

# TPUC3

Total Protein Urine/CSF Gen.3

cobas®

## Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
08058679190	Total Protein Urine/CSF Gen.3 (650 tests)	System-ID 2112 001 <b>cobas c 303, cobas c 503</b>
Materials required (but not provided):		
03121305122	C.f.a.s. PUC (5 x 1 mL)	Code 20489
03121313122	Precinorm PUC (4 x 3 mL)	Code 20240
03121291122	Precipath PUC (4 x 3 mL)	Code 20241
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001

## English

### System information

TPU3: ACN 21122 (Urine)

TPC3: ACN 21123 (CSF)

### Intended use

In vitro test for the quantitative determination of protein in human urine and cerebrospinal fluid on Roche/Hitachi **cobas c** systems.

### Summary

Protein measurements in urine are used in the diagnosis and treatment of disease conditions such as renal or heart diseases, or thyroid disorders, which are characterized by proteinuria or albuminuria. Cerebrospinal fluid (CSF) protein measurements are used in the diagnosis and treatment of conditions such as meningitis, brain tumors and infections of the central nervous system.<sup>1</sup>

Urine is formed by ultrafiltration of plasma across the glomerular capillary wall. Proteins with a relative molecular mass > 40000 are almost completely retained, while smaller substances easily enter the glomerular filtrate. Most CSF protein originates by diffusion from plasma across the blood-CSF barrier. Elevated levels occur as a result of increased permeability of the blood-CSF barrier or with increased local synthesis of immunoglobulins.

Turbidimetric methods using trichloroacetic acid (TCA) or sulfosalicylic acid (SSA) precipitate proteins in the sample depending on their size; the resulting turbidity may be unstable and flocculate. Reagents of dye-binding methods such as Coomassie blue and pyrogallol red-molybdate react with proteins depending on their amino acid composition, but may stain glass and plastic ware. Due to their reaction mechanisms all methods, turbidimetric and colorimetric, exhibit different sensitivities to various proteins, especially to protein fragments such as Bence Jones proteins<sup>2</sup> and small proteins such as  $\alpha$ 1-microglobulin.

The Roche Diagnostics Urinary/CSF Protein assay is based on the method described by Iwata and Nishikaze,<sup>3</sup> later modified by Luxton, Patel, Keir, and Thompson.<sup>4</sup> In this method, benzethonium chloride reacts with protein in a basic medium to produce a turbidity that is more stable and evenly distributed than that observed with the SSA or TCA methodologies. This assay shows an underrecovery of  $\gamma$ -globulin compared to albumin of about 30 %<sup>5</sup> and no interference from magnesium ions due to the addition of EDTA.

### Test principle

Turbidimetric method.

The sample is preincubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity.

### Reagents - working solutions

**R1** Sodium hydroxide: 677 mmol/L; EDTA-Na: 74 mmol/L

**R3** Benzethonium chloride: 32 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:



### Danger

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

H412 Harmful to aquatic life with long lasting effects.

### Prevention:

P273 Avoid release to the environment.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

### Response:

P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. + P331

P303 + P361 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. + P353

P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. + P310 Immediately call a POISON CENTER/ doctor.

P305 + P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. + P338 Continue rinsing. Immediately call a POISON CENTER/ doctor. + P310

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.

Urine

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## Total Protein Urine/CSF Gen.3



Use random or 24-hour urine specimens. Use no preservatives. Refrigerate specimen during collection.

### CSF

No special additives are required. Blood in a CSF specimen invalidates the protein value.<sup>1</sup>

Samples for urinary/CSF protein should be collected before fluorescein is given or at least 24 hours later.<sup>6</sup>

**Note:** Urine, CSF and control samples with a protein concentration above 7000 mg/L must not be measured with TPUC3 as this may clog the instrument lines.

### Stability:<sup>7</sup>

Urine:	1 day at 15-25 °C
	7 days at 2-8 °C
	1 month at (-15)-(-25) °C
CSF:	1 day at 15-25 °C
	6 days at 2-8 °C
	> 1 year at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Non centrifuged samples may produce elevated results.

