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TOTAL PROTEIN (BIURET)

CE

STABLE LIQUID REAGENT

Cat. No.:	41951,41951S	41953	41991,41991S
	1x250 ml	20x20 ml	2x500 ml

Reagent kit for the quantitative determination of total protein concentration in serum. Biuret method.

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 polypeptides 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.

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

Reference values

Serum total protein **62-80 g/l (6,2-8,0 g/dl)**
It is recommended that each laboratory should assign its own normal range.

Reagents
1.Reagent(R1)

Potassium iodide	30 mmol/l
Potassium sodium tartrate	100 mmol/l
Copper sulphate	30 mmol/l
Sodium hydroxide	3.8 mol/l

2. Total protein standard
Ready for use.For details please check the insert.
Available only in Cat. No.: 41991S and 41951S.

Safety instructions:
Reagent 1:
X, Irritative
R36/38 Irritating to eyes and skin
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

Sample
Serum free of haemolysis.

Note
This reagent contains sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.
For greater accuracy a serum blank correction is recommended when assaying turbid or lipaemic samples.

PROCEDURE

Preparation and stability of working reagent
The reagents are ready for use.
If the absorbance of working reagent is higher than 0.1 at 546 nm the reagent can not be used.

Assay conditions

Wavelength:	546 nm (530-580) nm
Temperature:	37 °C
Cuvette:	1 cm light path
Read against:	reagent blank
Method:	endpoint (increasing)

Pipette into cuvette

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and read the absorbance (A) after a ten-minute incubation.

Calibration (37°C, Biuret method)
S1: Distilled water
S2: Total protein standard Cat. No.: 51911 or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I or
Randox Calibration Serum Level II
Calibration frequency
Two-point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{sample}}{A_{standard}} xC_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzers.

Linearity
The test is linear up to 120g/l (12,0 g/dl) protein concentration.
Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.18 g/l (0,018 g/dl) Protein concentration at 546 nm.
Precision

	Reproducibility		
Sample	Average conc. (g/l)	SD	CV%
Sample I.	50.1	0.462	0.92
Sample II.	51.8	0.172	0.33

Correlation
Comparative studies were done to compare our reagent with another commercial Total Protein reagent on 60 human samples.
The results from these studies are detailed below.
Correlation coefficient: r=0.9957
Linear regression: y (g/l)= 0.994x+0.525
(x= other commercial reagent, y= own reagent).

Specificity
Bilirubin 855 µmol/l (50mg/dl), lipid 1000mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.
NOTE
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography
Gornall A. et al.: J. Biol. Chem. 177, 751 (1949)
Weichselbaum, P.E.: Am. J. Path. 16, 40 (1946)