



Protein in urine and CSF Pyrogallo red. Colorimetric

Quantitative determination of total urinary and CSF protein

For *in-vitro* diagnostic use only.
Store at 2-8°C

PRINCIPLE OF THE METHOD

Protein react in acid solution with pyrogallol red and molybdate to form a colored complex.

The intensity of the color formed is proportional to the protein concentration in the sample¹².

CLINICAL SIGNIFICANCE

In healthy persons, the urine contains no protein or only a trace amount of protein; normally the glomeruli prevent passage of protein from the blood to the glomerular filtrate. Glomerular injury causes increased permeability to plasma proteins, resulting in proteinuria, which refers to the presence of protein in the urine.

A persistent finding of proteinuria is the single most important indication of renal disease.

Elevated concentration of protein in cerebro-spinal fluid (CSF) can be caused by infections and intracranial pressure^{1,5,6}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R	Pyrogallol red	50 mmol/L
	Sodium molybdate	0.04 mmol/L
PROTEIN CAL	Albumin/Globulin aqueous primary standard 1000 mg/L	

PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 598 \geq nm 0.30.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 598 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Urine 24 h: Stability 8 days at 2-8°C.
- Cerebrospinal fluid (CSF): Stable 4 days at 2-8°C.

PROCEDURE

1. Assay conditions:
Wavelength: 598 nm
Cuvette: 1 cm light path
Temperature 37°C / 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard (μ L)	--	20	--
Sample (μ L)	--	--	20

4. Mix and incubate for 5 min at 37°C or 10 min at room temperature (15-25°C).
5. Read the absorbance (A) of the samples and Standard, against the Blank. The color is stable for at least 30 minutes.

CALCULATIONS

Urine 24 h

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 1000 \times \text{vol. (L) urine 24 h} = \text{mg protein/24 h}$$

CSF

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 1000 = \text{mg/L protein in the sample}$$

1000 (Standard Conc.)

QUALITY CONTROL

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁵

Urine:	< 100 mg/24 h (< 150 mg/24 h in pregnancy)
CSF:	Children 300 -1000 mg/L
	Adults 150 - 450 mg/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: Up to *linearity limit* of 4000 mg/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Accuracy: Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis.

A list of drugs and other interfering substances with protein determination has been reported by Young et. al¹.

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

1. Orsonneau JL et al. An improved Pyrogallol Red-Molybdate Method for Determining Total Urinary Protein. Clin Chem 1989 (35):2233-2236.
2. Koller A. Total serum protein. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1316-1324 and 418.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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