### 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 urine and CSF

#### Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/505 nm		
Reagent pipetting		Diluent (H <sub>2</sub> O)	
R1	75 µL	–	
R3	30 µL	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	4.5 µL	–	–
Decreased	4.5 µL	25 µL	50 µL
Increased	4.5 µL	–	–

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

### Calibration

#### Application for urine (ACN 21122)

Calibrators	S1: H <sub>2</sub> O
	S2-S6: C.f.a.s. PUC
Calibration mode	Non-linear

Calibration frequency	Full calibration
	- after reagent lot change - as required following quality control procedures

#### Application for CSF (ACN 21123)

Calibrators	S1: H <sub>2</sub> O
	S2-S6: C.f.a.s. PUC
Calibration mode	Non-linear
Calibration frequency	Full calibration
	- after reagent lot change - as required following quality control procedures

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

Traceability:<sup>8</sup> This method has been standardized against a primary standard traceable to NIST.

### Quality control

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

Urine: Precinorm PUC, Precipath PUC

CSF: Precinorm PUC, Precipath PUC

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 mg/L (mg/dL, g/L).

Conversion factors:	mg/L x 0.1 = mg/dL
	mg/L x 0.001 = g/L

To calculate 24-hour urine protein excretion:  
mg/L x total volume (liters per 24 hours) = mg/day.

### Limitations - interference

High dose hook-effect: No false result without a flag was observed up to a total protein concentration of 100000 mg/L.

#### Urine

Criterion: Recovery within  $\pm 10$  % of initial value at a total protein concentration of 120 mg/L.

Icterus: No significant interference up to a conjugated bilirubin concentration of 342 µmol/L or 20 mg/dL.

Hemolysis: Hemoglobin interferes.<sup>9</sup>

Urea: No significant interference from urea up to a concentration of 1300 mmol/L (7809 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>10</sup>

Exception: Levodopa, methyldopa and Na<sub>2</sub>-cefoxitin cause artificially high total protein results and calcium dobesilate causes artificially low protein results.

Other: Patient samples containing > 8 g/L of organically bound iodine from Radiopaque media (e.g. Hexabrix) may have falsely elevated results.

High levels of homogentisic acid can be found in urine of patients with the rare genetic disorder Alkaptonuria.<sup>11</sup> Homogentisic acid in urine samples at concentrations > 0.6 mmol/L can cause false results.

The administration of gelatin-based plasma replacements can lead to increased urine protein values.

### CSF

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## Total Protein Urine/CSF Gen.3



Criterion: Recovery within  $\pm 10\%$  of initial value at a total protein concentration of 450 mg/L.

Icterus: No significant interference up to an I index of 15 for conjugated bilirubin (approximate conjugated bilirubin concentration: 15 mg/dL).

Hemolysis: Hemoglobin interferes.<sup>9</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

40-2000 mg/L

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 = 40 mg/L

Limit of Detection = 40 mg/L

Limit of Quantitation = 40 mg/L

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 urine/CSF samples.

### Expected values

#### Urine<sup>12</sup>

24 h: < 140 mg/24 h\*

Random: < 150 mg/L\*

\* Values obtained from centrifuged samples

#### CSF

Reference range acc. to Tietz:<sup>13</sup> 150-450 mg/L

Reference range acc. to Thomas:<sup>14</sup> 200-400 mg/L\*

\* calculated by unit conversion factor

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.

#### Urine

<i>Repeatability</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mg/L</i>	<i>mg/L</i>	<i>%</i>
Precinorm PUC	202	5.52	2.7
Precipath PUC	1373	5.64	0.4
Human urine 1	80.4	3.52	4.4
Human urine 2	329	5.05	1.5
Human urine 3	486	5.72	1.2
Human urine 4	994	5.36	0.5
Human urine 5	1644	7.46	0.5

<i>Intermediate precision</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mg/L</i>	<i>mg/L</i>	<i>%</i>
Precinorm PUC	202	6.14	3.0
Precipath PUC	1373	7.63	0.6
Human urine 1	74.8	3.88	5.2
Human urine 2	329	5.55	1.7
Human urine 3	499	6.94	1.4
Human urine 4	994	7.65	0.8
Human urine 5	1644	10.2	0.6

