BT 312 Analytical Biotechnology Lab

Aim: Determination of Protein concentration by Lowry Method

Theory/Principle: The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic 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 assay solution should be maintained at 10 - 10.5.

Procedure:

1. Dilute the sample at a ratio of 1:25 (20uL sample in 480uL of milliQ).
   NOTE: Dilution is an essential step at this particular ratio.
2. To the diluted sample (500uL), add 700uL of Lowry's reagent (Preparation mentioned below) in dark.
3. Incubate for 20 minutes in dark.
4. After incubation, add 100uL of Folin and ciocalteus reagent (Preparation mentioned below) in dark.
5. Incubate for 30 mins in dark.
6. After incubation (aliquot 200uL in 96 well plate, quadruplicates), take reading at 750nm.

Reagents Preparation

Lowry's Reagent

Lowry Reagent A
2% sodium carbonate in 0.1N sodium hydroxide

Dissolve 0.4gm sodium hydroxide and 2gm of sodium carbonate in 100mL of milliQ.

Lowry Reagent B
1% Sodium Potassium Tartarate in milliQ

Dissolve 0.1gm Sodium Potassium Tartarate in 10mL of milliQ.

Lowry Reagent C
0.5% of copper sulphate in milliQ.

Dissolve 0.05gm of copper sulphate in 10mL of milliQ

Lowry's Reagent – Lowry reagent A + Lowry reagent B + Lowry reagent C in 100:1:1 ratio.

Folin and ciocalteu reagent
45% Folin-ciocalteu reagent and 55% milliQ.

NOTE: Prepare Lowry's reagent and Folin-ciocalteu reagent fresh before use.

BSA stock solution: 1mg/ml

Tabulation:

Sl. No.	Conc. Of BSA	Volume of BSA (ul)	milliQ (ul)	Lowry's Reagent (ul)	Folin Ciocalteu Reagent (ul)	A_{750} (nm)
A	1 mg/ml					
B	500 ug/ml	250 (A)	250	700	100	
C	250 ug/ml	250 (B)	250	700	100	
D	125 ug/ml	250 (C)	250	700	100	
E	62.5 ug/ml	250 (D)	250	700	100	
	Unknown Sample	-	-	700	100	

Calculation:

Prepare a standard curve of absorbance versus micrograms protein and determine the slope y/x from the standard curve, which gives the A_{750} per unit of protein (µg). Hence determine the amount of protein in the unknown sample.