



3830 Valley Centre Dr. #705275  
San Diego, CA. 92130  
Tel: (858) 450-0065

## CSF/URINE TOTAL PROTEIN (COLORIMETRIC)

Catalog Number: BQ 038CR

CSF/Urine Total Protein reagent is used for the quantitative determination of total protein, using manual or automated procedure, in human urine or cerebrospinal fluid.

### INTRODUCTION

Measurement of Urine Protein is becoming increasingly important in the detection of renal pathology. <sup>1</sup> Proteinuria (increased amounts of protein in urine) can occur in increased glomerular permeability, defective tubular reabsorption and abnormal secretion of protein into the urinary tract. <sup>2</sup> Albuminuria (increased amounts of albumin in urine) has been recognized as an early indicator of renal damage in diabetes, that can be reversed if detected and treated sufficiently early. <sup>3</sup>

The measurement of CSF total protein and specific protein is used to detect increased permeability of the blood/brain barrier (the capillary endothelium of vessels of the central nervous system) to plasma proteins or to detect increased intrathecal secretion of immunoglobins. <sup>1</sup>

### PRINCIPLE

The total protein test for urine is based on the procedure developed by Watanabe et al<sup>4</sup> which is a dye-binding colorimetric method utilizing pyrogallol red-molybdate complex, and modified<sup>5</sup> to equalize the reactivity of albumin and g-globulin, and provide good precision and linearity.

The pyrogallol red is combined with molybdenum acid, forming a red complex with maximum absorbance at 467 nm. When this complex is combined with protein in acidic conditions, a blue-purple color develops with an increase in absorption at 598 nm<sup>4</sup>.

### REAGENTS

1. CSF/Urine Protein Reagent: 2.4 mg/dL pyrogallol red, 0.96 mg/dL Sodium molybdate and surfactants in buffer solution.
2. Total Protein Standard, 100 mg/dL: Bovine serum albumin in aqueous solution with 0.1% sodium azide as preservative tested during manufacturing using standards that traceable to the National Institute of Standards and Technology (NIST) Reference Material # SRM927a.

### PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Exercise universal precautions required for handling of all laboratory reagents.
3. Standard contains sodium azide. May react with copper or lead plumbing to form explosive metal azide build up. Upon disposal, flush with large volume of water.

### REAGENT STORAGE AND STABILITY

Reagent and Standard are stable until the expiration date on the label, when stored at 2 – 8 °C.

### REAGENT DETERIORATION

1. Do not use if the reagent appears turbid or has precipitation.
2. Do not use if the reagent fails to obtain accurate results.

### MATERIALS PROVIDED

1. CSF/Urine Protein Reagent
2. Total Protein Standard (100 mg/dL)

### MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer capable of accurately measuring absorbance at 600 nm.
2. Test tubes with optical properties suitable for use at 600 nm.
3. Pipetting device for the accurate delivery 3.00 mL
4. Pipetting device for the accurate delivery 50 mL (0.050 mL)
5. Incubator capable of maintaining 37 °C
6. Timer
7. Test tubes/rack

### SPECIMEN COLLECTION AND PREPARATION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T.
2. Random urine or 24-hour urine specimens may be used (keep specimen on ice during collection<sup>6</sup>). First morning specimens is preferred for random specimens. Store at 2 – 4 °C for up to 24 hours. Stable frozen at -20°C for up to 1 year. No preservatives are required<sup>6</sup>.
3. CSF (lumbar) must be free from hemolysis. Centrifuge before analysis. CSF may be stored at 4 °C for <72 hours. Stable at -20°C for 6 months, or at -70 °C indefinitely. Specimens should not contain blood<sup>6</sup>.

### INTERFERING SUBSTANCES

1. It is recommended not to use urine specimens with added preservatives since some preservatives, such as HCl, Benzoic acid have shown to interfere in the protein assay, giving false low results<sup>4</sup>.
2. Some drugs and medications may interfere, see Fujita et al<sup>7</sup>

### PROCEDURE (MANUAL)

1. Set the temperature of the incubator to 37 °C.
2. Warm CSF/Urine Protein Reagent to 37 °C.
3. Label test tubes: Blank, Standard, Sample, Control, etc...
4. Pipette 3.0 mL of CSF/Urine Protein Reagent into all tubes
5. To tube labeled BLANK add 50 mL of water. To tube labeled STANDARD add 50 mL of standard. To tube(s) labeled SAMPLE add 50 mL of sample(s).
6. Mix all tubes by inversion and place them into the incubator (37°C) for 10 minutes.
7. After 10 minutes, set spectrophotometer wavelength at 600 nm and the absorbance reading to zero with BLANK.(Wavelength range: 580-630nm)
8. Read and record absorbance of STANDARD and SAMPLE versus BLANK as reference.
9. To calculate concentration(s) of sample(s) see CALCULATIONS section.

### QUALITY CONTROL

The validity of the reaction should be monitored by use of normal and abnormal controls with known values for each assay.

