Total Protein – Method Biuret

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182512	n° 10 vials x 60 mL (R.A)
	REF B75182513	n° 12 vials x 20 mL (R.A)
CHEMILAB	REF B81180151	n° 3 vials x 26 mL (R.A)
	REF B81180152	n° 10 vials x 31 mL (R.A)

INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of Total Proteins in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Plasma proteins are mainly albumin and globulins. Most plasma proteins are synthesized by the liver, except immunoglobulins. Many factors can modify proteinemia. Increase of the plasmatic volume (salt retention, hyperhydration) or its reduction (dehydration) induce mild hypoproteinemia or mild hyperproteinemia, respectively. For a normal plasmatic volume, hypoproteinemia can occur in severe protein insufficiencies (malabsorption, maldigestion, dietary insufficiency), or renal (increased protein loss) and hepatic diseases (impaired protein synthesis). Hyperproteinemia can reveal hyperimmunoglobulinemia (multiple myeloma, infection). In practice, total proteins measurement is mostly indicated to help diagnose liver and kidney disorders, or to determine nutritional status.

PRINCIPLE OF THE METHOD

Method Biuret. The determination of Total Proteins can be performed both with physical and chemical methods. Sclavo Diagnostics bases its determination on the chemical method of biureto-tartrate. Copper ions in alkaline medium react with substances containing two or more peptide groups forming a complex of violet color, this reaction is indicated by the term of biuret reaction. The intensity of the colour is directly proportional to the concentration of proteins in the sample.

Storage and stability

= storage temperature 15-25°C

Stored unopened at the indicated temperature avoiding direct light, evaporation and contamination of any kind, reagents are stable until the expiration date printed on the label.

Concentrations

Reagent:			
	Conc.	U.M.	
Sodium hydroxide	0.5	mol/L	
Potassium iodide	7.50	mmol/L	
Copper sulphate	15.9	mmol/L	
K-Na tartrate	46.8	mmol/L	*GHS05

* Warning: **DANGER -** *Contains Sodium Hydroxide (CAS - 1310-73-2)*

H314 - Causes severe skin burns and eye damage.
H412 - Harmful to aquatic life with long lasting effects.
P101 - If medical advice is needed, have product container or label at hand.
P102 - Keep out of reach of children.
P103 - Read carefully and follow all instructions.
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 - Immediately call a POISON CENTER/doctor.
P321 - Specific treatment (see on this label).
P501 - Dispose of contents/container in accordance with local/ regional/national/international regulations.

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2.Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements.
Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE

Quality control

Control sera with a known titer of Total Protein are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration

For calibration use the "Calibrator serum Sclavo" code B35181702.

Traceability

The Total Protein traceability is reported in the package insert supplied with the "Calibrator Serum."

SAMPLE

Type of sample and storage

Serum or plasma samples can be used. The present method can be used to titrate sera with a high lipid content and sera with hemoglobin concentrations up to 250 mg/dL. When plasma samples are used, the results obtained are up to 0.4 g/dL higher than those found in serum samples. This difference is due to the presence of fibrinogen in plasma.

REAGENT PREPARATION

Liquid reagent ready for use. After opening the reagent is stable for 30 days if protected from direct light. Do not mix different batches, operate away from direct light Slight variations in colour from batch to batch, do not affect the test results.

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R/C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	540 nm
Temperature:	37°C
Reaction	End point (increasing reaction)

Technique

Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Blank	Calibr. serum	Sample
Reagent	µL	1000	1000	1000
Calibr. serum	µL	-	10	-
Sample	µL	-	-	10
Blank	µL	10	-	-

Mix well and let stand for 10 minutes before reading.
Read the absorbance of sample and calibrator serum against reagent blank. Final colour is stable for at least 2 hours protected from light.
The reaction volumes may be varied proportionally without alteration of results.

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Results:

Manual Method

Calculation of Total Protein concentration:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator serum}} \times \text{Calibrator serum Concentration} = \text{Total Protein g/dL}$$

Automation

The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

REFERENCE RANGE

Serum or plasma:

- 6.2 – 8.5 g/dL (62 - 85 g/L)

Each laboratory must establish its own normal values based on its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE

Linearity

The method is linear up to 14.1 g/dL (141g/L). If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Recovery

Commercial control sera were analyzed with the kit following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean	DS	CV%	Recovery
Low	5	4.05	0.094	2.33	100.8 %
High	5	6.44	0.167	2.60	100.5 %

Precision

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	g/dL	6.75	0.08	1.18	30
High	g/dL	4.70	0.07	1.49	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	g/dL	7.05	0.071	1.01	20
High	g/dL	4.80	0.071	1.48	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 0.35 g/dL in the conditions established for this test.

Comparison between methods

The method was compared with a similar commercially available method, analyzing 30 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	0.647
Slope	0.909
Correlation Coeff. (R)	0,998

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

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4. **Ritzmann SE, tucker ES III,** Protein analysis in disease – current concepts. Workshop manual. Chicago 1979 American Society of Clinical pathologists.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
6. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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