Quantitative Proteins Estimation by Lowry method

Amal Alamri

Biochemical research often requires the quantitative measurement of protein concentrations in solutions. Several techniques have been developed; Since the amino acid content varies from protein to protein, no single assay will be suitable for all protein.

In four of the five methods, chemical reagents are added to protein solutions to develop a color whose intensity is measured in a spectrophotometer

Methods of Quantitative Proteins Estimation

Method	Sensitivity	Time	Principle	Interferences	comments
Biuret	LOW 1-20 mg	20-30min	Peptide bonds+ alkaline cu^{2+}→ Purple complex	Zwitterionic buffers, Some amino acids	Similar color with all proteins. Destructive to protein samples.

Method	Sensitivity	Time	Principle	Interferences	comments
Lowry	High ~ 5 µg	Slow 40-60min	1)Biuert Reaction 2)Reduction of phosphomolybdate-phosphotungstate by Try and Trp	Ammonium sulfate ,glycine, Zwitterionic ,buffers, mercaptans	Time-consuming. Color varies with proteins. Critical timing of procedure. Destructive to protein samples.

Method	Sensitivity	Time	Principle	Interferences	comments
Bradford	High ~ 1 µg	Rapid 15 min	λ max of coomassie day shift from 465 nm to 595 nm when protein-bound	Strongly basic Buffers, detergents TritonX-100 ,SDS	Stable color which varies with proteins. Reagent commercially available. Destruction to protein samples Discoloration of glassware.
Bicinchoninic acid assay (BCA)	High 1 µg	Slow 60 min	1)Burit reaction 2)Copper complex with BCA; λ_{max} =562 nm	EDTA,DTT , Ammonium sulfate	Compatible with detergents. Reagents commercially available. Destructive to Protein samples.

Method	Sensitivity	Time	Principle	Interferences	comments
Spectrophoto-metric (A280)	Moderate 50-100 µg	Rapid 5-10 min	Absorption of 280-nm light by Aromatic residues	Purines , pyrimidines , Nucleic acids	Useful for monitoring column eluents. Nucleic acid absorption Can be corrected. Nondestructive to protein samples. Varies with proteins.

Proteins Estimation by Lowry method

Objective: to determine the concentration of protein by Lowry method

Principle:

The method combines the reactions of Cu++ with the peptide bonds under alkaline conditions (Biuret test)with the oxidation of aromatic protein residues.

The Lowry method based on the reaction of Cu^{+}, produced by the oxidation of peptide bonds, with Folin–Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid). The reaction mechanism is not well understood, but involves reduction of the Folin reagent and oxidation of aromatic residues (mainly tryptophan, also tyrosine).

Materials:

Chemicals:

- protein sample(unknown)
- Sodium carbonate
- Sodium hydroxide
- Sodium potassium tartarate
- Sodium dodecyl sulphate (SDS)
- Copper sulphate ($CuSO_4.5H_2O$)
- Folin-Ciocalteu Reagent

Solutions:

- Albumin standard (200 µg/ml)
- Reagent A
- Reagent B
- Reagent C

Reagent A:

Na_2CO_3 , NaOH , sodium potassium tartarate , SDS in water and make up to 100 ml. Store it at room temperature.

Reagent B:

$CuSO_4.5H_2O$, Dissolve $CuSO_4.5H_2O$ in a little volume of water and make up to 10 ml. Store at room temperature.

Reagent C:

100 parts of reagent A + 1 part reagent B. Take 100 ml reagent A and add 1ml reagent B.

Folin-Ciocalteu reagent:

Dilute commercial reagent by 1: with water. Prepare fresh.

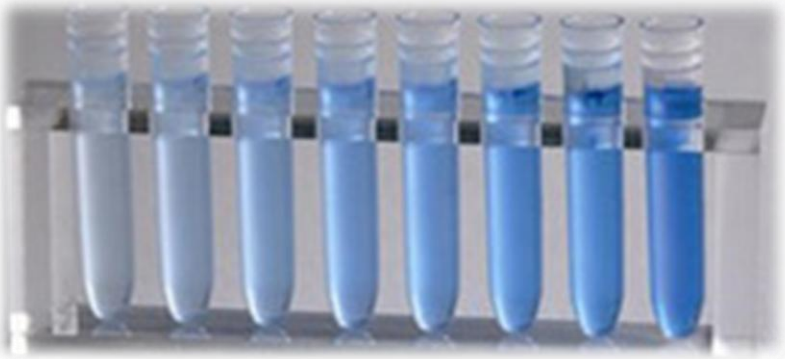
- **Equipment and glassware:**
- Refrigerated centrifuge & tubes
- Spectrophotometer
- Test tubes
- Glass cuvettes

Method

Set up 8 tubes as follows:

Tube	Water (ml)	albumin standard (ml)	unknown (ml)	reagent C
A(blank)	1.0	-	-	3ml
B	0.8	0.2	-	3ml
C	0.6	0.4	-	3ml
D	0.4	0.6	-	3ml
E	0.2	0.8	-	3ml
F	-	1.0	-	3ml
G	-	-	1.0	3ml
H	-	-	1.0	3ml

- Add 0.3 ml of Folin-Ciocalteu reagent. (Add this reagent to one tube at a time and immediately after adding it mix well).
- Let the tubes stand at room temperature for 45 min.
- Read absorbance at 660 nm against the blank.



Results :

Tube	Albumin concentration (µg/ml) X-values	A_{660} Y-values
A	0	
B	40	
C	80	
D	120	
E	160	
F	200	
G		
H		

Plot a standard curve for absorbance at 660 nm against casein concentration (µg/ml). From the standard curve obtain the concentration of unknown sample.

Thank You