MICROPROTEIN REAGENT KIT
MD-101104

Intended Use
For the quantitative determination of total protein in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
The presence of protein in urine is a very sensitive indicator of renal disorders. There are four ways by which increased amounts of protein can occur: increased glomerular permeability; defective tubular re-absorption; increased plasma concentration of an abnormal, low molecular weight protein; and abnormal secretion of protein into the urinary tract.[1] 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 early.[2]
Various methods have been described for the determination of protein concentrations in biological fluids. These methods are based on colorimetric, turbidimetric, electrophoretic, or immunologic procedures.[3,4] The dye binding methods are characterized as having good precision and sensitivity. The Coomassie Blue method[5] is very sensitive, but the reagents stain glassware and plastic.
This method is based on the procedure developed by Fujita[6] and Watanabe.[7] It is a sensitive dye binding, colorimetric method employing Pyrogallol Red. The method seldom stains cuvettes or plastic tubing and can be automated.
Pyrogallol Red is combined with molybdenum acid at a low pH. When the complex is combined with protein, a blue-purple color is formed. The increase in absorbance at 600 nm is directly proportional to the protein concentration in the sample.

Reagents
MICROPROTEIN REAGENT: Contains Buffer, Pyrogallol Red 0.067 mmol/L, sodium molybdate stabilizer 0.153 mmol/L, surfactants, and preservative.

Reagent Stability and Storage
Store Microprotein Reagent refrigerated (2-8°C). Reagent and is stable until the expiration date shown on the labels.
The reagent should be clear. Do not use if turbid. Failure to achieve assayed control values may indicate deterioration of reagent. Do not use if the reagent absorbance at 600 nm is less than 0.100.

Cautions
Microprotein Reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed. May be harmful if inhaled or swallowed. Do not pipette by mouth. Avoid contact with skin and eyes. In case of contact, flush the area with water. Seek immediate medical attention for eyes.

Instrumentation
Microprotein Reagent Kit for use on Mission Diagnostics Affirm C200* and Beckman AU680 Analyzers. Refer to instrument procedure instructions in the instrument manual provided with the specific analyzer.

Specimen Collection and Handling
Tests are performed on 24-hour samples. The urine should not be collected during periods of exercise because of its effect on albumin concentration. Protein determinations should be performed with fresh specimens. If test cannot be performed with fresh urine, specimens may be stored at –20°C for up to one year.[8]
NOTE: Hemoglobin can increase recovered total protein values, DO NOT USE samples containing blood. Handle all urine samples as if potentially infectious! Specimens containing visible particulate matter should be clarified by centrifugation prior to testing.

Quality Control
Standard practice for Quality Control should be applied to this procedure. Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. Mission Diagnostics provides Quality Controls and Calibrators to meet your program needs and which conform to NLCP Guidelines[9]:

MD-101204 – Microprotein Calibrator

Specificity, Limitations, and Interferences
Microprotein Reagent Kit is for the detection of total protein. False positives may be resulted from improperly stored samples and bacterial growth.
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.[7] Bilirubin to a level of 20 mg/dl and Ascorbic acid to a level of 3.0 mg/dl have been found not to interfere with the assay. (Less than 3.0% deviation for samples in the range of 130.0 - 132.0 mg/dl and less than 17% for samples measured in the range of 9.2 - 12.5 mg/dl) Hemoglobin being a protein, will increase recovered total protein values. pH variation was found to have no effect on the total protein determination. The effects of specific gravity variation was not evaluated. Some drugs and medications may interfere, see Fujita.[6]

* Also known as Zybio EXC200 Analyzer

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Typical Performance Characteristics
The following performance data was obtained using the Affirm C200 and Beckman AU680 Analyzers. Other instruments may yield different performance data.

Linearity
The following results were obtained on Affirm C200 and Beckman AU680 Analyzers using the MISSION Microprotein Reagent Kit on samples containing approximately 10, 20, 38, 73, 146, and 290 mg/dL protein. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
9.884	0.103	1.0
19.030	0.124	0.7
37.748	0.304	0.8
75.384	0.492	0.7
146.208	0.653	0.4
272.100	1.315	0.5

Precision
Studies performed on Affirm C200 and Beckman AU680 Analyzers. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	**SD**	**CV%**	**Mean** (mg/dL)	**SD**	**CV%**
8.27	0.22	2.7	73.091	1.669	2.3
277.9	1.8	0.6	266.690	8.725	3.3

Assay Range
The Microprotein procedure has an analytical measuring range of 0 - 250 mg/dL. Samples that exceed 250 mg/dL should be diluted with deionized water, reanalyzed and the result should be multiplied by the appropriate dilution factor.

Analytical Specificity
Cross contamination studies have not been performed on Affirm C200 and Beckman AU680 Analyzers. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions
For the data presented in this insert, studies using this reagent were performed on Affirm C200 and Beckman AU680 Analyzers using the parameters listed below.

Calibration
Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Method Parameters

Analyzer Specific Settings

Method type:	Endpoint
Slope:	positive
Units	mg/dL
DOM wavelength	600
SUB wavelength	800
Sample volume	2
Reagent volume	200
Blank rxn read (cycles)	7 – 9
Sample rxn read (cycles)	18 – 22

Calibration Settings

Calibration Type	Linear
Reagent Blank required	No
Calibrator 1	5
Calibrator 2	50

Analytical Measuring Range (AMR)

Range: (low)	0
Range: (high)	250

BIBLIOGRAPHY
1. Tietz, N.W., Textbook of Clinical Chemistry. W.B. Saunders, Philadelphia p. 608, 1986.
2. Viberti, G.C., Pickup, J.C., Jarrett, R.J., Keon, H., N Engl J Med. 300:638-41 1979.
3. Grant, G.H., Kachmer, J.F.: Fundamentals of Clinical Chemistry. N.W. Tietz, Editor, W.B. Saunders, Philadelphia, pp. 358-374, 1976.
4. Cannon, D.C., Olitzky, I., Inkpen, J.A.: Clinical Chemistry – Principles and Technics, 2nd Ed., R.J. Henry, D.C. Cannon, J.W., Winkleman, Editors, Harper & Row, New York, pp. 442-431, 1974.
5. Pesce, M.A., Strande, C.S., Clin Chem 19:1265-1267, 1973.
6. Fujita, Y., Mori, I., Kitano, S. Color reaction between pyrogallol red-molybdenum (VI) complex and protein. Bunseki Kagaku 32: E379-E386, 1983.
7. Watanabe, N., Kamel, S., Ohkubo, A., Yamakna, M., Clin Chem 32:1551-1554, 1986.
8. Tietz, N.W.; Clinical Guide to Laboratory Tests, W.B. Saunders, Phil. P.470, 1990.
9. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November, 2002.

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