POINTE SCIENTIFIC, INC.
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Microalbumin Reagent Set

Intended Use
For the quantitative determination of low levels of albumin in urine by immunoturbidimetric assay. For *in vitro* diagnostic use.

Introduction
A small amount of protein is excreted daily into the urine of healthy individuals. The excreted proteins are mucoproteins, most of which are filtered out of the uriniferous tubules and the glomeruli. Albumin, a protein of molecular weight of 50,000, is not easily filtered out and is excreted into the urine (microalbuminuria).[1,2] This makes albumin excretion into the urine a useful indicator of early glomerular disease.

Microalbuminuria is a condition characterized by increased urinary excretion of albumin in the absence of overt nephropathy.[3,4] Microalbumin has been reported in several studies to predict development of diabetic nephropathy and its mortality risk in diabetic patients.[2,5,6,7,8,9] Because microalbuminuria may be reversible if diabetes is well controlled, the early detection of microalbumin may be very beneficial in treatment programs for diabetes.

Albumin in urine has been measured by a variety of methods. Many of these lack the sensitivity to be used for microalbumin measurements. Radioimmunoassay (RIA) and immunoturbidimetric assay provide the sensitivity required.[10] This Microalbumin uses an immunoturbidimetric format.

Principle
When a sample is mixed with anti-human albumin goat antiserum, agglutination is caused by the antigen-antibody reaction. The turbidity is measured at 340nm and 700nm and albumin in the sample is quantitatively determined.

Characteristics
1. No reagent preparation such as dissolving, mixing, or dilution.
2. No sample dilution up to 30 mg/dl (300ug/ml).
3. Long reagent stability after opening (1 month).
4. Good correlation with RIA.
5. No interference from drugs.
6. No prozone effect in ordinary measuring range.
7. Compatible with most clinical chemistry auto-analyzers.

Kit Composition
Reagents (Liquid stable)

R1: Buffer Reagent, pH 7.6
100mM Tris (hydroxymethyl) aminomethane

R2: Antiserum Reagent, pH 7.6
20% Anti-human albumin, goat antiserum
100mM Tris (hydroxymethyl) aminomethane

Warnings and Precautions
1. FOR IN VITRO DIAGNOSTIC USE.
2. Not to be used internally in humans or animals.
3. Normal precautions exercised in handling laboratory reagents should be followed.
4. Do not mix or use reagents from one test kit with those from a different lot number.
5. Do not use reagents past their expiration date stated on each reagent container label.
6. Do not pipette by mouth. Avoid ingestion and contact with skin.
7. Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.

Reagent Preparation
Reagents are ready to use and do not require reconstitution.

Storage and Handling
All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used up to the expiration date on the package and bottle labels.

Reagent Stability
Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard. If the absorbance of isotonic saline is greater than 0.1 or if the absorbance of the 5.0 mg/dl calibrator is smaller than 0.15, the reagents should not be used. Opened reagents can be used for 1 month if stored at 2-8°C.

Specimen Collection and Preparation
The specimen should be a fresh or a 24-hour urine. Urine specimens should be stored refrigerated (2-8°C). The specimens may be stored refrigerated up to two weeks or frozen at -70°C for at least 5 months.[11]

Automated Analyzer Application
Suitable for two-reagent automated analyzers that use a two-point calibration method. Measurements of absorbance are to be made with a spectrophotometer able to accurately read absorbance at 340 and 700nm. Refer to the instrument manual from the manufacturer regarding the following:
a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics and specifications
e) Operation instructions
f) Calibration procedures including materials and/or equipment to be used
g) Operational precautions and limitations
h) Hazards
i) Service and maintenance

Materials Provided
1.	Reagent 1 (R1) Buffer Reagent	4x20ml
2.	Reagent 2 (R2) Antiserum Reagent	2x10ml

Materials Required but not Provided
1. Calibrators: Microalbumin Multi-Calibrator Set, 6 Calibrators; Approx. values: 0, 0.5, 1.0, 5.0, 10.0, 30.0 mg/dl (For actual values see vial labels)
2. Spectrophotometer: capable of accurate absorbance reading at 340 and 700nm with appropriate cuvettes.
3. Pipettes: capable of accurately dispensing the required volumes
4. Test Tubes: glass or plastic
5. Water Bath: capable of maintaining 37°C

Procedure (Automated)
Refer to specific instrument application instructions.

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Calibration Curve
For albumin concentrations less than 10 mg/dl, a two-point calibration curve can be made using a saline blank (0mg/dl) and an albumin standard. When a sample with a higher albumin concentration (>10mg/dl) is assayed, it is recommended that a multi-point calibration curve using the microalbumin multi-calibrator set be made. It is recommended that a calibration curve be made each day.

Quality Control
A quality control program is recommended for all clinical testing laboratories. It is recommended that control urines, both normal and abnormal, be run with each batch of samples to monitor the procedure.
The values obtained for controls should fall within the manufacturers specified range. A laboratory may establish its own control urine by assaying the urine a sufficient number of times to generate a valid mean and acceptable range.

Calculations
Albumin levels are determined using the prepared calibration curve.

Limitations
The measurable range for this albumin test kit is between 0.5 mg/dl and 30 mg/dl. If albumin concentrations are greater than 30 mg/dl, dilute 1 part sample with 4 parts isotonic saline including 0.5% Tween 20 and re-assay. Multiply the result by 5 to compensate for the dilution.

Performance
1. Sensitivity; when saline blank is used as a sample, the absorbance is below 0.05. When a calibrator containing 5 mg/dl of human albumin is assayed, the absorbance (after subtracting the absorbance value for the saline blank) is within range of 0.131-0.525.
2. Specificity: When a urine sample with a known value is assayed, it is within ± 10%.
3. Precision: When a sample containing 5 mg/dl of human albumin is repeatedly assayed 20 times, the absorbance C.V. is less than 5%.
4. Precision Assay:

Within Run (N=20)			Run to Run (N=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
0.96	0.05	5.2	0.97	0.11	11.3
5.47	0.12	2.2	5.26	0.22	4.2

Assay Range
0.5-30 mg/dl or 5-300 ug/ml (multi-point calibration)
0.5-10 mg/dl or 5-100 ug/ml (two-point calibration)

5. Correlation: A comparison of this Microalbumin and a Kamiya Microalbumin Test Kit was performed on a Hitachi 717 automated analyzer. The test results provided the following data. All values are expressed in mg/dl of albumin.
y =1.0738x + 0.01555
r = 0.995, (n=74, range = 0.9-20.7)

x = Kamiya Test Kit	y = this Microalbumin test kit
x = min = 0.900	y min = 1.000
x max = 20.7	y max = 22.7
x mean = 5.88	y mean = 6.33

Interferences
Ascorbic acid: No interference up to 200 mg/dl (Less than 5%)
Glucose: No interference up to 3.0 g/dl (Less than 15%)
Uric Acid: No interference up to 100 mg/dl (Less Than 5%)
Creatinine: No interference up to 300 mg/dl (Less than 8%)
Creatine: No interference up to 100 mg/dl (Less than 5%)
Calcium: No interference up to 30.0 mM (Less than 8%)
NaCl: No interference up to 900 mg/dl (Less than 15%)
Mg: No interference up to 30 mM (Less than 5%)
KCl: No interference up to 300 mg/dl (Less than 5%)
Urea: No interference up to 3.0 g/dl (Less than 8%)

Expected Values
The expected value for Microalbumin is 30-300 mg/24 hours. [9] Each laboratory should establish its own expected values using this kit.

References

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