Ex : 1

mili (m) 10^{-3}
Micro (μ) 10^{-6}
Nano (n) 10^{-9}
pico 10^{-12}

concentration expression

most common

Molarity
(one component sol)

$$M = \frac{moles}{V(L)}$$

Normality
used in reactions as:
① Nutralization (acid-base)
② Redox (Oxidation - Reduction)

$$N = n M$$

M → molarity
n → No. of replacemeble ions (H^+/OH^-)

persent
→ w/w
g (solut) = X
g (solvent) = 100-X

→ v/v
v (solut) = X
v (solvent) = 100-X

→ w/v
g (solut) = X
v (solvent) = required to hit Tot V (q.s)

→ mg%
(of clinical laboratories)
mg (solut) = X
v (solvent) = to q.s

X conc.
↓
when sol contains mixture (more than one chemical)

نعم، في العادة عندما نقول "ethanol %70"، تعني أن المحلول يحتوي على:
- 70 جزء من المذاب (الإيثانول)
- 30 جزء من المذيب (عادةً الماء)

ولكن طريقة التعبير تعتمد على نوع النسبة المستخدمة:

1. نسبة الحجم/الحجم (v/v):
   - تعني 70 mL من الإيثانول + 30 mL من الماء (إجمالي 100 mL من المحلول).
   - هذه هي النسبة الأكثر شيوعًا عند تحضير الكحول للاستخدام الطبي أو المختبري.
2. نسبة الوزن/الوزن (w/w):
   - تعني 70 g من الإيثانول + 30 g من الماء (إجمالي 100 g من المحلول).
   - تُستخدم هذه الطريقة في بعض التطبيقات الكيميائية.
3. نسبة الوزن/الحجم (w/v):
   - تعني 70 g من الإيثانول مذابًا في ماء ليصل الحجم الكلي إلى 100 mL.

لذا، إن لم يكن هناك توضيح إضافي، فإن "ethanol %70" غالبًا ما تعني 70 mL من الإيثانول لكل 100 mL من المحلول (v/v).

Dilutions :

- The process of changing of a sol, with the total amount of solute remains unchanged, only it's conc. is decreased.

$$C_1V_1 = C_2V_2$$

- when can I use $C_1V_1 = C_2V_2$?

* for diluting conc. sol (percent, molar, normality, X factor)
* not with serial type of dilutions
* it's very easy to use, but can be a large source of error if calculations not preformed correctly

* doesn't change No. of moles $M_1V_1 = M_2V_2$

The "X" Factor

- labelling as 10X → tells that the stock sol is ten times more concentrated that it needs to be for use thus : Dilution is needed

concentrated stock sol (e.g 10X buffer) :

① The working concentration : conc. of each of the chemicals when the sol is at the correct conc. to use → 1X
② All conc. stock sol that use the X factor → uses the working conc. as 1X
③ 1X can be 2X or 5X --- etc

V of stock needed = $\frac{\text{V of 1X needed}}{\text{conc. factor}}$] this is bullshit —use this→ $C_1V_1 = C_2V_2$

[A] one to ten (1 to 10) dilution (to interpreting it we have two methods)

① one part of concentrate in ten parts of final sol (1ml of concentrate and 9 ml solvent) 1 : 10 → Tot = 10 ml

② one part of concentrate to ten parts of solvent (1 in 11) e.g: 1 mL of concentrate and 10 ml vehicle (solvent) with final v = 11 mL 1 : 10 → Tot = 11 mL

* which one better ?
1 : 10 → one part of sample + nine parts of diluent → V of each part = $\frac{\text{Tot. V}}{\text{Tot. parts (X)}}$

Note :
Always add small amount to larger amount →

ex: 1 : 10 → 100 mL
V (parts) = $\frac{100}{10}$ = 10 mL
↓
X-1 = 10-1 = 9
to
large (9 ml) ← 1 ml small

Last but not least → **Serial dilution**

* when to use it ?

- is used when you need a volume or amount that is too small to measure , no balances to measure it } A problem

solve : two options :

① prepare larger volumes than what you need → which is a waste

✓② make a small volume of a higher conc. and then perform multiple dilution steps (serial dilution) to reach the required conc.

