TOTAL PROTEIN U/CSF LS Mono

IVD

CE

mtd DIAGNOSTICS

Quantitative determination of Total Protein in Urine or Cerebrospinal Fluid. Colorimetric method (Pyrogallol Red). Endpoint.

REF **CC1290** R1:4x60 mL + R2: 1x3 mL (standard)

METHOD AND PRINCIPLE

Proteins, in acid solution and in the presence of molybdate, bind to Pirogallol Red to form a colored complex whose color intensity, measured photometrically at 600 nm, is directly proportional to the protein concentration present in the sample under examination.

CLINICAL SIGNIFICANCE

Normally, the proteins present in the blood (globulins, albumins, etc.), because of their size, can not pass the kidney filter, but in various pathological conditions involving the kidney, these are no longer able to restrain them, allowing their loss in the urine. Normal urinary protein excretion is less than 150 mg per day. Protein urinary excretion of protein greater than 150 mg per day is called proteinuria. A proteinuria that persists beyond a single measurement should not be ignored and should be evaluated by the doctor. Sometimes patients have obvious symptoms such as: edema (swelling of the legs or body), blood in the urine (haematuria) or pus (pyuria), but in many cases patients with proteinuria have no symptoms.

The presence of proteins in the urine can be associated with various other conditions and pathologies, including:

Acute Glomerulonephritis, Focal Glomerulonephritis, Amyloidosis, IgA Dependent Nephropathy, Cardiac Disease (Pericarditis, Heart Failure), Multiple Myeloma, Leukemia, Malaria, Sickle Cell Anemia, Rheumatoid Arthritis, Sarcoidosis, Systemic Lupus Erythematosus, Heavy Metal Poisoning, Mesangial Proliferation Glomerulonephritis, Renal infection, Bladder cancer, Potentially toxic kidney drugs, Goodpasture syndrome, Polycystic kidney, Urinary tract infection.

In addition to these pathological circumstances, sensitive increases in urinary protein concentrations can also be associated with fairly physiological conditions (this is referred to as transient proteinuria). Exposure to cold or intense heat, fever, severe emotional stress and strenuous physical exercise (both sporting and working) can in fact significantly increase the amount of protein found in the urine sample. Pregnancy may also be associated with mild proteinuria, although significant protein concentrations should cause an ongoing urinary infection to suspect or the development of pre-eclampsia.

Orthostatic proteinuria is a relatively common disease in children and young adults, which is associated with significant losses of protein in the urine during standing (orthostatic). Presumably, this condition is linked to the increase in pressure on the renal glomeruli, which also forces the passage of proteins between the meshes of these filters. In the lying position (clinostatic), the pressure decreases and the loss of protein is reduced; doctors consider this disorder to be of benign origin, given that in the great majority of cases it spontaneously regresses with growth. The orthostatic proteinuria is diagnosed through a collection of the urine divided into 2 samples: one obtained in an upright position and one obtained at night, after the young patient has rested for a few hours and has emptied the bladder before going to bed.

High concentrations in CSF can be caused by intra-cranial infections and pressures. In this case further laboratory tests are necessary and urgent

REAGENT COMPOSITION

Reagent (R1)

Succinate buffer, pH 2.5	60 mmol/L
Pyrogallol red	0.06 mmol/L
Sodium molybdate	0.04 mmol/L
Detergents	< 2 %

Reagent (R2)

Standard (Proteins):	value on label

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light.

Do not freeze the reagents. Store Reagent 1 at 15° - 25°C, Reagent 2 (standard) at 2-8° C.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Blank Absorbance (Abs) at 600 nm > 0.200 in 1cm cuvette.

After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

SPECIMEN

24 hours-urine, CSF (cerebrospinal fluid).

Collect urine without any additive.

Stability in urine: 1 day at 20–25°C ; 2 days at 2-8 °C

Stability in CSF: 1 day at 20° - 25°C ; 6 days at 2° - 8° C ; 1 year at -20°C

Discard contaminated specimens.

PROCEDURE

Wavelength:	600 nm (578– 620)
Temperature:	37° C
Measurement:	against distilled water

Pipette as follow:

Reagent R1	1000 µL
Sample, Std / Cal /H_2O	20 µL

Mix, incubate 5 minutes and read Absorbance within 30 minutes.

CALCULATION

Urine - CSF

$$\text{Total Protein} = \frac{\text{Abs Sample} - \text{Abs Blank Reagent}}{\text{Abs Std/Cal} - \text{Abs Blank Reagent}} \times \text{Concentr. Std/Cal}$$

Urine 24h

Total Protein (mg/24 h) = Total Protein (mg/L) x Vol (L) urine/24 h

Total Protein (mg/24 h) = Total Protein (mg/dL) x Vol (L) urine/24 h x 10

Conversion Factors:

mg/dL x 10 = mg/L

mg/dL x 0.01 = g/L

mg/L x 0.1 = mg/dL

mg/L x 0.001 = g/L

CALIBRATION

Results will depend on the accuracy of the instrument calibration, assay settings, the reagent/specimen ratio and the temperature control.
Use **MTD Diagnostics** standard provided with the kit.

EXPECTED VALUES

In occasional urinary samples, normally there are no proteins but occasionally it is possible to find them in the range 0 - 200 mg/L (0 – 20 mg/dL). In this case the test must be repeated on the 24-hour urine.

24 hours urine :

- Adults < 100 mg/24h
- Pregnant < 150 mg/24h

CSF :

- Children 300 -1000 mg/L
- Adults: 150 - 450 mg/L

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 178; S.D. = 5.23; CV = 2.94%
High Level: Samples = 20; Average = 1560; S.D. = 27.6; CV = 1.77%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

1.02 x + 2.20 r = 0.990

SENSITIVITY: 20 mg/L (2 mg/dL)

LINEARITY: 20 – 3000 mg/L (2-300 mg/dL)

SPECIFICITY / INTERFERENCES

Bilirubin (< 5 mg/dL) does not interfere. Haemoglobin may affect the results. Other drugs and substances may interfere. Positive interferences in urine of patients under treatment with aminoglycosids-gentamicine or tobromycine-reported with other pyrogallol tests have been shown to have no influence with this specific formulation.
CSF contaminated by red cells from a traumatic lumbar puncture or intracerebralhemorrhage will increase protein concentrations by ≈ 10 mg/L for every 1000 erithrocytes.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS

The products do not contain dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

Boege F. Urinary proteins. In: Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 382-400.
Felgenhauer K. Laboratory diagnosis of neurological diseases. In: Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 1308-26.
Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 477-540.
Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogallol red-molybdate method for determining total urinary protein. ClinChem 1989;35:2233-6.
Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K et al. Urinary protein as measured with a pyrogallol red-molybdate complex. Manually and in a Hitachi 726 automated analyzer. ClinChem 1986;32:1551-4.