TOTAL PROTEIN

BIURET METHOD

Hemezyme

GENUINE QUALITY, RELIABLE RESULTS

SAFETY PRECAUTIONS AND WARNINGS:

This reagent is for In vitro diagnostic use only.

INTENDED USE:

This reagent kit is intended for "in vitro" quantitative determination of TOTAL PROTEIN concentration in serum. Biuret Method.

CLINICAL SIGNIFICANCE:

The human body contains countless different protein (50% in cells). Not only is the variety of proteins seemingly infinite, so are their variations of concentration in health and disease, their distribution within the body, their functions, their compositions and their structures. Most plasma proteins with the exception of immunoglobulins and hormonal proteins are synthesized in liver . They function as major components of cells, are involved in transport, enzyme catalysis, homeostatic control, hormonal regulation, blood coagulation, immunity, growth and repair, and heredity.

PRINCIPLE:

Cupric ions in an alkaline solution react with the peptide bonds of proteins and polypeptide containing at least two peptide bonds to produce a violet colored complex. The absorbance of the complex at 546 nm is directly proportional to the concentration of protein in the sample.

$$Cu^{++} + \text{Protein} \xrightarrow{OH^-} \text{Cu-Protein complex}$$

REAGENT COMPOSITION:

Reagent 1: Biuret reagent
Total Protein standard : 6 gm/dl (store at 2-8°C)

MATERIALS REQUIRED BUT NOT PROVIDED:

- Clean and dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:

Serum is free of hemolysis..

STABILITY OF REAGENT:

When Stored tightly closed at room temperature protected from light and contaminations prevented during their use; reagent is stable up to the expiry date stated on the label.

WORKING REAGENT:

The Reagent is ready for use

GENERAL SYSTEM PARAMETERS:

REACTION TYPE	End Point
WAVE LENGTH	546 nm
LIGHT PATH	1 cm
REACTION TEMPERATURE	37°c
BLANK / ZERO SETTING	Reagent
REAGENT VOLUME	1 ml
SAMPLE VOLUME	10 µl
INCUBATION TIME	5 Minutes
STANDARD CONCENTRATION	6.0 gm/dl
LOW NORMAL	6.2 gm/dl
HIGH NORMAL	8.0 gm/dl
LINEARITY	12 gm/dl

ASSAY PROCEDURE:

1.Take three clean, dry test tubes labeled B (blank), S (standard), T (test). 2.
2.Set the instrument to zero with the blank, aspirate the standard to factor.
3.Then aspirate the test sample one by one to read the result.

	BLANK	STANDARD	SAMPLE
REAGENT	1ml	1ml	1ml
STANDARD		10 µl	
SAMPLE			10 µl

Mix and read the optical density (A) after a 5-minute incubation at 37°C.

CALCULATION:

$$\text{Total Protein (gm/dl)} = \frac{\text{OD of Sample}}{\text{OD of Standard}} \times \text{Conc. of Standard}$$

LINEARITY:

Reagent is Linear up to 12 gm/dl.

Dilute the sample appropriately and re-assay if Total Protein Concentration exceeds 12 gm/dl. Multiply result with dilution factor.

REFERENCE NORMAL VALUE:

Serum Total Protein 6.2-8.0 gm/dl

QUALITY CONTROL:

For accuracy it is necessary to run known controls with every assay..

LIMITATION & PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperatures as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during the assay process.

BIBLIOGRAPHY:

Gournall A et al Journal of Biol.Chem 177 (1949), 751.
Tietz, N.W., Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia, PA (1970), 302.

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