Aim 24

To Estimate the Protein Content in the Biological sample by Biuret / Lowry Method

Requirements

1. Biological sample (soaked gram seeds, pulses)
2. 10 mM Phosphate buffer.
3. Rest reagents and procedure is same mentioned in above two experiments.

Procedure

1. 5 g of biological sample is taken.
2. Use pestle mortar to homogenize and the protein is extracted in nearly 30 ml of 10 mM Phosphate buffer, pH 7.0 containing 0.1 M NaCl.
3. The homogenized suspension is centrifuged at 10,000 rpm for 5 min.
4. Take volume of the supernatant (let Z ml) and determine the protein content after suitable dilution (10X or 50X or more) depending upon the protein content in the given sample.
5. Calculation of the protein content can be done by Lowry/Biuret method.

IMPORTANT

In this case, take a control tube, in which all the reagents as the test are involved but in control tube, when reaction stops then add the test sample whereas in the test sample tube, add test sample in the starting. Note down the reading of the test against the control so that the colour and non-specific

absorbance contributed by the biological sample could get subtracted. Take separate control for different test samples and also prepare different controls for different test volumes.

Calculations

Consider, we have diluted the 1.0 ml of supernatant to 50 ml (Solution - A)

From this, 0.1 ml (from Solution - A) taken for proteins estimation by Lowry method.

Suppose we get O.D. - x

ml of diluted supernatant (solution A) contains y µg protein.

So 50 ml will contain = (y/0.1) x 50 µg protein

Since, we have diluted 1 ml of protein extracted supernatant to 50 ml.

So, 1 ml protein extracted supernatant contains (50y/0.1) µg protein

Z ml will contain = (50y/0.1) x Z µg protein

= (50Yz/0.1) µg protein/5 g Biological sample

= (50Yz/0.1x 5) µg protein/5 g Biological sample

= 100yZ µg protein/g sample

So, protein content per g of biological sample is 100y Z µg.