Atlas Medical CE

# Protein in urine and CSF
# Pyrogallo red. Colorimetric
# Quantitative determination of total urinary and CSF protein

IVD *For in -vitro diagnostic use only*

Store at 2-8 °C

## INTENDED USE
For the quantitative determination of total urinary and CSF protein.

## INTRODUCTION
In healthy persons, urine contains no protein or only a trace amount of protein; normally glomeruli prevent the passage of protein from blood to the glomerular filtrate. Glomerular injury causes increased permeability to plasma proteins, resulting in proteinuria, which refers to the presence of protein in urine. A persistent finding of proteinuria is the single most important indication of renal disease. Elevated concentrations of protein in cerebro-spinal fluid (CSF) can be caused by infections and intracranial pressure. Clinical diagnosis should not be based on a single test result; it should integrate clinical and other laboratory data.

## PRINCIPLE
Protein reacts in acid solution with pirogallol red and molybdate to form a colored complex.
The intensity of the color formed is proportional to protein concentration in sample.

## MATERIALS
## REAGENTS

| | | |
|---|---|---|
| **R1** | Pyrogallol red<br>Sodium molybdate | 50 µmol/L<br>0.04 mmol/L |
| **PROTEIN CAL** | Albumin/Globulin aqueous primary standard | 1000 mg/dL |

## EQUIPMENTS NEEDED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 600±20 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

## PREPARATION
- Reagents provided are ready to use.

## STORAGE AND STABILITY
- All 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 nm ≥ 0.70.

## SAMPLES
- Urine protein is stable for 8 days at 2-8°C.
- Cerebrospinal fluid (CSF) protein is stable for 4 days at 2-8°C.

## PROCEDURE
1. Assay conditions:
   Wavelength:……598 nm
   Cuvette light path:……1 cm
   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 (µL) | -- | 20 | -- |
| Sample (µL) | -- | -- | 20 |

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

## CALCULATIONS
**Urine 24 h**

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 1000 \times V = \text{mg/24-h}$$

V=Liters urine/24-h
1000= mg/L standard

**Urine 24 h**

$$\frac{(A)\text{Sample} - (A)\text{Blank}}{(A)\text{Standard} - (A)\text{Blank}} \times 1000 \times \text{Vol.(L) Urine 24 h} = \text{mg protein /24 h}$$

**CSF**

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

Samples with concentrations higher than 400 mg/dl should be diluted 1:1 with saline and assayed again. Multiply the result by 2.

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

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

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

## PERFORMANCE CHARACTERISTICS
**Measuring range**: From detection limit 9.44 mg/L to linearity limit of 4000
mg/L.If the concentration is greater than linearity limit, dilute the sample to half with NaCl 9 g/L and multiply the result by 2.
**Precision:**

| | Intra-assay (n=20) | | | Inter-assay (n=20) | | |
|---|---|---|---|---|---|---|
| Mean (mg/dL) | 220 | 536 | 1014 | 216 | 499 | 1018 |
| SD | 3.7 | 4.0 | 5.2 | 18.3 | 26.1 | 166.1 |
| CV (%) | 1.68 | 0.75 | 0.51 | 8.47 | 5.23 | 16.32 |

**Sensitivity:** 1mg/L = 0,00026 (A).
**Accuracy:** Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were as follows:
Correlation coefficient $(r)^2$: 0,9338
Regression equation: y = 0,4294x – 5.4159

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

**INTERFERENCES**

Hemolysis.

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

**NOTES**

1. Currently samples accepted are: 24-h collection; overnight (8-12-h) collection; 1-2-h collection, or first morning sample. Because of the high intraindividual and diurnal variation, at least three separate samples should be assayed.
2. To increase the sensitivity in the normal range, test 50 µL of sample, and dilute the standard 1:4 (1+3) with saline. Use the new concentration of 50 mg/dL for the calculations.

**REFERENCES**


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**PPI1462A01**
**Rev B (24.10.2019)**


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|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |