

## Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack (s) can be used
08058652190	Total Protein Gen.2 (1050 tests)	System-ID 2111 001 <b>cobas c 303, cobas c 503</b>
Materials required (but not provided):		
10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302
11333127122	Precipath Protein (3 x 1 mL)	Code 20303
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001

## English

## System information

TP2: ACN 21110

## Intended use

In vitro test for the quantitative determination of total protein in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary<sup>1</sup>

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and in bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values.

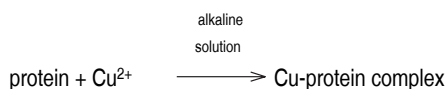
Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency).

Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma proteins can be due to a change in the percentage of one plasma protein fraction. Often in such cases the amount of total protein does not change. The A/G ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosus as well as in certain acute and chronic inflammations. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.

Test principle<sup>2</sup>

## Colorimetric assay

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.



The color intensity is directly proportional to the protein concentration which can be determined photometrically.

## Reagents - working solutions

- R1** Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L
- R3** Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L; potassium iodide: 61 mmol/L; copper sulfate: 24.3 mmol/L

R1 is in position B and R3 is in position C.

## Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



## Warning

H290 May be corrosive to metals.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H411 Toxic to aquatic life with long lasting effects.

## Prevention:

P264 Wash skin thoroughly after handling.

P273 Avoid release to the environment.

P280 Wear protective gloves/ eye protection/ face protection.

## Response:

P337 + P313 If eye irritation persists: Get medical advice/attention.

P390 Absorb spillage to prevent material damage.

P391 Collect spillage.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

## Reagent handling

Ready for use

## Storage and stability

Shelf life at 15-25 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer:

26 weeks

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K<sub>2</sub>-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

<i>Stability:</i> <sup>3</sup>	6 days at 20-25 °C
	4 weeks at 4-8 °C
	1 year at -20 °C

The total protein concentration is 4 to 8 g/L lower when the sample is collected from a patient situated in the recumbent position rather than upright.<sup>4</sup>

#### Materials provided

See "Reagents – working solutions" section for reagents.

#### Materials required (but not provided)

See "Order information" section

General laboratory equipment

#### Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

#### Application for serum and plasma

##### Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/546 nm		
Reagent pipetting		Diluent (H <sub>2</sub> O)	
R1	59 µL	18 µL	
R3	21 µL	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	1.3 µL	–	–
Decreased	1.3 µL	25 µL	50 µL
Increased	1.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

#### Calibration

Calibrators	S1: H <sub>2</sub> O
	S2: C.f.a.s.
Calibration mode	Linear

Calibration frequency

Automatic full calibration  
- after reagent lot change

Full calibration  
- as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against SRM 927.

#### Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

#### Calculation

**cobas c** systems automatically calculate the analyte concentration of each sample in the unit g/L (g/dL).

Conversion factor:  $\text{g/L} \times 0.1 = \text{g/dL}$

#### Limitations - interference

Criterion: Recovery within  $\pm 10\%$  of initial value at a total protein concentration of 66 g/L (6.6 g/dL).

Icterus<sup>5</sup>: No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis<sup>5</sup>: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid)<sup>5</sup>: No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Dextran: No significant interference from dextran up to a concentration of 30 mg/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>6,7</sup>

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>8</sup>

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

#### ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet for information. For further instructions refer to the operator's manual.

#### Limits and ranges

##### Measuring range

2.0-120 g/L (0.2-12 g/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

#### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

Limit of Blank	= 2.0 g/L (0.2 g/dL)
Limit of Detection	= 2.0 g/L (0.2 g/dL)
Limit of Quantitation	= 3.0 g/L (0.3 g/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95<sup>th</sup> percentile value from  $n \geq 60$  measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration total protein samples.