#### CSF

<i>Repeatability</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mg/L</i>	<i>mg/L</i>	<i>%</i>
Precinorm PUC	1615	9.06	0.6
Precipath PUC	242	9.60	4.0
Human CSF 1	130	9.52	7.3
Human CSF 2	357	7.72	2.2
Human CSF 3	501	7.16	1.4
Human CSF 4	1087	8.56	0.8
Human CSF 5	1715	10.9	0.6

<i>Intermediate precision</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mg/L</i>	<i>mg/L</i>	<i>%</i>
Precinorm PUC	1615	10.7	0.7
Precipath PUC	242	11.3	4.7
Human CSF 1	130	9.88	7.6
Human CSF 2	357	9.57	2.7
Human CSF 3	503	8.29	1.6
Human CSF 4	1063	13.0	1.2
Human CSF 5	1715	12.4	0.7

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

**Method comparison**

Total protein values for human urine and CSF 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).

*Urine*

Sample size (n) = 77

Passing/Bablok <sup>15</sup>	Linear regression
$y = 0.952x + 19.3 \text{ mg/L}$	$y = 0.948x + 24.0 \text{ mg/L}$
$\tau = 0.983$	$r = 0.999$

The sample concentrations were between 40.8 and 1784 mg/L.

*CSF*

Sample size (n) = 75

Passing/Bablok <sup>15</sup>	Linear regression
$y = 0.968x + 31.3 \text{ mg/L}$	$y = 0.954x + 38.6 \text{ mg/L}$
$\tau = 0.990$	$r = 1.000$

The sample concentrations were between 43.4 and 1890 mg/L.

Total protein values for human urine and CSF 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).

*Urine*

Sample size (n) = 71

Passing/Bablok <sup>15</sup>	Linear regression
$y = 1.012x + 3.81 \text{ mg/L}$	$y = 1.011x + 6.01 \text{ mg/L}$
$\tau = 0.981$	$r = 0.999$

The sample concentrations were between 45.5 and 1879 mg/L.

*CSF*

Sample size (n) = 77

Passing/Bablok <sup>15</sup>	Linear regression
$y = 1.046x + 17.5 \text{ mg/L}$	$y = 1.029x + 25.4 \text{ mg/L}$
$\tau = 0.987$	$r = 1.000$

The sample concentrations were between 89.8 and 1926 mg/L.

**References**

- 1 Tietz NW. Fundamentals of Clinical Chemistry, 3rd ed. Pa: WB Saunders Co 1987:336.
- 2 Boege F. Bence Jones-Proteine. J Lab Med 1999;23(9):477-482.
- 3 Iwata J, Nishikaze O. New micro-turbidimetric method for determination of protein in cerebrospinal fluid and urine. Clin Chem 1979;25(7):1317-1319.
- 4 Luxton RW, Patel P, Keir G, et al. A micro-method for measuring total protein in cerebrospinal fluid by using benzethonium chloride in microtiter plate wells. Clin Chem 1989;35(8):1731-1734.
- 5 Hohnadel DC, Koller A. Urine protein total. In: Pesce AJ, Kaplan LA, editors. Methods in clinical chemistry, St. Louis, Mosby 1987.
- 6 Koumantakis G. Fluorescein Interference with Urinary Creatinine and Protein Measurements. Clin Chem 1991;37/10:1799.
- 7 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.
- 8 Standard Reference Materials from NERL, traceable to NIST (National Institute of Standards and Technology).
- 9 Yilmaz FM, Yücel D. Effect of Addition of Hemolysate on Urine and Cerebrospinal Fluid Assays for Protein. Clin Chem 2006;52:152-153.
- 10 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.
- 11 Phornphutkul C, Introne WJ, Perry MB, et al. Natural History of Alkaptonuria. N Engl J Med 2002;347(26):2111-2121.

- 12 Junge W, Wilke B, Halabi A, et al. Reference Intervals for Total Protein in Collected and Random Urine using the Benzethonium Chloride Method [Abstract]. Clin Chem 2006;52:157.
- 13 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;518-523.
- 14 Thomas L. Labor und Diagnose, 6. Auflage, TH-Books Verlagsgesellschaft mbH, Frankfurt/Main 2005;930-934.
- 15 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):

 CONTENT

Contents of kit



Volume for reconstitution

 GTIN

Global Trade Item Number

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Additions, deletions or changes are indicated by a change bar in the margin.

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
[www.roche.com](http://www.roche.com)

+800 5505 6606

