TOTAL PROTEIN (BIURET)

INTENDED USE
Bioline TOTAL PROTEIN is intended for the quantitative in vitro diagnostic determination of total protein in human serum and plasma.

CLINICAL SIGNIFICANCE
In human plasma, albumin accounts for 50 to 60 % of total proteins; the reminder fraction mainly contains globulins (α1, α2, β and γ). Most plasmatic proteins are synthesized by the liver, except immunoglobulins. Increase of the plasmatic volume (salt retention syndrome, intoxication with water…) or its reduction (dehydration related to vomiting, diarrhoea…) induce respectively relative hypo proteinemia and relative hyper proteinemia.
For a normal plasmatic volume, abnormal total protein rates only occur in the even of disorder affecting the concentration of albumin or immunoglobulins. Thus, severe proteinic insufficiency (malabsorption, maldigestion, dietary insufficiency), renal and hepatic diseases result in hypoproteinemia. . Hyper proteinemia can be seen, for example, in case of hyper immuneglobulinemia (multiple myeloma, infection).

METHOD AND PRINCIPLE

Proteins+Cu^{2} Alkaline solution Colored complex

REAGENT COMPOSITION

Potassium iodide	6	mmol/L
Potassium sodium tartrate	21	mmol/L
Copper sulfate	6	mmol/L
Sodium hydroxide	490	mmol/L
Standard:	**6**	**g/dl**

WARNING AND PRECAUTION: Reagent may be corrosive to metals. Causes skin irritation. Causes serious eye irritation. Harmful to aquatic life with long lasting effects.Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses,if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. Absorb spillage to prevent material damage.
For more information, refer to the Safety Data Sheet (SDS).
The standard contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these reagents always flush with copious amounts of water to prevent azide build-up.

- The standard should be immediately and tightly capped to prevent contamination and evaporation.
- Take normal precautions and adhere to good laboratory practice.
- Use clean or single use laboratory equipment only to avoid contamination.

REAGENT PREPARATION
The reagent and standard are ready to use.

REAGENT STORAGE AND STABILITY
Reagents are stable until the expiration date on their respective labels, when properly stored at 2 - 8°C and protected from light.

REAGENT DETERIORATION

- The reagent and standard solution should be clear. Cloudiness would indicate deterioration.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.

SPECIMEN COLLECTION AND STABILITY
Specimen
Serum: Lithium heparinized plasma.
Do not use other specimens
Samples are stable for 7 days at 2-8°C and at least 2 months at-20°C.For longer storage, freeze samples at-70°C

- According to Good Laboratory Practice, venipuncture should be performed prior to the administration of drugs.
- Samples must be free from haemolysis and lipemia.

INTERFERENCE
Unconjugated Bilirubin: No significant interference upto 30.0 mg/dL.
Conjugated Bilirubin: No significant interference upto 29.5 mg/dL
Glucose: No significant interference upto 507 mg/dL.
Turbidity: No significant interference up to 263 mg/dL triglycerides equivalent.
Hemoglobin: No significant interference up to 300 mg/dL.
Dextran: Induces falsely high results at therapeutic concentrations

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.
Wavelength 546nm
Temperature 37°C
Read against reagent blank

	Blank	Standard	Test
Reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix and read the optical density (OD) after 5 minutes of incubation. The final colour is stable for 20 minutes.

CALCULATION
Total Protein (g/dl) = $\frac{\text{A Sample}}{\text{A Standard}} \times n$
Where n = Std Concentration (g/dl)

CALIBRATION
The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results. It is recommended to establish a linearity curve up to 12 g/dl with other available commercial standard solutions to verify the performance of instruments and reagents.

LIMITATION
The reagent is linear to 12 g/dl.

1. Samples with values above 12g/dl should be diluted 1:1 with isotonic saline and re-run. Multiply final results by two.
2. Grossly lipemic serums require a "sample blank."Add 0.02ml (20µl) of sample to 2.5m1 saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the corrected reading.

QUALITY CONTROL
To ensure adequate quality, (normal control) and (abnormal control) should be used. These controls must be performed and validated before the patient samples are assayed. The control frequency must be at least once a day, after each calibration.

EXPECTED VALUES

Serum:

6.0 - 8.3g/dL

60 - 83 g/L

PERFORMANCE CHARECTERSTICS

Linearity is from 0.20 to 12.0 g/dL (2.0 to 120.0 g/L).

-**Precision**

Intra assay

	Sample 1	Sample 2
Mean (g/dL)	6.66	4.91
SD	0.3	0.2
CV%	3.1	3.6

Inter assay

	Sample 1	Sample 2
Mean (g/dL)	6.52	4.83
SD	0.24	0.22
CV%	3.7	4.5

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-**Correlation**

A comparative study has been performed between on 100 human sera samples.

The sample concentrations were between 0.27 and 11.25 g/dL (2.7 and 112.5 g/L) The parameters of the linear regressions are as follows:

Correlation coefficient: (r) = 0.997

Linear regression: y = 0.993 x + 0.05 g/dL (0.5 g/L)

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**546 nm**
Reaction Direction / Type	**Increasing / Positive**
Reagent Blank	**Yes**
Sample Vol.	**10 µL**
Reagent Vol.	**1000 µL**
Incubation	**5 min.**
Reagent Blank Abs.(Max)	**< 0.100 Abs**
Calibration Method	**1- Point**
Standard (Conc.)	**6.0 g/dL**
Linearity	**12.0 g/dL**
Decimal Places	**1**
Temp.	**37 °C**
Unit	**g/dL**
Ref. Low (Male / Female)	**6.0 g/dL**
Ref. High (Male / Female)	**8.3 g/dL**

REFERENCE

1. Scherwin, J.E, Liver function. Clinical Chemistry: Theory, Analysis, Correlation, 4thEd., Kaplan, L.A,Pesce, A.J., Kazmierczak, S.C., (Mosby Inc.edsStLouisUSA),(2003),492 and appendix.
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3. Guder, W.G., et al., Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples.(2002). WHO/DIL/LAB/99.1Rev.2.
4. Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline. CLSI(NCCLS) document EP17-A (2004), **24** (34).