



Total Protein

Colorimetric method for determination of total proteins
in serum and plasma

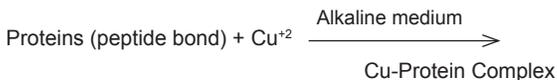
SUMMARY

Proteins are macromolecular organic compounds widely distributed in the body and are essential for life. They act as structural and transport elements and also appear as enzymes, hormones, antibodies, coagulation factors, etc. In plasma, proteins help to maintain the circulating fluid volume, transporting relatively insoluble substances, and they act in the inactivation of toxic compounds and in the defense against invasive agents.

The determination of total proteins is useful to monitor changes caused by different diseases. Under pathological conditions such as renal loss, malnutrition, long-term infections, etc., hypoproteinemias may appear; while hyperproteinemias are observed with multiple myeloma, bacterial endocarditis and hemoconcentration of diverse origins.

PRINCIPLE

In alkaline medium, the peptide bonds of proteins react with cupric ion forming violet-purple complex of Biuret. The absorption intensity of this complex at 540 nm is directly proportional to the concentration of total protein in the sample. The potassium sodium tartrate present in the reagent inhibits the formation of copper hydroxide, thus avoiding precipitation. Potassium iodide prevents the reduction of cupric ion to cuprous oxide.



PROVIDED REAGENTS

A. Reagent A: 600 mmol/L sodium hydroxide, 127.6 mmol/L potassium-sodium tartrate.

B. Reagent B: 600 mmol/L sodium hydroxide, 127.6 mmol/L potassium-sodium tartrate, 120 mmol/L potassium iodide, 48.9 mmol/L copper sulfate.

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus**
- Saline solution (9 g/L NaCl).

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

The reagents are for diagnostic "in vitro" use. The Provided Reagents are corrosive. H315+H320: Causes skin and eye irritation. H314 Causes severe skin burns and

eye damage. P262 Do not get in eyes, on skin, or on clothing. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P280 Wear protective gloves/protective clothing/eye protection/face protection.

Use the reagents keeping the standard work precautions in the clinical chemistry laboratory.

All reagents and samples must be discarded according to local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in a refrigerator (2-10°C) until the expiration date shown on the box. Once opened, they must not remain uncapped and unrefrigerated for long periods. Avoid contamination. Protect from light.

A slight discoloration of the Reagent B can be observed without changes in the reagent's performance.

SAMPLE

Serum or plasma

a) Collection: obtain the sample in the usual way.

b) Additives: in case of using plasma as a sample, we recommend the use of heparin or EDTA (Wiener lab's **Anticoagulant W**) as anticoagulants for collection.

The samples with precipitants should be centrifuged prior to testing.

c) Known interfering substances: no interference is observed with dextran up to 30 g/L, hemoglobin up to 750 mg/dL, triglycerides up to 4000 mg/dL, bilirubin up to 20 mg/dL (200 mg/L). Refer to the literature of Young for the effects of drugs in this method.

d) Stability and storage instructions: the sample should be preferably fresh. It may be stored up to one week in the refrigerator (2-10°C) or for 6 months at -20°C.

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring stated volumes.
- Automatic analyzer

PROCEDURE

(Automatic analyzer)

Following there is a general procedure for Total Protein in an automatic analyzer. When implementing the technique on a particular analyzer, follow its work instructions. In a cuvette held at the required temperature, place:

Sample or Calibrator	3 μ L
Reagent A	120 μ L
Incubate for 120 seconds at 37°C. Measure at 540 nm primary wavelength and 700 nm secondary wavelength.	
Reagent B	40 μ L
Incubate for 300 seconds at 37°C. Measure at 540 nm primary wavelength and 700 nm secondary wavelength. Wiener lab analyzers. automatically calculate the analyte concentration for each sample.	

CALIBRATION

Calibrador A plus is processed in the same way as the samples and the corresponding factor is calculated based on it. The concentration values of total proteins vary batch to batch. Refer to the values in the instruction manual of Wiener lab's Calibrador A plus.

