**Microalbumin**

For the Quantitative Determination of Albumin in Urine

| Cat. No. KAI-019 / KAI-057 |

**INTENDED USE**

For the quantitative determination of human albumin in urine by immunoturbidimetric assay. Measurement of albumin in urine aids in the diagnosis of kidney dysfunction. **FOR IN VITRO DIAGNOSTIC USE.**

**INTRODUCTION AND SUMMARY**

A small amount of protein is excreted daily into the urine of healthy individuals. The excreted proteins are mucopolysaccharides, most of which are filtered out of the urinary tubules and the glomeruli. Albumin, a protein of molecular weight of 50,000 daltons, is not easily filtered out and is excreted into the urine (microalbuminuria). 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. Microalbuminuria has been shown to be the earliest stage of diabetic nephropathy in type I diabetes and a marker for development of nephropathy in type II diabetes. Early detection of microalbuminuria may be beneficial in diabetes treatment programs because early detection and management has been shown to reduce the risk and slow progression of end-stage renal disease.

Albumin in urine has been measured by a variety of methods. The **K-ASSAY® Microalbumin assay** uses an immunoturbidimetric format which provides the necessary sensitivity required for accurate determination of urinary microalbumin.

**PRINCIPLE OF TEST**

When a sample is mixed with anti-human albumin goat antiserum, agglutination is caused by the antigen-antibody reaction. The turbidity is measured at 340 nm and 700 nm and albumin in the sample is quantitatively determined by comparison to a standard calibration curve of known concentrations.

**KIT COMPOSITION**

Reagents (Liquid Stable)

- R1: Buffer Reagent, pH 7.6
- Tris(hydroxymethyl)aminomethane (100 mM)
- R2: Antiserum Reagent, pH 7.6
- Anti-human albumin goat antiserum (20%)
- Tris(hydroxymethyl)aminomethane (100 mM)

**WARNINGS AND PRECAUTIONS**

FOR **IN VITRO DIAGNOSTIC USE. Rx only.**

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

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. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

Controls that contain bovine serum albumin (e.g. Bio-Rad Liqui-check Urine Chemistry Control and Lyphochek Quantitative Urine Control) may show false value shifts with different lots of **K-ASSAY® Microalbumin reagent. It is recommended that controls not containing animal material (such as the **K-ASSAY® Microalbumin Urine Control, Cat. No. K37C** be used with this reagent.**

**REAGENT PREPARATION**

Reagents are ready to use and do not require reconstitution.

**STORAGE AND HANDLING**

All reagents should be stored refrigerated (2-8°C). All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

**REAGENT STABILITY**

Opened reagents can be used for 1 month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

**SPECIMEN COLLECTION AND PREPARATION**

The specimen should be a fresh or a 24-hour urine specimen. The specimens may be stored refrigerated at 4-8°C up to 1 month or frozen at -20°C for up to 6 months.

**AUTOMATED ANALYZER APPLICATION**

Suitable for two-reagent automated analyzers that use a two-point or multi-point calibration method. Measurements of absorbance are to be made with a spectrophotometer able to accurately read absorbance at 340 and 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use of function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

**PROCEDURE**

**Materials Supplied**

- **KAI-019, Microalbumin**
  - Reagent 1 (R1) Buffer Reagent 4 x 20 mL
  - Reagent 2 (R-2) Antiserum Reagent 2 x 10 mL
- **KAI-057, Microalbumin (L)**
  - Reagent 1 (R1) Buffer Reagent 3 x 80 mL
  - Reagent 2 (R-2) Antiserum Reagent 1 x 80 mL

**Materials Required But Not Supplied**

- Reagent 1 (R1)
- Reagent 2 (R-2)
- Buffers
- CAPS
- Sodium azide
- Sodium hydroxide
- Water
- Standard (STD.)
- Calibration standards
- Microalbumin
- Distilled water
- pH meter
- UV/Vis spectrophotometer
- Hitachi 917
- Hitachi 737

**Calibration**

Calibrators: **K-ASSAY® Microalbumin Calibrator, Cat. No. KAI-020C**

**Assay Procedure**

**Note:** Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 917): Sample 7 µL

- Reagent 1 (Buffer Reagent) 210 µL 37°C, 5 min.
- Reagent 2 (Antiserum Reagent) 70 µL 37°C, 5 min.
- 2-point endpoint, 340 / 700 nm

**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 in order to monitor the procedure. Two levels of quality control material of known values should be run according to state, federal, and accreditation requirements or whenever there are questionable results or instrument performance, after analyzer maintenance or manufacturer’s service, with every new lot of reagent, and at least a minimum of every 30 days for opened vials to check storage conditions.

**Automated Method (Example)**

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Roche / Hitachi 917</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST</td>
<td>(MALB)</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT END) (10)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(700) / (340)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(7.0) / (0.0) / (0.0)</td>
</tr>
<tr>
<td>REAGENT VOL (R1)</td>
<td>(210) / (0)</td>
</tr>
<tr>
<td>REAGENT VOL (R2)</td>
<td>(0) / (0)</td>
</tr>
<tr>
<td>REAGENT VOL (R3)</td>
<td>(70) / (0)</td>
</tr>
<tr>
<td>REAGENT VOL (R4)</td>
<td>(0) / (0)</td>
</tr>
<tr>
<td>ABS LIMIT (SLOPE)</td>
<td>(32000) / (INCREASE)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(32000) / (34) (LOWER)</td>
</tr>
<tr>
<td>CALIB. TYPE</td>
<td>(SPLINE)</td>
</tr>
<tr>
<td>POINT</td>
<td>(6)</td>
</tr>
<tr>
<td>SPAN POINT</td>
<td>(6)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>S1ABS RANGE</td>
<td>(-32000) / (32000)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>a- (1.0) b- (0.0)</td>
</tr>
<tr>
<td>UNIT</td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(1) (1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(2) (2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(3) (3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(4) (4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(5) (5)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(6) (6)</td>
</tr>
</tbody>
</table>

*1-6: Input concentration of calibrators (using two decimal places [X.XX]).

