ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

URINE PROTEIN

INTENDED USE
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[1]. 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[2]. 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[3].

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[1]

PRINCIPLE
The total protein test for urine is based on the procedure developed by Watanabe et al[4] which is a dye-binding colorimetric method utilizing pyrogallol red-molybdate complex, and modified[5] to equalize the reactivity of albumin and γ-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[4].

REAGENT COMPOSITION
1. CSF/Urine Protein Reagent: 2.4 mg/dL pyrogallol red, 0.96 mg/dL Sodium molybdate and surfactants in buffer solution.
2. CSF/Urine 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.

WARNINGS AND 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. CSF/Urine 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 devices for the accurate delivery 3.0 mL and 50 µL
4. Incubator capable of maintaining 37°C
5. Timer
6. 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[6]). 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[6].
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[6].

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[4].
2. Some drugs and medications may interfere, see Fujita et al[7]

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, etc…
4. Pipette 3.0 mL of CSF/Urine Protein Reagent into all tubes.
5. To tube labeled BLANK add 50 µL of water. To tube labeled STANDARD add 50 µL of standard. To tube(s) labeled SAMPLE add 50 µL 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
It is recommended that controls be included in each set of assays. Commercially available control material with established CSF/Urine protein values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

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:

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

or

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

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:

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

$$= 113 \text{ mg/day}$$

PROCEDURAL 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)[6].
3. In premature infants, CSF protein values >130 mg/dL may occasionally be observed[6].

NOTE : If greater sensitivity is desired for normal or marginally elevated specimens, a 100 µl (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 [6]
(<1 mo.)	20-80 mg/dL [6]
(>1 mo.)	15-40 mg/dL[6]
CSF (lumbar) (adult)	8-32 mg/dL [6]
Random urine	under 10 mg/dL [8]
Urine (24 hours)	28-141 mg/day [8]

PERFORMANCE CHARACTERISTICS

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 (ΔAbs. = 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

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