Expt. BL 301

Total Protein Estimation by Lowry’s Method

Objective

To determine the concentration of proteins by Lowry’s method.

Reagents Required

1. BSA stock solution (1mg/ml).

2. Analytical reagents:

   (a) 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water.)

   (b) 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartarate solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a)

3. Folin - Ciocalteau reagent solution (1N) Dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water)

Principle

The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins. Most proteins estimation techniques use Bovin Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity and ready availability. The method is sensitive down to about 10 µg/ml and is probably the most widely used protein assay despite its being only a relative method, subject to interference from Tris buffer, EDTA, nonionic and cationic detergents, carbohydrate, lipids and some salts. The incubation time is very critical for a reproducible assay. The reaction is also dependent on pH and a working range of pH 9 to 10.5 is essential.
**Procedure**

1. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ml.

2. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well.

3. This solution is incubated at room temperature for 10 mins.

4. Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm.

5. Plot the absorbance against protein concentration to get a standard calibration curve.

6. Check the absorbance of unknown sample and determine the concentration of the unknown sample using the standard curve plotted above.

<table>
<thead>
<tr>
<th>BSA (ml)</th>
<th>Water (ml)</th>
<th>Sample conc. (mg/ml)</th>
<th>Sample vol (ml)</th>
<th>Alk. CuSO₄ (ml)</th>
<th>Lowry reagent (ml)</th>
<th>O.D. 600 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4.75</td>
<td>0.05</td>
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<tr>
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<td>4.5</td>
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<tr>
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<tr>
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<td>1.0</td>
<td>0.2</td>
<td>2</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Write short notes on the following points in the report:

1. Beer-Lamberts law


**References**


2. Hartree E.E. (1972). Anal. Biochem. 48:422 (This modification makes the assay linear over a larger range than the original assay)