BT 210 BIOCHEMISTRY LAB

Hartree-Lowry Method Of Protein Estimation

Theory/Principle:

The Lowry assay (1951) is an often-cited general use protein assay. For some time it was the method of choice for accurate protein determination for cell fractions, chromatography fractions, enzyme preparations, etc. The Hartree version of the Lowry assay, a more recent modification that uses fewer reagents, improves the sensitivity with some proteins, is less likely to be incompatible with some salt solutions, provides a more linear response, and is less likely to become saturated. **.**

Under alkaline conditions the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion which reacts with folin reagent. Phospomolybdotungstate of folin reagent is reduced to heteromolybdenum blue by the copper-catalysed oxidation of aromatic amino-acids, tyrosine and tryptophan present in proteins.The intensity of the colour depends on the amount of the aromatic amino acids present and will thus vary for different proteins.The lowry method is sensitive to pH changes and therefore the pH of the assay should be maintained at 10-10.5.

Methodology:

a)Materials Required:

(i) **Equipments:** Spectrophotometer.

Glass or polystyrene cuvettes

Water Bath

(ii) **Chemicals/reagents:**

- Sodium potassium tartrate.$4H_2O$
- Sodium carbonate
- NaOH
- Copper sulfate
- Folin-ciocalteau reagent
- Bovine serum albumin(BSA)

(iii) **Glass wares and others:**

- Test-tubes
- Pipettes
- Graduated cylinder

b)Procedure:

- **REAGENT A:**The reagent consists of

  2gm Sodium potassium tartarte.$4H_2O$ (7mM Na-K Tartrate)

  100gm Sodium carbonate (0.81M Sodium carbonate)

  500 ml 1N NaOH (0.5N NaOH)

  H_2O upto 1Lt.The reagent can be kept for 2-3 months

- **REAGENT B:**The reagent consists of:

  2 gm Sodium potassium tartrate.$4H_2O$ (70mM Na-K Tartrate)

  1gm Copper Sulfate (40mM $CuSO_4$)

10ml 1N NaOH

90ml H_2O.The reagent can be kept for 2-3 months

- **REAGENT C:**

1 vol Folin-Ciocalteau reagent diluted with15 vols of water

- **BSA stock solution**:0.3mg/ml

c)Assay:

1.Prepare a series of dilutions of 0.3mg/ml bovine serum albumin in the same buffer containing the test sample to give concentrations of 30 to 150μg/ml.

2. Tube 1 is used as blank and tubes 2 through 6 are for standard calibration curve for protein. Tubes7-10 are for unknown samples (Table1).To 1 ml of blank,each dilution of standard and test sample and add 0.9 ml Reagent A in separate tubes and mix.

3.Incubate the tubes at 50ºC for 10min and cool to room temperature

4.Add 0.1ml reagent B to each tube, mix and incubate for 10min at room temperature.

5.Rapidly add 3 ml Reagent C to each tube, mix, incubate at 50ºC for10min and cool to room temperature.

6.Incubate the tubes for 30-60 mins at room temperature. Measure absorbance at 650 nm using UV-Vis spectrophotometer.

Table1:

Sl No	PROTEIN (μl)	PROTEIN (μg)	DH_2O (μl)	ReagentA (ml)	ReagentB (ml)	ReagentC (ml)	A_{650} (nm)
1	-	-	1000	0.9	0.1	3	
2.	100	30	900	0.9	0.1	3	
3.	200	60	800	0.9	0.1	3	
4	300	90	700	0.9	0.1	3	
5.	400	120	600	0.9	0.1	3	
6.	500	150	500	0.9	0.1	3	
7.	1000	-	-	0.9	0.1	3	
8.	Unknown Sample (A)	-	-	0.9	0.1	3	
9.	Unknown Sample (B)	-	-	0.9	0.1	3	
10.	Unknown Sample (C)	-	-	0.9	0.1	3	

Calculation: Prepare a standard curve of absorbance versus micrograms protein and determine the slope y/x from the standard curve, which gives the A_{650} per unit of protein(μg).Hence determine the amount of protein in the unknown sample.

References:

1.Lowry,OH,NJ Rosbrough,AL Farr,and RJ Randall.(1951).J.Biol.Chem.**193**:265.

2.Hartree,EF.(1972)Anal Biochem.**48**:422-427