

**EX.NO:**

**DATE:**

## **ESTIMATION OF PROTEIN BY LOWRY'S METHOD**

### **AIM**

To estimate the amount of protein present in the given unknown solution.

### **PRINCIPLE**

The blue colour developed by the reduction of phosphomolybdic and phosphotungstic components in the folins - ciocalteau reagent by the amino acid tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of protein with cupric tartarate are measured in this method

### **REAGENT REQUIRED**

#### **STOCK STANDARD SOLUTION**

250mg of bovine serum albumin (BSA) is weighed and dissolved in distilled water and made up to 100 ml in a standard flask.

#### **WORKING STANDARD SOLUTION**

10ml of stock standard is diluted to 100ml with distilled water in a standard flask.

#### **ALKALINE COPPER REAGENT**

1. Solution A: 2%  $\text{Na}_2\text{CO}_3$  in 0.1N of NaOH

2. Solution B: 0.5% copper sulphate in 1% sodium potassium tartrate

Mix the solution A and B just before use.

#### **FOLINS – CIOCALTEAU**

The following contents are refluxed gently in a 1.5-litre flask. 100gm of sodium tungstate, 25gm sodium molybdate, 700ml of water, 50 ml of 85% phosphoric acid and 100 ml of concentrated HCl, 150g of lithium sulphate, 50ml of water and few drops of bromine water

are added. The mixture is boiled for 15min without condenser to remove excess bromine. Cooled and diluted to 1 litre and filtered. The reagent should have no green tinge.

## PROCEDURE

In a series of test tube 0.2, 0.4, 0.6, 0.8, 1.0ml of working standard solution are pipetted out into the test tubes. 0.1 to 0.2 ml of the unknown solution is pipette in other two test tubes. The tubes are made up to 1 ml with distilled water. And a tube with 1ml of water is used as a blank. Add 4.5ml of alkaline copper reagent and 0.5ml of folin's reagent (1:2 dilution), mix well and incubate at room temperature in the dark for 30 min. The blue colour developed is measured at 660nm. A standard graph is drawn from which the amount of protein in the given solution is calculated.

## RESULT:

The amount of protein present in the given unknown sample is \_\_\_\_\_mg.

## TABULATION:

| S.No                                                 | Contents                                  | B   | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | T1  | T2  |
|------------------------------------------------------|-------------------------------------------|-----|----------------|----------------|----------------|----------------|----------------|-----|-----|
| 1                                                    | Volume of working standard (ml)           | 0.0 | 0.2            | 0.4            | 0.6            | 0.8            | 1.0            | --  |     |
| 2                                                    | Concentration of working standard (µg)    | 00  | 50             | 100            | 150            | 200            | 250            |     |     |
| 3                                                    | Volume of test (ml)                       | --  | --             | --             | --             | --             | --             | 0.1 | 0.2 |
| 4                                                    | Volume of distilled water (ml)            | 1.0 | 0.8            | 0.6            | 0.4            | 0.2            | --             | 0.9 | 0.8 |
| 5                                                    | Volume of alkaline copper reagent (ml)    | 4.5 | 4.5            | 4.5            | 4.5            | 4.5            | 4.5            | 4.5 | 4.5 |
| <b>Incubation in room temperature for 15 minutes</b> |                                           |     |                |                |                |                |                |     |     |
| 6                                                    | Volume of folins-ciocatlteau reagent (ml) | 0.5 | 0.5            | 0.5            | 0.5            | 0.5            | 0.5            | 0.5 |     |
| <b>Incubation in room temperature 30 minutes</b>     |                                           |     |                |                |                |                |                |     |     |
| 7                                                    | O.D at 660nm                              | 0.0 |                |                |                |                |                |     |     |

## **CALCULATION**

### **Stock Standard Calculation**

Stock standard contains 250mg protein in 100ml water.

10ml of stock is diluted to 100ml with distilled water.

10 ml of stock standard contains =  $250/100 \times 10 = 25\text{mg}$ .

Therefore ---ml corresponds to ---- $\mu\text{g}$ .

### **Unknown Calculation:**

----ml of unknown corresponds to -----O.D

From the graph ---- OD corresponds to ----- $\mu\text{g}$  of protein.

Therefore 1ml of unknown  $Y \times 5 = \text{-----}$   $\mu\text{g}$  of protein.

## **Results**

The amount of protein in the given unknown sample was found to be ..... mg/dl.