Total Protein Reagent
Biuret Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-25°C
Linear Range	:	Up to 150 g/L (15 g/dL)
Specimen Type	:	Serum, plasma
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP Authorized Representative
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
Corrosion

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Total Protein in human serum or plasma on both manual and automated clinical chemistry systems.

CLINICAL SIGNIFICANCE[1]
Total protein is useful for monitoring gross changes in protein levels caused by various disease states. It is usually performed in conjunction with other tests such as serum albumin, liver function tests or protein electrophoresis. An albumin/globulin ratio is often calculated to obtain additional information.
Increased levels are found in dehydration, multiple myeloma and chronic liver diseases, whilst decreased levels are found in renal disease and terminal liver failure.

METHODOLOGY
Methods which have been devised for the determination of total protein include measurement of specific gravity, refractive index, absorbance of light in the ultraviolet region and reaction of proteins with Folin and Ciocalteau's reagent.
Historically total protein was first determined by the Kjeldahl method which still remains as a reference method. The biuret reaction has been in use since the end of the 19th century and is the method of choice in clinical laboratories because of its simplicity, rapidity and reliability. Many modifications of the biuret method have been proposed, the reagent used in this procedure is based on the work of Goodwin, et al,[2] and Flack and Woollen.[3]
The peptide bonds of protein react with the copper II ions in alkaline solution to form a blue-violet complex (the so-called biuret reaction), each copper ion complexing with 5 or 6 peptide bonds.[4] Tartrate is added as a stabilizer whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 520-560 nm. For bichromatic analysers the blank wavelength should be set to 600-700 nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
Copper II Sulphate	12 mmol/L
Potassium Sodium Tartrate	32 mmol/L
Potassium Iodide	30 mmol/L
Sodium Hydroxide	600 mmol/L
pH 13.5 ± 0.1 at 20°C	

Hazard Symbol: Corrosion
Signal Word: Danger
Hazard Statements
H314 Causes severe skin burns and eye damage
Precautionary Statements - Prevention
Do not breathe dust/fume/gas/mist/vapors/spray
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Precautionary Statements - Response
*I*mmediately call a POISON CENTER or doctor/physician
Specific treatment (see supplemental first aid instructions on this label)
Eyes
*I*F IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
Immediately call a POISON CENTER or doctor/physician
Skin
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
Inhalation
*I*F INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Immediately call a POISON CENTER or doctor/physician
Ingestion
IF SWALLOWED: Rinse mouth. DO NOT induce vomiting
Precautionary Statements - Storage
Store locked up
Precautionary Statements - Disposal
Dispose of contents/container to an approved waste disposal plant
Refer to the product Safety Data Sheet for additional information.

REAGENT PREPARATION
The reagent is supplied ready for use.

STABILITY AND STORAGE
When stored between 2-25°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Indications of Reagent Deterioration:
- Turbidity;
- Presence of a precipitate;
- Reagent Absorbance >0.200 at 540 nm; and/or
- Failure to obtain control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING[2]
Serum: Use non-haemolysed serum.
Plasma: Use heparin.
Storage: Total Protein samples may be stored for at least 7 days at room temperature (18-25°C) and for at least 1 month at 4°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 540 nm (520 nm - 560 nm).
- Analyser specific consumables, eg: samples cups.
- Normal and Abnormal assayed controls.
- Calibrator or a suitable aqueous Total Protein standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature		37°C
Primary Wavelength		540 nm (520-560 nm)
Assay Type		Endpoint
Direction		Increase
Sample : Reagent Ratio		1:50
eg:	Sample Vol	5 µL
	Reagent Vol	250µL
Incubation Time		600 seconds
Reagent Blank Limits		Low 0.0 AU
(540nm, 1cm lightpath)		High 0.2 AU
Linearity		150 g/L (15 g/dL)
Analytical Sensitivity		5.5 ΔmA per g/L
(540nm,1cm lightpath)		(0.055 ΔA per g/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Total Protein} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of calibrator	=	0.319
Absorbance of unknown	=	0.396
Value of Calibrator	=	58 g/L (5.8 g/dL)

Thermo SCIENTIFIC

$$\text{Total Protein} = \frac{0.396}{0.319} \times 58 = 72 \text{ g/L}$$

$$\text{Total Protein} = \frac{0.396}{0.319} \times 5.8 = 7.2 \text{ g/dL}$$

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. S.I. Unit conversion factor: g/L x 0.1 = g/dL.

CALIBRATION
Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.
However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-
- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:-
- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:-
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are sill out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on a well maintained automated Clinical Chemistry analyser. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 265 mg/dL.
   Free Bilirubin: No interference from bilirubin up to 211 µmol/L (12.3 mg/dL).
   Conjugated Bilirubin: No interference from bilirubin up to 211 µmol/L (12.3 mg/dL).
   Lipaemia: When measured bichromatically no interference from lipaemia, measured as an absorbance at 630 nm, up to 1.046 AU.
2. Young DS[5] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

60 - 83 g/L (6.0 - 8.3 g/dL)[4]

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.[6]

PERFORMANCE DATA
The following data was obtained using the Total Protein Reagent on a well maintained automated Clinical Chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION
Imprecision was evaluated over a period of 20 days using two levels of commercial controls and following the NCCLS EP5-T procedure.[7]

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (g/L)	58	49
Mean (g/dL)	5.8	4.9
SD (g/L)	0.8	0.6
SD (g/dL)	0.08	0.06
CV (%)	1.4	1.3

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (g/L)	58	49
Mean (g/dL)	5.8	4.9
SD (g/L)	1.9	1.6
SD (g/dL)	0.19	0.16
CV (%)	3.2	3.2

METHOD COMPARISON
Comparison studies were carried out using a similar commercially available Total Protein reagent as a reference. Serum and plasma (Heparin) samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	60
Range of sample results	21-92 g/L (2.1-9.2 g/dL)
Mean of reference method results	71.8 g/L (7.18 g/dL)
Mean of Total Protein reagent results	71.5 g/L (7.15 g/dL)
Slope	0.955
Intercept	2.9 g/L (0.29 g/dL)
Correlation coefficient	0.9844

LINEARITY
When run as recommended, the assay is linear between 0 and 150 g/L (0 - 15 g/dL).

ANALYTICAL SENSITIVITY
When run as recommended the sensitivity of this assay is 5.5 ΔmA per g/L (0.055Δ A per g/dL).

REFERENCES

1. Tietz N.W, (Ed.), Textbook of Clinical Chemistry, W.B Saunders 1986; p579.
2. Goodwin J.F., et al, Automation in Anal. Chem, Technicon Syumpolsia 1965, p.315-320.
3. Flack C.P. and Woollen J.W., Clin. Chem., 30, 559 (1984).
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (4th Ed.) Burtis, Ashwood & Bruns (Eds), Elsevier Saunders, 2005; 2293.
5. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3:292-301.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
7. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Laboratory Devices. NCCLS; 1984, NCCLS Publication EP5-T.

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REF

Reorder Information

Catalogue No.	Configuration
TR34026/1700-500	2 x 250 mL