* A serial solution is: a stepwise series of dilutions which start with a small amount of starting material and amplifies the dilution factor serially by using diluted material as a source of subsequent dilutions

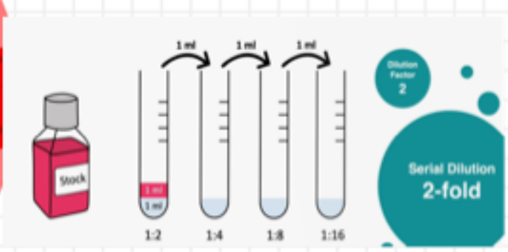
→ Advantages : ① saving reagents / spaces
② used in experiments which require standard curve
③ avoid having to pipette very small volumes

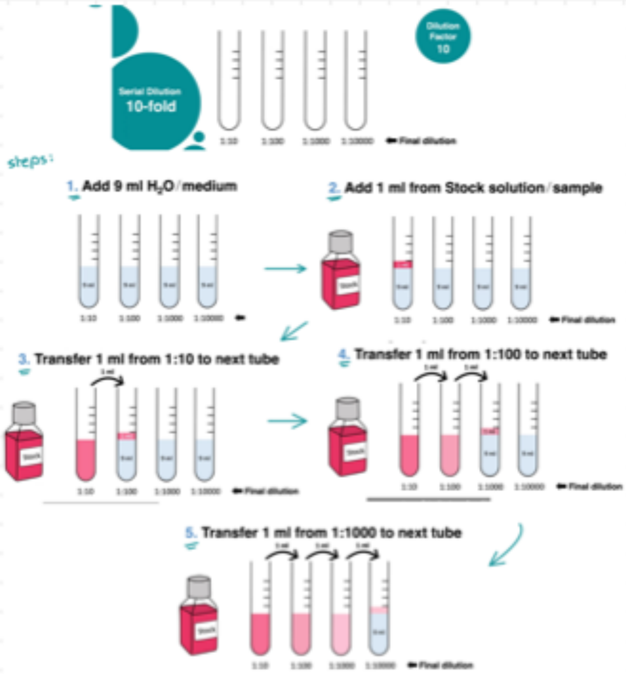
How to serial dilution a solution ?

two methods

① two-fold

② Ten-fold





* The total dilution factor (DF) for any of the resulting solutions can be calculated by :

$$DF_{total} = DF1 * DF2 * DF3 * DF4 \quad \text{(in this ex 4)}$$

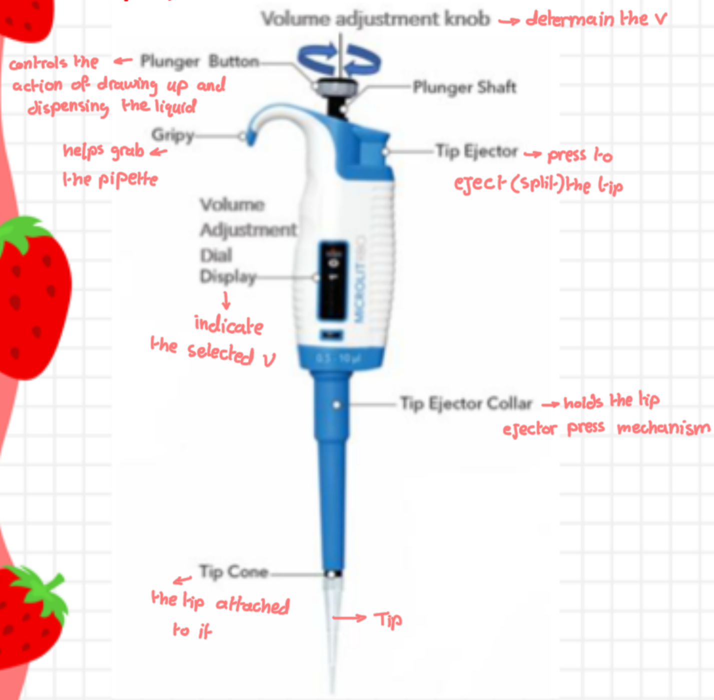
1000 * 2 * 2 * 2 = 8000
(1000: particular dilution; 2 * 2 * 2: all dilutions factors)

* we use the DF_{tot} to calculate the conc. from the stock :

$$\frac{1000\,000}{8000} = 125 \text{ ng/ml}$$

Ex : 3 Micropipette

parts



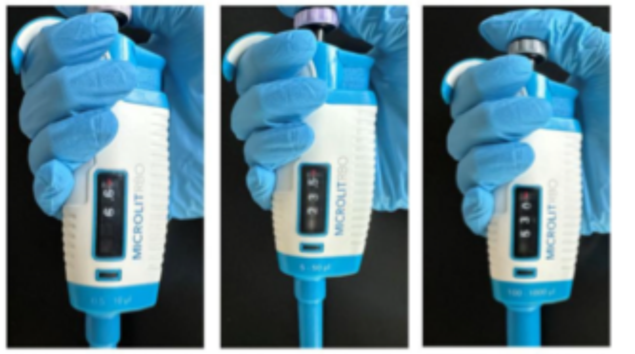
size

B. Different sizes of micropipettes

The micropipettes in this laboratory come in three different sizes each of which measures a different range of volumes. The three sizes are P10, P50 and P1000. Micropipette are named based on the upper range volume.