#### Expected values

g/L

*Expected values according to Josephson<sup>9</sup>*

Adults 66-87 g/L\*

\* calculated by unit conversion factor

*Expected values according to Tietz<sup>10</sup>*

Umbilical cord 48-80 g/L

Premature 36-60 g/L

Newborn 46-70 g/L

1 week 44-76 g/L

7 months-1 year 51-73 g/L

1-2 years 56-75 g/L

> 3 years 60-80 g/L

Adults (ambulatory) 64-83 g/L

*Expected values according to Australasian Association of Clinical Biochemists<sup>11</sup>*

Adults 60-80 g/L

g/dL

*Expected values according to Josephson<sup>9</sup>*

Adults 6.6-8.7 g/dL

*Expected values according to Tietz<sup>10</sup>*

Umbilical cord 4.8-8.0 g/dL

Premature 3.6-6.0 g/dL

Newborn 4.6-7.0 g/dL

1 week 4.4-7.6 g/dL

7 months-1 year 5.1-7.3 g/dL

1-2 years 5.6-7.5 g/dL

> 3 years 6.0-8.0 g/dL

Adults (ambulatory) 6.4-8.3 g/dL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

#### Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

#### Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute)

EP05-A3 requirements with repeatability ( $n = 84$ ) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c 503** analyzer.

Repeatability	Mean	SD	CV
	g/L	g/L	%
PCCC1 <sup>a)</sup>	49.5	0.244	0.5
PCCC2 <sup>b)</sup>	73.0	0.358	0.5
Human serum 1	4.61	0.140	3.0
Human serum 2	32.1	0.224	0.7
Human serum 3	61.4	0.292	0.5
Human serum 4	72.9	0.394	0.5
Human serum 5	103	0.588	0.6

Intermediate precision	Mean	SD	CV
	g/L	g/L	%
PCCC1 <sup>a)</sup>	49.7	0.396	0.8
PCCC2 <sup>b)</sup>	73.0	0.495	0.7
Human serum 1	4.83	0.196	4.1
Human serum 2	32.4	0.326	1.0
Human serum 3	61.4	0.396	0.6
Human serum 4	72.9	0.453	0.6
Human serum 5	103	0.686	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c 503** analyzer(s) are representative for **cobas c 303** analyzer(s).

#### Method comparison

Total protein values for human serum samples obtained on a **cobas c 503** analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Sample size ( $n$ ) = 74

Passing/Bablok <sup>12</sup>	Linear regression
$y = 1.010x - 0.0180 \text{ g/L}$	$y = 1.010x - 0.0639 \text{ g/L}$
$\tau = 0.975$	$r = 0.999$

The sample concentrations were between 8.43 and 116 g/L.

Total protein values for human serum samples obtained on a **cobas c 303** analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Sample size ( $n$ ) = 74

Passing/Bablok <sup>12</sup>	Linear regression
$y = 1.012x - 0.536 \text{ g/L}$	$y = 1.015x - 0.785 \text{ g/L}$
$\tau = 0.979$	$r = 1.000$

The sample concentrations were between 7.50 and 117 g/L.

#### References

- 1 Brobeck JR, ed. Physiological Basis of Medical Practice, 9th ed. Baltimore, MD: Wilkins and Wilkins 1973;4-7.
- 2 Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol 1946;10:40-49.
- 3 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.
- 4 Koller A. Total serum protein. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, theory, analysis, and correlation St. Louis: Mosby Company 1984;1316-1319.




- 5 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 6 Breuer J. Report on the Symposium "Drug Effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 7 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- 8 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 9 Josephson B, Gyllenswärd C. The Development of the Protein Fractions and of Cholesterol Concentration in the Serum of Normal Infants and Children. Scandinav J Clin Lab Investigation 1957;9:29.
- 10 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;518-523.
- 11 Tate JR, Sikaris KA, Jones GRD, et al. Harmonising adult and paediatric reference intervals in Australia and New Zealand: An evidence-based approach for establishing a first panel of chemistry analytes. Clin Biochem Rev 2014; Nov 35(4):213-35.
- 12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see [dialog. Roche.com](http://dialog. Roche.com) for definition of symbols used):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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