ANA BIO ISP Total Protein

(Biuret Method)

For Miura Instruments

Intended Use
Total protein is a reagent kit used for the determination of total protein in serum or plasma based on Biuret method. The reagents are for *in vitro* diagnostic use.

Principle
Proteins react with cupric ions under alkaline pH to produce a coloured complex. The intensity of the colour is directly proportional to the protein concentration in specimen.

Proteins $\xrightarrow{Cu^{2+}, \text{Alkaline pH}}$ Blue color complex

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent	Cupric Sulphate	15.9 mmol/L
	Potassium iodide	7.5 mmol/L
	K-Na tartrate	46.8 mmol/L
	NaOH	0.5 mol/l
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 15° - 25°C and is stable till the expiry date indicated on the label.

Reagent Preparation
Liquid reagent ready for use. After opening the reagent is stable for 30 days if closed, stored at 2° - 8°C, and protect from direct light and contamination. Do not mix different batches.

Specimen collection and preservation
Blood should be collected in a clean dry container. Plastic or siliconized container should be avoided as it may prolong clotting time. Serum or plasma should be separated from the cells within 60 minutes. For plasma separation following anticoagulants may be used.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- HEPARIN 200 IU/ml of blood
- OXALATE 3 mg/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Proteins are stable in the serum and plasma for 7 days when stored at 2° - 8°C and for a month when stored at -20°C or frozen.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Total Protein
Method Code	TP
Type	End-Point
Unit	g/dl
Filter F1	546 nm
Blank in	Used

Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	4	µl
Final Incubation	300	Sec.

Normal range
Guidance value : 6.3 - 8.4 g/dl
Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
The reagent is linear up to 18 gm / dl. For higher value, dilute sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.

Quality Control
To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
Total Protein added to a serum matrix containing known amounts of Total Protein gave an average recovery of 97%.

Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 40 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 500 mg/dl.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	g/dl	5.35	0.04	0.64	20
High	g/dl	6.96	0.05	0.66	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	g/dl	5.35	0.03	0.57	60
High	g/dl	6.96	0.05	0.66	60

Sensitivity
At 546 nm a concentration of 0.16 g/dl of Total Protein can estimate.

References

1. Stirkland R.D., et al. Anal, Chem. 33, (1961).
2. Henry, R.J., et al, "Clinical Chemistry- principles and technics" Harper & Row, II ed. (1974).

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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