction	Final Concentration
10X Standard *Taq* Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
Taq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR
Nuclease-free water	to 25 µl	to 50 µl	

How to prepare detergents percents

Type	Chemicals
ionic	sodium dodecyl sulfate (SDS), deoxycholate, cholate
non-ionic	triton X-100, DDM, digitonin, tween 20, tween 80
zwitterionic	CHAPS
chaotropes	urea

Some Useful Links For Concentration Calculations

Estrogens

Estrogens direct the development of the female phenotype in embryogenesis and during puberty by regulating gene transcription and, thus, protein synthesis. It also induces the production of gonadotropins which, in turn, induce ovulation. Exposure to estradiol increases breast cancer incidence and proliferation.

β-Estradiol
C18H24O2. *Molecular Weight* 272.38
Desired []: 0.1-1 nM
How much Estradiol you need???

http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/mass-molarity-calculator.html

Chemicals – Technical Library

Mass Molarity Calculator

Calculate Mass Required for Molar Solution

Formula weight: 58.44 g/mol

Desired final volume: 100 milliliters (mL)

Desired concentration: 155 millimolar (mM)

Mass = 0.9058200000000001 g

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The mass molarity calculator tool calculates the mass of compound required to achieve a specific molar concentration and volume. To dilute a solution of known molarity, please use the Solution Dilution Calculator. To dilute a solution of concentrated acid or base of known w/w% strength, please use the Acid & Base Molarity Calculator.

Acid & Base Molarity Calculator | Mass Molarity Calculator | Solution Dilution Calculator

http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/molarity-calculator.html

Chemicals - Technical Library

Normality & Molarity Calculator

Acid and Base Solution Preparation

Select acid or base: Hydrochloric Acid HCl

Density: 1.2 g/mL

Formula weight: 36.46 g/mol

Weight percentage: 37.0 % w/w

Desired final volume: 100 mL

Desired concentration: 1 Molar

Results based on your selection:

Your stock solution of **Hydrochloric Acid** is calculated to be **12.178 M** based on a density of **1.2** g/mL, a formula weight of **36.46** g/mol, and a concentration of **37%** w/w.

To make a **1 M** solution, slowly add **8.212** mL of your stock solution to **25** mL deionized water.
Adjust the final volume of solution to **100** mL with deionized water.

View Hydrochloric Acid (CAS# 7647-01-0) Products

Reset | Calculate | Print This Page

Acid & Base Molarity Calculator	Mass Molarity Calculator	Solution Dilution Calculator

Dilute Solution of Known Molarity

Stock concentration:	12.178	molar (M)
Desired final volume:	100	milliliters (mL)
Desired concentration:	1	molar (M)
Required volume =	8.211528986697322	mL

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