Micropipette Size	Lower – upper volume range
P-10	0.5-10μl
P-50	5-50μl
P-1000	100-1000μl

general : lower limit = 0.1 upper limit



P-10, range (0.5-10 μL) Display: 6.6 μL

P-50, range (5-50 μL) Display: 23.5 μL

P-1000, range (100-1000 μL) Display: 530 μL

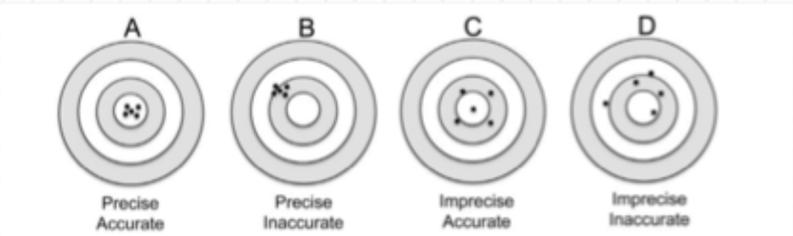
Volume is read from bottom to top.

* Note :
  - Trying to dispense less than the lower value of the range will result in inaccurate liquid mesurements
  - Trying to dispense over the upper range will completely fill the tip and allow the liquid to enter into the pipette body

Accuracy and precision

Accuracy → depends on the micropipette delivering the correct volume

precision → results are reproducible



Gravimatric method : the process in which manufacturers determain the accuracy and precision of micropipt using them to transfer defined volumes of distilled water then weighed on an analytical balance

(Density of water at 25°C = 1 g/ml)

$1 = D = \frac{m}{V} \longrightarrow m = V$

Accuracy

- the closeness of the dispensed volume to the true (standard / nominal) volume as set on pipette

$$\%\,Error\,(\%\,Accuracy) = \frac{\text{Experimantal value (mean)} - \text{Standard (nominal)}}{\text{Standard}} \times 100$$

precision

- provides info about reproducibility of your measurement "scatter" of individual measurement around the mean without any reference to a standard
- other name : coefficient of variation (cv%)

$$\%RSD = \frac{\text{sample S.D}}{\text{mean}} \times 100$$

How pipetting technique contributes to error :

1. Unnecessary tip wiping → lead to material loss
2. choosing wrong pipetting mode
3. working too fast
4. pipetting at an angle
5. using wrong pipette tips
6. if pipette is dropped → تخرب

بس و الله ♡

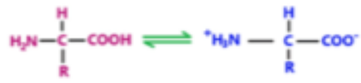
تقيلة الدم

Ex: 4

Protein is an important macronutrient essential for survival. They are an important constituent of cells and hence are present in all living bodies. Cells contain more than 3000 different proteins that play a variety of structural and functional roles in the cell.

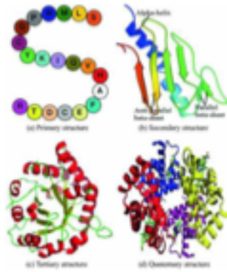
10-35% of calories should come from protein. Protein is found in meats, poultry, fish, meat substitutes, cheeses, milk etc. They contain carbon, hydrogen, oxygen, nitrogen, phosphorus and Sulphur in some cases. Proteins are large biological molecules of alpha-amino acids.

Amino acids are the main building blocks of proteins. They are relatively small molecules that characterized by presence of **α-amino group, carboxylic group and a unique R group**. the amino group is attached to alpha carbon, which are crystalline in nature and exist as zwitterions (متوازن كهربائي) (it has both positive and negative)



✓ Although amino acids share the general structure, they differ in the polarity, size solubility and electrical charge.

Amino acids form a specific type of linkage known as **peptide linkage**, amino acid molecules undergo a condensation reaction (Dehydration), the condensation of the **α-carboxyl group** of one amino acid and **an α-amino group** of another. The products formed are classified as; depending on the number of amino acid molecules involved in the condensation reaction.



