Spectrum For Diagnostic Industries

Total protein

IVD

REF.	Pack size
143 02 125	(2 x 125 ml) 250 tests
143 04 250	(4 x 250 ml) 1000 tests

Intended Use

Total protein reagent is intended for the in-vitro quantitative and diagnostic determination of total protein in human serum or plasma.

Introduction

Plasma proteins are mainly synthesized in the liver and are involved in the maintenance of normal water distribution between tissues and blood, as well as acid-base balance. Due to some pathological conditions, both total protein level and the ratio of different fractions may change independently of one another. Hyperproteinemia may be detected during dehydration associated with diarrhea or vomiting. The total protein levels also increase in multiple myeloma. Hypoproteinemia may occur as a result of prolonged low protein diet and in some pathological conditions such as nephrotic syndrome, bleeding, sprue and salt retention.

Method

Colorimetric method (Biuret reagent).

Principle

In alkaline medium the copper reacts with the peptide bonds of proteins to form the characteristic pink to purple biuret complex. Sodium potassium tartarate prevents copper hydroxide precipitation, and potassium iodide prevents the autoreduction of copper.

Protein + Cu^{2+} $\xrightarrow{Alkaline\ pH}$ Cu – protein complex

The color intensity is directly proportional to the protein concentration.It is determined by measuring the increase in the absorbance at 546nm.

Reagents

Reagent

Sodium hydroxide	750	mmol/L
Copper sulfate	12.0	mmol/L
Sodium potassium tartarate	40.9	mmol/L
Potassium iodide	19.8	mmol/L

(C)-Corrosive contains caustic materials.
R34 Causes burns.
S26-45 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice in case of accident or if you feel unwell, seek medical advice immediately.

Standard Total protein
6.0 g/dL

Reagents preparation, storage and stability

Total protein reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles. The reagents are stable at 15 – 25 ^{O}C. Only the standard needs to be kept refrigerated at (2 - 8^{O}C).Once opened, the reagent is stable for 6 months and the standard is stable for 3 months at the specified temperature.

Deterioration

Do not use The total protein regents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Specimen collection and preservation

Use serum or plasma (EDTA or heparin) for the test. Usually plasma results are higher due to fibrinogen. The serum or plasma should be separated from the cells within 4 hours .
Stability : 1 day at 15 – 25 ^{O}C ; 4 weeks at 4 – 8 ^{O}C;
1 year at -20 ^{O}C

Procedure

Wavelength	Hg 546 nm (530 – 570 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 50
Temperature	15 – 25 ^{O}C
Zero adjustment	Reagent blank
Incubation time	10 minutes at 15 – 25 ^{O}C
Reagent Blank Limits	Low 0.00 AU High 0.2 AU

	Reagent blank	Standard	Specimen
Reagent(R)	1.0 ml	1.0 ml	1.0 ml
Standard	------	20 µl	-------
Specimen	------	------	20µl

Mix and incubate for 10 minutes at room temp. Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank within 30 minutes.

Calculation

$$\text{Serum protein conc. (g/dL)} = \frac{A_{specimen}}{A_{standard}} \times 6$$

Note: For turbid highly icteric sera, prepare a serum blank by adding 20 µl serum to 1 ml saline into a labeled test tube. Read absorbance of serum blank at 540 nm vs water and subtract serum blank absorbance from test absorbance before calculating results.

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run

Sensitivity

When run as recommended, the minimum detection limit of this assay is 1.0 g/dL.

Linearity

The reaction is linear up to total protein concentration of 12 g/dL. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interference

Hemolysis
No interference up to hemoglobin level of 7.5 g/L.

Icterus
No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia
No significant interference.

Drugs
Sera from patients receiving dextran may cause artificially high levels due to turbidity during color development. This positive bias can be minimized by centrifuging the reaction mixture before reading the absorbance.

Expected Values

1- Adults	6.6 – 8.7 g/dL
2- Children	
a) > 1 year	6.0 – 8.0 g/dL
b) < 1 year	4.8 – 7.6 g/dL
3- Newborns (< 4 weeks)	4.6 – 6.8 g/dL
4- Prematures	3.4 – 5.0 g/dL

Performance characteristics

A comparison between Total protein reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.978 was obtained.

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (g/dL)	5.2	7.23
SD	0.12	0.15
CV%	2.47	2.2

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (g/dL)	5.7	7.32
SD	0.19	0.21
CV%	2.53	2.4

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Tietz NW : Fundamentals of Clinical Chemistry: 2 nd ed. NW Tietz,editor, 1994.
2. Gornall AG, Bardawill CJ, David MM: Determination of serum protein by means of the biuret reagent. J Biol Chem.
3.Schultze HE, Heremans JF:Molecular biology of human protein. Elsevier publishing company, Amsterdam, 1966

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use
LOT	Batch Code/Lot number
REF	Catalogue Number
	Consult instructions for use
	Temperature Limitation
	Use by/Expiration Date
	CAUTION. Consult instructions for use
	Manufactured by

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