**ESTIMATION OF PROTEIN – LOWRY’S METHOD**

**INTRODUCTION**

Protein can be estimated by different method as described by Lowry’s and also by estimating the free nitrogen content. No method in 100% sensitive, hydrolyzing the protein and estimating the amino acid alone with give the extract qualification. The method developed by Lowry’s in all sensitive enough to give a moderately constant to enzyme extract is usually determined by this method.

**AIM:**

To estimate the amount of protein present in the 100ml of the given sample.

**PRINCIPLE:**

The method is based on reaction of proteins with alkaline copper sulphate followed by Folin-Ciocalteau reagent (a solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid) which produce blue colored complex. The blue color is developed due to reaction of alkaline copper sulphate with the protein (as in the biuret reaction) and the reduction of phosphomolybdate- phosphotungstate by the amino acids tyrosine and tryptophane present in the protein. The intensity of the color depends upon the numbers of peptide bonds· and these aromatic amino acids, hence the quantity of the protein. The blue color developed by the revelation of phosphomolybdic phosphotungstic compound in the folin-ciocalteau reagent by amino acid. Tyrosine and tryptophan present the protein plus the color developed by the biuret reaction measured in the Lowry’s method.

**MATERIALS REQIIRED:**

1. **SOLUTION A**: 2% Sodium carbonate in 0.1N Sodium hydroxide

2. **SOLUTION B**: 0.5% Copper sulphate in 1% Potassium sodium tartarate.

3. **SOLUTION C**: Mix 50ml of solution A and 1ml solution B

4. **SOLUTION D**: Folin’s-ciocalteau reagent commercially available in ratio 1:2 with distilled water.

5. **PROTEIN SOLUTIONS [STOCK STANDARD]**

Weigh accurately 50 mg of bacteria serum albumin (BSA) and dissolved in distilled water and make up to 50ml in a standard flask. 1mg/1ml

6. **WORKING STANDARD**

Dilute 10ml of the solution [stock] to 100ml of distilled water in a standard flask in 1ml of solution contain 100µg of the proteins.

7. **PHOSPHATE BUFFER** (0.1 M, pH 7 .5)- Dissolve 8.899 g of Na2HPO4.2H2O, adjust pH to 7.5 with orthophosphoric acid and make up volume to 500 ml.

8. **SAMPLE:** Protein extract- 500mg plant leaves

**EXTRACTION OF PROTEIN FROM SAMPLES:**

Extraction is usually carried out with buffer used for the enzyme assay. Grind 500 mg leaf tissues in 5 ml of the phosphate buffer in a chilled mortar pestle. Centrifuge the homogenate at 5000 rpm for 1 0 min. Take out the clear supernatant in another tube and make up the volume to 5 ml with the phosphate buffer. This is protein extract of the given sample

**PROCEDURE**

1.Pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 ml of the working standard in to series of test tubes.

2.Pipetted out 0.2 ml of the sample extract in two other test tubes.

3.Made up the volume to 1 ml in all test tubes with distilled water, test tubes with 1 ml of water serves as a blank.

4.Add 5 ml of solution C to each test tube including the blank. mixed well and follow to stand for 10 minutes.

5.Then added 0.5 ml of solution D. mixed well and incubated at room temperature in a dark room for 30 minutes.

6.Blue color developed was measured at 660nm using colorimetric method.

7.Calculate the quantity of protein in the sample using the standard curve

**RESULT**

The amount of protein present in the given sample \_\_\_\_\_ mg

**STOCK STANDARD SOLUTION:** Concentration: 1mg/ml

50 mg of BSA in 50 ml of distilled water.

**WORKING STANDARD SOLUTION:** Concentration: 100µg/ml

10ml of the stock made upto 100ml with distilled water.

Weight of the sample taken = I 00 mg.

Total volume of the sample extract = 5 ml

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PARTICULARS** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **T1** | **T1** |
| 1 | Volume of working standard (ml) | - | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | - | - |
| 2 | Concentration of working standard (µg) | - | 20 | 40 | 60 | 80 | 100 | - | - |
| 3 | Volume of Unknown (ml) | - | - | - | - | - | - | 0.2 | 0.2 |
| 4 | Volume of Water  (ml) | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0.0 | 0.8 | 0.8 |
| 5 | Volume of Solution C(ml) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Allow to stand at room temperature for 10 minutes | | | | | | | | | |
| 6 | Volume of solution D(ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Kept the test tubes at room temperature in dark for 30 minutes. The blue color developed was read at 660nm | | | | | | | | | |
| 7 | Optical Density at 660 nm |  |  |  |  |  |  |  |  |

**CALCULATION:**

Optical density \_\_\_\_\_\_\_\_\_ corresponds to \_\_\_\_\_\_ µg of protein

0.2 ml of unknown corresponds to \_\_\_\_\_ µg of protein

Hence

5 ml of the sample extract contains (obtained from 100 mg sample)= x/0.2 X5 µg protein

So

1 g sample contains x/0.2 X5/100x1000 µg protein

= \_\_\_\_\_\_ mg of protein

The amount of protein present in the given sample = \_\_\_\_\_ mg