① **Dipeptide:** they are the products formed by the condensation of two molecules of alpha-amino acid.

② **Tripeptide:** they are formed by the condensation of three molecules of alpha amino acid.

③ **Polypeptide:** If a large number of molecules of amino acids combine, the formed product is called **a polypeptide**.

Proteins differ primarily from each other in their amino acid sequence. There are about 20+ amino acids here. Some amino acids are not produced by the body and are delivered by diet. They are called amino acids, which are **essential**.

Detection based on the following:

1. Presence of peptide bond: Biuret reaction
2. The nature of protein (coagulation by heating, precipitation by strong acid and heavy metals)
3. Presence of certain amino acids: color reactions of amino acids

Qualitative test

- protein (two tests)
  1. Biuret test
  2. Heat Denaturation
- Amino acid (five tests)
  1. Ninhydrin
  2. Millon
  3. Hopkin cole
  4. Lead-sulfur
  5. Nitroprusside

Test Name	Principle	Procedure	Positive Result	Negative Result	Image/Color Description
Biuret Test	Detects peptide bonds in proteins using $CuSO_4$ in alkaline medium. A violet color indicates presence of protein.	1. Add 15 drops of test solution to test tube. 2. Add 5 drops of 20% NaOH, mix well. 3. Add 2 ml of 0.2% $CuSO_4$. Warm if necessary.	Violet color (indicates protein with ≥2 peptide bonds).	No color change.	Test tube with violet solution indicates presence of proteins. Expected color: Violet (positive)
Heat Denaturation Test	Detects protein stability under heat. Denatured proteins precipitate.	1. Take 5 ml of test solution. 2. Heat to boiling. 3. Observe for precipitation.	White precipitate (denatured protein).	No precipitate (protein absent or stable).	White cloudy solution or sediment at bottom of test tube indicates denaturation. Expected color: Cloudy or precipitate (positive)

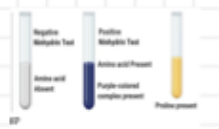
Biuret Test

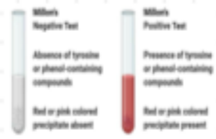
Negative biuret test

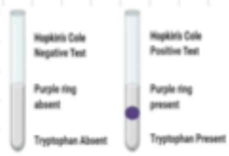
positive biuret test (polypeptide chain chelates with a copper ion)

Denaturation test (heating)

Test Name	Principle	Procedure	Positive Result	Negative Result	Image/Color Description
Ninhydrin Test	Reacts with free $-NH_2$ groups in amino acids to give purple/blue or yellow color.	1. Add 2 ml of sample + 0.5 ml 0.1% Ninhydrin. 2. Heat in water bath for 5 minutes. 3. Cool and observe color.	Purple/blue (amino acids with α-NH_2). Yellow (proline or hydroxyprolin e).	No color change.	Purple or yellow colored solution in test tube based on amino acid type. Expected color: Purple or yellow (positive)
Millon's Test	Specific for tyrosine or phenol-containing proteins. Gives red precipitate.	1. Add 2 ml sample + 2 ml Millon's reagent. 2. Heat in water bath if no immediate precipitate. 3. Observe.	Red or pink precipitate (tyrosine present).	No red precipitate.	Brick red or pink solid at bottom of test tube. Expected color: Red or pink (positive)
Hopkins-Cole Test	Detects tryptophan using glyoxylic acid and H_2SO_4; gives purple ring.	1. Mix 2 ml sample with 2 ml Hopkins-Cole reagent. 2. Add conc. H_2SO_4 down the side to form 2 layers. 3. Observe interface.	Purple ring at interface (tryptophan present).	No ring.	Thin purple ring at junction of two liquid layers. Expected color: Purple ring (positive)
Lead Sulfur Test	Detects sulfur-containing amino acids by forming black lead sulfide.	1. Add 2 ml sample + 2 ml 20% NaOH. 2. Boil, cool, add 20% lead acetate dropwise. 3. Observe.	Black precipitate (sulfur amino acids like cysteine).	No precipitate.	Black particles suspended or settled in solution. Expected color: Black (positive)
Nitroprusside Test	Detects cysteine by forming red complex with nitroprusside in alkaline medium.	1. Add 2 ml sample + 0.5 ml nitroprusside. 2. Add 0.5 ml 20% NaOH. 3. Observe color.	Red color (cysteine present).	No red color.	Red solution in test tube indicates cysteine. Expected color: Red (positive)