### CALIBRATION

Use the aqueous protein standard (traceable to NIST # SRM927a) provided in the kit for calibration. The concentration of total protein should be validated by comparison with commercially available standard. For automated instrument, refer to appropriate application specification.

## CALCULATIONS

Values are derived by the following equation:

$$\text{Urine Protein (mg/24 hours)} = \text{Au/As} \times \text{Cs} \times \text{V}$$

or

$$\text{Protein (mg/dL)} = \text{Au/As} \times \text{Cs}$$

Where Au and As are the absorbance values of unknown and standard, respectively, Cs is the concentration of the standard (mg/dL) and V is the 24 hours urine volume in dL.

Example:

$$\begin{aligned} \text{Urine Total Protein (mg/day) [ 24 hours ]} &= \frac{0.020}{0.195} \times 100 \times 11 \\ &= 113 \text{ mg/day} \end{aligned}$$

## PROCEDURE LIMITATIONS

1. Samples that exceed the linearity limit (200 mg/dL) should be diluted with an equal volume of isotonic saline and rerun. Multiply the result by two to compensate for the dilution.
2. In urine, intense exercise will elevate the result (<250 mg/day)<sup>6</sup>.
3. In premature infants, CSF protein values >130 mg/dL may occasionally be observed<sup>6</sup>.

NOTE : If greater sensitivity is desired for normal or marginally elevated specimens, a 100 ml (0.100 mL) sample may be used. In this case, dilute the standard with equal volume distilled or deionized water and use this as 50 mg/dL Standard instead of the 100 mg/dL standard in test.

## EXPECTED VALUES

CSF (newborn)	40 – 120 mg/dL <sup>6</sup>
(< 1 mo.)	20 – 80 mg/dL <sup>6</sup>
(> 1 mo.)	15 – 40 mg/dL <sup>6</sup>
CSF (lumbar) (adult)	8 – 32 mg/dL <sup>6</sup>
Random urine under	10 mg/dL <sup>8</sup>
Urine (24 hours)	28 – 141 mg/day <sup>8</sup>

## PERFORMANCE

1. Linearity: 0-200 mg/dL
2. Sensitivity: Base on instrument absorbance resolution of 0.001, this procedure has a sensitivity of 0.58 mg/dL ( $\Delta\text{Abs.} = 0.001$ ).
3. Comparison: A comparison study was performed between the procedure described (Y) and a similar established technique (X) on an Abbott VP chemistry analyzer. Urine samples of 83 range from 0.1-163.9 mg/dL and CSF samples of 79 range from 4-139 mg/dL. Lower 95% confidence interval point (at 14 mg/dL) = 15.27 mg/dL. Upper 95% confidence interval point (at 14 mg/dL) = 15.43 mg/dL.

Sample	Correlation Coefficient	Sample Size	Regression Equation	Total Error
Urine	0.995	83	$Y = 0.96X + 4.86$	3.02
CSF	0.996	79	$Y = 0.95X + 4.86$	2.20

4. Precision: The tests were performed according to NCCLS guideline #EP5-T. 3 different urine samples were used for both the within-run and run-to-run precision studies. Each sample was subjected to 21 replicated assay for the run-to-run precision study. For the within run precision study, each sample was subjected to 4 replicated assays per day for 5 days (total 20 assays).

	Within-run			Run-to-Run		
	#1	#2	#3	#1	#2	#3
Mean (mg/dL)	7.41	56.1	114.6	7.04	60.3	114.1
S.D. (mg/dL)	0.30	0.8	0.9	0.68	0.9	1.0
CV. (%)	4.04	1.5	0.8	9.67	1.6	0.9
No. of assays	21	21	21	20	20	20

## REFERENCES

1. Carone FA, Peterson Dr., Oparil S, Pullman TN. Renal tubular transport and catabolism of proteins and peptides. Kidney Int; 16: 271-8; (1979).
2. Teitz, N.W.: Textbook of Clinical Chemistry. W.B. Saunders Co. pp. 602-613 (1986).
3. Viberti GC, Pickup JC, Jarrett RJ, Keon H. Effect of control of blood glucose on urinary excretion of albumin and B2-microglobulin in insulin-dependent diabetics. N Engl J Med; 300: 638-41 (1979).
4. Watanabe N, Kamei S, Ohkubo A, et al. Urinary protein as measured with a Pyrogallol red-molybdate complex, manually and in Hitachi 726 automated analyzer. Clin Chem; 32: 1551-4 (1986).
5. Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogallol red-molybdate method for determining Total Urinary Protein. Clin Chem; 35: 2233-6 (1989).
6. Tietz, N.W.: Clinical guide to laboratory tests; 470-473 (1990).
7. Fujita y, Mori I, Kitano S. Color Reaction Between Pyrogallol Red-Molybdenum (VI) Complex and Protein. Benseki Kagaku; 32: 379-86 (1983).
8. Tietz, N.W.: Fundamental of clinical chemistry (1976): 356, 358, 369.