Parameters for other automated analyzers are available.
the manufacturer’s specified range. However, due to differences in assays and analyzers used to assay a control by the control manufacturer, a laboratory may establish its own control ranges by assaying the controls a sufficient number of times to generate a valid mean and acceptable range.

**CALCULATIONS**

Albumin levels are determined using the prepared calibration curve.

**LIMITATIONS OF PROCEDURE**

If albumin concentrations are greater than the highest calibrator value, use 1 part sample with 4 parts of one of the following: isotonic saline including 0.05% Tween 20, isotonic saline, or deionized water. Deionized water is an acceptable diluent for diluting high value samples using an analyzer’s auto-rein feature. Re-assay and multiply the result by 5 to compensate for the dilution. If dilution of low value samples is needed, use 1 part sample with 4 parts isotonic saline including 0.05% Tween 20.

**PERFORMANCE**

**Precision**

The within-run, between-run, and total precision for the K-ASSAY® Microalbumin assay was determined using packaged reagents, human urine samples, and a Roche / Hitachi 917 analyzer in accordance with CLSI EP5-A2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>0.311</td>
<td>0.987</td>
<td>27.250</td>
</tr>
<tr>
<td>Within Run S.D.</td>
<td>0.019</td>
<td>0.021</td>
<td>0.126</td>
</tr>
<tr>
<td>Within Run C.V. %</td>
<td>6.007</td>
<td>2.168</td>
<td>46.43</td>
</tr>
<tr>
<td>Between Run S.D.</td>
<td>0.012</td>
<td>0.014</td>
<td>0.063</td>
</tr>
<tr>
<td>Between Run C.V. %</td>
<td>3.840</td>
<td>1.411</td>
<td>22.99</td>
</tr>
<tr>
<td>Total S.D.</td>
<td>0.021</td>
<td>0.024</td>
<td>0.166</td>
</tr>
<tr>
<td>Total CV %</td>
<td>6.914</td>
<td>2.407</td>
<td>60.68</td>
</tr>
</tbody>
</table>

**Method Comparison / Correlation**

Testing was performed on a Roche / Hitachi 917 analyzer using unaltered, natural human urine samples and in accordance with the CLSI EP9-A2 guideline. A comparison of the K-ASSAY® Microalbumin and the Roche Tina-Quant Albumin assay was performed with the following results.

Linear Regression:

\[ y = 0.9149x + 0.0174 \]
\[ r = 0.9996 \]
\[ n = 91 \]
\[ x = \text{Roche Tina-Quant Albumin} \]
\[ y = \text{K-ASSAY® Microalbumin} \]

Minimum: 0.33 mg/dL
Maximum: 33.04 mg/dL
Mean: 6.918 mg/dL

**Bence-Jones Proteins**

Kappa Light Chain:
No interference \( \leq 30 \) mg/dL
Lambda Light Chain:
No interference \( \leq 30 \) mg/dL

**Administered Diuretics**

Furosemide:
No interference \( \leq 400 \) µg/mL
Trichlormethiazide:
No interference \( \leq 20 \) µg/mL

**Interference**

Testing was performed on a Roche / Hitachi 917 analyzer in accordance with the CLSI EP7-A2 guideline with the following results.

Criteria: Recovery within ±10% of initial value

- Acetone: No interference \( \leq 350 \) mg/dL
- Ascorbic Acid: No interference \( \leq 100 \) mg/dL
- Bilirubin: No interference \( \leq 66 \) mg/dL
- Calcium: No interference \( \leq 160 \) mg/dL
- Creatinine: No interference \( \leq 500 \) mg/dL
- Glucose: No interference \( \leq 2.000 \) mg/dL
- Hemoglobin: No interference \( \leq 300 \) mg/dL
- Urea: No interference \( \leq 4.200 \) mg/dL
- Uric Acid: No interference \( \leq 70 \) mg/dL
- Urobilinogen: No interference \( \leq 20 \) mg/dL

**Analytical Medications**

Acetaminophen: No interference \( \leq 0.2 \) mg/mL
Ibuprofen: No interference \( \leq 2.0 \) mg/mL

**Oral Diabetes Medications**

Glibenclamide: No interference \( \leq 15 \) µg/mL
Metformin Hydrochloride: No interference \( \leq 4.0 \) µg/mL

**EXPECTED VALUE**

The expected value for urinary albumin as per the literature is:

- \( < 2 \) mg albumin / dl urine
- \( < 20 \) mg albumin / L urine
- \( < 0.02 \) g albumin / L urine

For spot AM samples, the expected values are:

- \( < 0.03 \) mg albumin / mg creatinine

Microalbuminuria is typically defined as:

- 30-300 mg albumin / 24 hours

Due to population differences, each laboratory should establish its own expected values using this kit.

**REFERENCES**