



PROTEIN (MICRO)-PYROGALLOL RED Method, End Point

Intended Use

The reagents are used for the quantitative determination of Proteins in human urine and cerebrospinal fluid (CSF). For in-vitro diagnostic use only.

Introduction

The presence of proteins in urine is a very sensitive indicator of renal disorder. Proteinuria (increased amount of protein in urine) may occur due to increased glomerular permeability, defective tubular re-absorption and abnormal secretion of proteins into the urinary tract. Albuminuria (increased amount of albumin in urine) has been recognized as an early indicator of renal damage in diabetes that can be reversed if detected and treated early. The measurement of CSF total protein provides an indication of either increased permeability of the blood/brain barrier to plasma proteins or of increased intrathecal secretion of immunoglobulins.

Method

Pyrogallol Red Method, End Point.

Principle

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

Reagent Composition

Reagent 1:

Pyrogallol red	0.076 mmol/l
Sodium molybdate	0.056 mmol/l

Reagent 2:

Protein micro Standard	100 mg/dl
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Precautions

Following precautions should be taken:

- Avoid ingestion, do not pipette by mouth.

- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.

- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened Reagent 1 is stable till expiry as mentioned on the label when stored at 2 – 8°C temperature. Do not freeze. Standard Reagent 2 is stable till expiry mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 3 (Protein micro: 300 mg/dl) & Reagent 4 (Protein micro: 400 mg/dl) can be provided for linearity check with Reagent 2 (Protein micro: 100 mg/dl-Standard).

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagents should be clear solutions. Turbidity and/or precipitation may be because of deterioration.

Sample Collection and Storage

Urine or CSF can be used for the testing. CSF should be free from hemolysis and any cellular debris.

Preferably 24hr urine collection should be used for analysis. Urine specimen may be stored at 2-8°C for up to 24 hours or frozen up to 3 months until assayed and CSF specimen may be stored 2-8°C for several days or frozen up to 3 months.

General Assay Parameters

Mode	End Point
Wavelength 1 (nm)	600
Wavelength 2 (nm)	-
Blank with	Reagent
Sample Volume (µl)	5/10
Reagent 1(µl)	500/1000
Incubation Time (min)	10
Incubation Temperature (°C)	37
Normal Low	Refer to the insert
Normal High	Refer to the insert
Linearity (mg/dl)	Upto 400
Standard Conc. (mg/dl)	100
Units	mg/dl

PROCEDURE

One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

	Blank	Standard	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Dist. Water	10 µl	-	-
Reagent 2	-	10 µl	-
Sample	-	-	10 µl

Mix well & incubate for 10 min. at 37°C. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 600 nm.

Calculation

Protein micro concentration in the sample can be calculated using the following formula:

$$\text{Protein Micro (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.180} \times 100 = 111.11 \text{ mg/dl}$$

Calculation for 24 hour urinary protein:

1. Measure the 24 hour urine volume (ml).
2. Divide the volume by 100 to determine the volume expressed in deciliters (dl).
3. Multiply the 24 hour urine volume in dl by the protein concentration in mg/dl as determined by the test.
4. The resulting value is the amount of protein is expressed as mg/24 hours.

If protein micro concentration exceeds 400mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

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

Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.
2. It is recommended not to use urine specimens with added preservatives since some added preservatives such as HCl and benzoic acid have been shown to interfere in the protein assay, giving false low results. Some drugs and medications may also interfere.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for protein micro.

Performance

Linearity Limit: 400 mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	4.02	10.99
S.D.	0.08	0.08
C.V. %	1.9	0.72

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	4.05	10.98
S.D.	0.07	0.06
C.V. %	1.70	0.57

References

1. Tietz, N.W., Textbook of Clinical Chemistry. W.B. Saunders Philadelphia608,1986.http://www.tecodiag.com/Admin/pdf/345_P_61100B%20Package%20Insert.pdf SYNC HRON CX@4CE/CX@7 Clinical Systems Chemistry Information Manual, Brea, CA, Beckman Coulter, Inc. (1992). http://www.beckman.com/literature/ClinDiag/9282%20p4%20M-TP%2011_02.pdf
2. Wiechelman, K., Braun, R. and Fitzpatrick, J. (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. Anal Biochem. 175, 231-237. wolfson.huji.ac.il/purification/PDF/Protein_Quantification/PIERCE_BCA.pdf

Pack Presentation

Product Code/ Catalogue No.	Pack Size*	Reagent 1	Reagent 2
KGPMI101.3.1	1x25ml	1x25ml	1x2ml
KGPMI101.3.2	2x50ml	2x50ml	1x2ml

*Pack size may vary on market demand.

Revision No: (Ver: KGPMI101.3/1)

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Symbols

Following symbols are used in the labeling of KEE GAD kits:



Catalogue No.



Batch No.



CE Mark



Read instructions



In Vitro Diagnostics



Storage temperature



Expiry Date



Content



Product Name



Manufactured By



Manufactured by:

KEE GAD Biogen Pvt. Ltd.

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