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known total proteins concentration.

REFERENCE VALUES

Adults: 6.4 - 8.3 g/dL (64 - 83 g/L)
 Cord blood: 4.8 - 8.0 g/dL (48 - 80 g/L)
 Premature infants: 3.6 - 6.0 g/dL (36 - 60 g/L)
 Newborns: 4.6 - 7.0 g/dL (46 - 70 g/L)
 1 week: 4.4 - 7.6 g/dL (44 - 76 g/L)
 7 months - 1 year: 5.1 - 7.3 g/dL (51 - 73 g/L)
 1-2 years: 5.6 - 7.5 g/dL (56 - 75 g/L)
 > 3 years 6.0 - 8.0 g/dL (60 - 80 g/L)

This range is given as guidance only. It is recommended that each laboratory establish its own reference intervals in its patient population.

UNITS CONVERSION

Total Proteins (g/dL) x 10 = Total Proteins (g/L)

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

To preserve the integrity of the reagents avoid any kind of contamination, using only perfectly clean and dry micro-pipettes for measurement.

We recommend using Wiener lab's **Standatrol S-E 2 niveles**, as quality control material, since different values to the specified range may be obtained by using controls from other trademarks, depending on the method or system used.

PERFORMANCE

a) Precision: based on protocol EP5-A2 from CLSI, the following coefficients of variation were obtained: intra-assay precision (CV_i) and total precision (CV_t):

Level	CV _{wr}	CV _t
4.4 g/dL	0.36%	0.96%
6.7 g/dL	0.65%	1.16%
7.3 g/dL	0.57%	0.88%

b) Linearity: the linearity was evaluated using a protocol based on EP6-A from CLSI. The reaction is linear up to 12.0 g/dL. For higher values †dilute the sample 1+4 parts with saline solution (NaCl 9 g/L), repeat the determination and multiply the result by the dilution factor.

c) Quantification limit: was evaluated using a protocol based on the EP17-A from CLSI. The quantification limit is 2.5 g/dL.

PARAMETERS FOR AUTOMATED ANALYZERS

For programming instructions refer to the corresponding applications of the Wiener lab analyzers for the Total Protein method. Use Wiener lab's **Calibrador A plus** for calibration.

WIENER LAB PROVIDES

3 x 125 mL Reagent A
 1 x 125 mL Reagent B
 (Cat. N° 1999736)

REFERENCES

- Dumas, B; Bayse, D; Carter, R; Peters, T schaffer, R.- A candidate reference method for determination of total protein in serum. I. development and validation. - Clin. Chem. 27/10:1642, 1981.
- Glick, M; Ryder, K; Jackson, S. - Graphical comparisons of interferences in clinical chemistry instrumentation. - Clin. Chem. 32:470, 1986.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.
- Tholen, D; Kallner, A; Kennedy, J; Krouwer, J; Meier, K. - Evaluation of precision of quantitative measurement methods; Approved Guideline - Second Edition. Vol. 24 N° 25, 2004.
- Tholen, D; Kroll, M; Astles, J; Happe, T; Krouwer, J; Lasky, F. EP6-A. - Evaluation of the Linearity of quantitative Measurement Procedures: A Statical approach; Approved Guideline. - Vol. 23 N° 16, 2003.
- Tholen, D; Linnet, K; Kondratovich, M; Armbruster, D; Garret, P; Jones, R; Kroll, M; Lequin, R; Pankratz, T; Scasellati, G; Schimmel, H; Tsai, J. EP17-A. - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. - Vol. 24 N° 34, 2004.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Authorized representative in the European Community



"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution



Contents



Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use



Calibrator



Control



Positive Control



Negative Control



Catalog number

 Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
<http://www.wiener-lab.com.ar>
Dir. Téc.: Viviana E. Cétola
Biochemist
A.N.M.A.T. Registered product
PM-1102-78



Wiener lab.

2000 Rosario - Argentina