ANA BIO CLR MICROPROTEIN

(Pyrogallol Red Method)

Intended Use

Microprotein is a reagent kit used for quantitative determination of Proteins in human urine and cerebrospinal fluid (CSF).

Principle

Pyrogallol red ion in the presence of molybdate ion reacts with the proteins in urine/ CSF sample to form a blue-purple color complex.

Pyrogallol Red + Protein + Molybdate ⟶ Blue-purple Complex

Intensity o the color formed is directly proportional to the concentration of microproteins in sample. The intensity is measured at 600 nm.

Reagents Provided

1. Microprotein reagent – Ready to use liquid reagent
2. Standard – Microprotein (100 mg/dl)

Reagent storage, stability and handling

Microprotein reagent and standard are stable till the expiry date indicated on the bottle label when stored at 2° - 8℃.

Specimen collection and preservation

Urine or CSF can be used for the testing. CSF should be free from hemolysis and any cellular debris. Preferably 24 hours urine collection should be used for analysis. Urine specimen may be stored at 2° - 8℃ for up to 24 hours or frozen up to 3 months until assayed and CSF specimen may be stored at 2° - 8℃ for several days or frozen up to 3 months.

Specimen collection and preservation

Reaction type	End point with standard
Reaction slope	Increasing
Incubation time	10
Wavelength	600 nm (570 – 630 nm)
Blank	Reagent Blank
Sample volume	10 µl (0.01 ml)
Reagent volume	1000 µl (1.0 ml)
Standard concentration	100 mg/dl
Factor calculation	100 mg/dl÷ Abs. of Std.
Low Normal	15 mg/dl
High Normal	45 mg/dl
Linearity	400 mg/dl

Assay guidelines for Manual procedure

Bring the reagent and standard to room temperature before performing the assay.

Reagents	**Blank**	**Standard**	**Sample**
Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	10 µl (0.01 ml)	-
Sample	-	-	10 µl (0.01 ml)

1. Mix thoroughly and incubate at 37 °C for 10 minutes.
2. Read the absorbance against reagent blank at 600 nm
3. The final color is stable for 20 minutes at room temperature.

Calculation

$$\text{Conc. in sample (mg/dl)} = \frac{\text{Sample OD}}{\text{Std. OD}} \times \text{Conc. of Std.}$$

Normal Range

Urinary excretion of protein is normally less than 200 mg/24 hour in the volume of 1000-1500 ml/24 hour. CSF in clinically healthy people ranges from 15-45 mg/dl and in newborns is 40-120 mg/dl.

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation

- The reagent is considered unsatisfactory and should not be used if it develops insoluble precipitation.
- The reagent is linear up to 400 mg/dl. For higher value, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.

Quality Control

To ensure the adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and function together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

Reference

1. Tietz, N.W., Textbook of Clinical Chemistry, (1986).
2. Wiechelman K., et al, "Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation.", An. Biochem., (1988).

Symbols

IVD	In Vitro Diagnostics.		Caution.		Keep away from sun light.		Date of Manufacture.
LOT	Batch No.		Read Instructions.		Fragile.		Product Expiry Date.
CONT	Content.		Storage Temperature.		Keep Dry.		Manufactured By.
REF	Catalogue No.						

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