Ex: 5

Assay: method to detect, quantify a particular type of molecule → example: Spectrophotometric

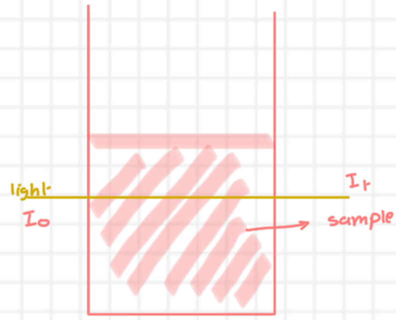
* The purpose of the protein assay (quantifications):
  1. determain the amount or concentration of a specific protein
  2. an array of different protins in a sample

* isolating and deceting protein is used for:
  1. clinical and research processes
  2. in clinical laboratory as part of a disease diagnosis (to quantify loss of protein, level, enzymes, untibodies)

Spectrophotometry: colorimetric reagent protein assay (relay on color changes)

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

Light is often treated as energy wave. The wavelength is expressed by lambda λ. And measured in nanometre= 10^{-9} m). The transfer of energy from a photon to a molecule is absorption. Light absorption may (A) result directly from the intrinsic chemical properties of the molecules of interest. Alternatively, they (B) may occur indirectly as a result of treating the molecules of interest with other compounds which react with them to create new chemicals that exhibit absorption. The wavelength at which a substance has its strongest photon absorption (highest point along the UV spectrum) is called Lambda max (λ max).



Spectrophotometry
- Single Beam → needs correction
- Double Beam → self correction (two regions)

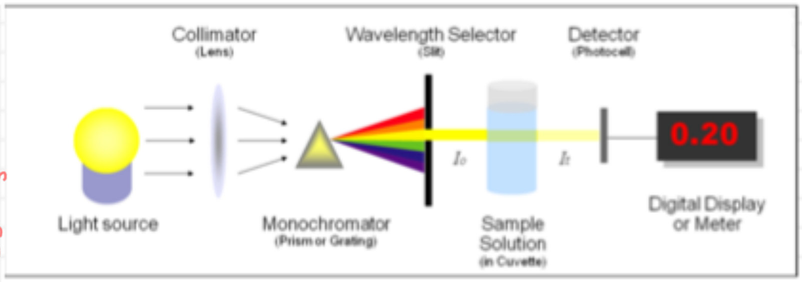


Figure 1: Components of spectrophotometer

Biochemical assays for biomolecules are usually based on absorption in the UV or visible region. In this experiment we will use the **ultraviolet-visible spectrophotometer (figure 1),** the UV range normally extends from 100 to 400 nm, with the visible range from approximately 400 to 800 nm (red to violet wavelength range).

Beer – Lambert law

– Tot amount of light absorbed at any particular wavelength (Absorbance) is determain by three factors:

determined by three factors: 1) the absorption characteristics of the molecules of interest; 2) the pathlength or distance through which the light must travel; and 3) the concentration of the absorbing molecule.

$$A = \epsilon C L$$

- A → Absorbance (no unit)
- ε → constant
- C → constant (mole/L)
- L → leangth (cm)

→ general form

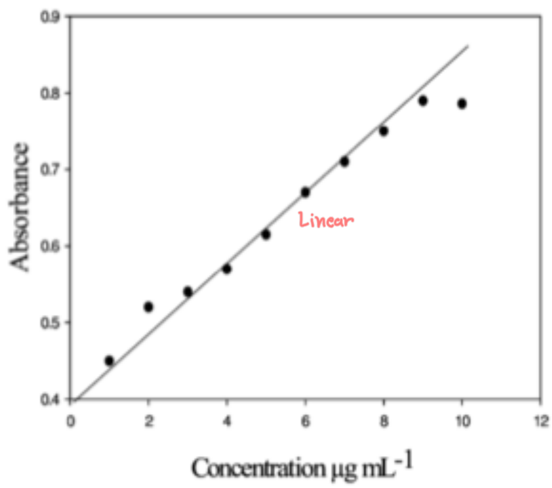
$$Y = (m) X$$

$$A = (\epsilon l)\, C \quad , \quad \text{slop}(m) = \epsilon l$$

Calibration Curve

A calibration curve, also known as a **standard curve**, is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration (for protein assay, sugar assays, and various types of assays).

You can find the slope and y-intercept of the linear graph to get the linear equation. By measuring the absorbance of the sample of unknown concentration, you can apply it to the equation to find the concentration (there is an example afterward).



$(y_2 - y_1) = m(x_2 - x_1)$

Calibration curve of absorbance against concentration using Beer's Lambert law.

Absorbtion with no molecule in sol (blank) $c = 0$ → $y = mx + 0$

Theoretically the Y-intercept should be zero for the Beer's Law Plot?

Because at zero concentration, there is no compound dissolved in solution, and the spectrophotometer is zeroed using the blank before taking the measurements.

All light absorption measurements are made relative to a blank (anything expect the sample) solution which contains all the components of the sample solution except the substance being analyzed

But practically, you may have y-intercept, if the plot is not linear or if the y-intercept deviates substantially from the origin, it indicates that the standards were improperly prepared, that there is an unknown interference in the sample.

* If we have two concentrations of the same solution, then they have the same molar extinction coefficient (E), and the length path (l) is 1 cm in most spectrophotometers.

A = (E l) C

(E l) = A/C

- $E \times l_{\text{(1st concentration)}} = E \times l_{\text{(2nd concentration)}}$
- $E_1 = E_2$
- $A/C_1 = A/C_2$

Therefore, the absorbance/concentration relationship can be applied using the same linear equation we got for the standard.

Go back to examples

Finally → Colorimetric reagent protin assay

- These assays are useful to quantify the amount of protein in a given sample, it's fast and easy to perform, don't require complex of hazardous chemicals (خطرة)
- A reagent that specifically absorbs a specific amount of light is attached to a specific protein, and then the amount of light is measured.

Tow main types of colorimetric reagent protein assay
(colorimetric: color change)

Copper chelation and detection of the reduced copper
↓
Bicinchoninic acid (BCA)

- used with: detergents
- limits: ① Incompatible (غير متوافق) with reducing agents
  ② chelators
- Assay range: 20 – 2000 µg/mL (25 µL) (sample v)
- Incubation time: 30 min
- Temp: 37°C
- Assay measurement (A max wavelength): λmax = 540 nm

protein–dye binding and associated color change
↓
Bradford (Commassie dye) for general use

- most common
- compatible (used with): samples containing buffer salts, metal ions, reducing agents, chelators
- Incompatible: samples containing detergents.
- range: 100 – 1500 µg/mL (20 µL)
- Incubation time: 5–10 min, shouldn't be incubated longer than 1h
- Temp: room temprature
- λmax = 595 nm

↓
principles (مبادئ)

• Use of Coomassie G-250 Dye in a colorimetric reagent for the detection and quantitation of total protein was first described by Dr. Marion Bradford in 1976. It is a colorimetric, spectrophotometric quantitative assay to measure protein concentration.

➔ **Chemistry of Bradford, Coomassie-based protein assays**

In an acidic environment, proteins bind to coomassie dye. This results in a spectral shift from the brown form of the dye to the blue form. The optimal wavelength to measure the blue color from the Coomassie dye–protein complex is 595 nm.

Development of color in Coomassie dye–based protein assays has been associated with the presence of certain basic amino acids—primarily arginine, lysine, and histidine—in the protein. Van der Waals forces and hydrophobic interactions also influence dye–protein binding. The number of Coomassie dye molecules bound to each protein is approximately proportional to the number of positive charges found on the protein.

⟹ خلاص زهقت

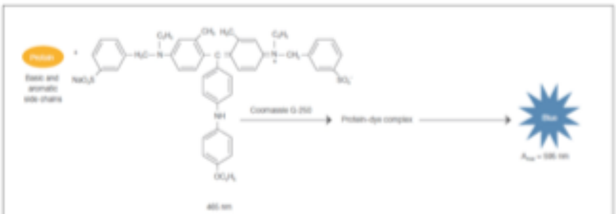


Figure: Reaction schematic for the Coomassie dye–based Bradford protein assays.

Free amino acids, peptides, and low molecular weight proteins do not produce color with Coomassie dye reagents. In general, the mass of a peptide or protein should be at least 3,000 Dalton for quantification with this reagent.

Adv ① Bovine serum albumin (BSA) which is used to generate the standard curve has a molecular mass of 66.5 KDalton and is used because it is widely available in high purity ② and it is inexpensive ③ (high).

disAdv The main disadvantage of Coomassie based protein assays is their incompatibility with surfactants/detergents at concentrations routinely used to solubilize membrane proteins ①. In general, the presence of a surfactant in the sample, even at low concentrations, causes precipitation of the reagent. In addition, the Coomassie dye reagent is highly acidic, so proteins with poor acid-solubility cannot be assayed with this reagent ②.