**K-ASSAY® 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 mucoproteins, most of which are filtered out of the urinary tubules and the glomeruli. Albumin, a protein of molecular weight of 59,000 daltons, is not easily filtered out and is excreted into the urine (microalbuminuria). 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 an anti-human albumin goat antisemur, 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
100 mM Tris(hydroxymethyl)aminomethane
20% Anti-human albumin, goat antisemur
100 mM Tris(hydroxymethyl)aminomethane

R2: Antiserum Reagent, pH 7.6

**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 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 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:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and/or equipment to be used
- Operational precautions, limitations, and hazards
- Service and maintenance information

**PROCEDURE**

**Materials Supplied**

**KAI-019 Microalbumin**

Reagent 1 (R1) Buffer Reagent 4 x 20 mL
Reagent 2 (R2) Antiserum Reagent 2 x 10 mL

**KAI-057 Microalbumin (L)**

Reagent 1 (R1) Buffer Reagent 3 x 80 mL
Reagent 2 (R2) Antiserum Reagent 1 x 80 mL

**Materials Required But Not Supplied**

Calibrators: **K-ASSAY®** Microalbumin Calibrator, Cat. No. KAI-020C, 6 Calibrators; Approx. values: 0.05, 1.0, 5.0, 10.0, 30.0 mg/dL (For actual values see Package Insert).

Two Reagent Clinical Chemistry Analyzer:

- Capable of accurately dispensing the required volumes
- Capable of maintaining 37°C

**Test Tubes:** plastic or glass (for short-term storage only)

**Assay Procedure**

An example of automated application (Hitachi 917):

- **Sample**: 7.0 µL
  - R1 (Buffer Reagent) 210 µL
  - R2 (Antiserum Reagent) 70 µL
- **2-point endpoint**, 340/700 nm
- **Point**: 37°C, 5 min.

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

**Automated Method (Example)**

**Chemistry Parameters for Automatic Analyzer (Hitachi 917):**

**STORAGE AND HANDLING**

**Parameters for other automated analyzers are available.**

**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 each new lot of reagent, and at a minimum of every 30 days for opened vials to check storage conditions.

The values obtained for controls should ideally fall within 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-run feature. Re-assay and multiply 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 with-in-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>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>0.311</td>
<td>0.967</td>
</tr>
<tr>
<td>Within Run S.D.</td>
<td>0.019</td>
<td>0.021</td>
</tr>
<tr>
<td>Within Run C.V.</td>
<td>6.007%</td>
<td>2.168%</td>
</tr>
<tr>
<td>Between Run S.D.</td>
<td>0.012</td>
<td>0.014</td>
</tr>
<tr>
<td>Between Run C.V.</td>
<td>3.840%</td>
<td>1.411%</td>
</tr>
<tr>
<td>Total S.D.</td>
<td>0.021</td>
<td>0.024</td>
</tr>
<tr>
<td>Total C.V.</td>
<td>6.914%</td>
<td>2.407%</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:

\[ y = 0.9149x + 0.0174 \]

\[ r = 0.9963 \]

\[ n = 91 \]

\[ x = \text{Roche Tina-Quant Albumin} \]

\[ y = K-\text{ASSAY® Microalbumin} \]

Lineararity

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP6-A1 guideline. A high albumin human urine sample was serially diluted with albumin free human urine to make 12 samples between 0.20 - 30.00 mg/dL and each sample run 5 times with the following results.

First order regression:

\[ y = 0.9962x + 0.0308 \]

\[ r = 0.9999 \]

Standard Error of Regression = 0.075

ASSAY RANGE

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-A2 guideline using native and diluted human urine samples with the following results.

- Limit of Blank (LoB) = 0.03 mg/dL
- Limit of Detection (LoD) = 0.05 mg/dL
- Limit of Quantitation (LoQ) = 0.20 mg/dL

Assay Range: 0.20 – 30.00 mg/dL

(using LoQ as lower limit and highest calibrator as upper limit)

INTERFERENCES

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
- Ascorbic Acid: No interference
- Bilirubin: No interference
- Calcium: No interference
- Creatinine: No interference
- Glucose: No interference
- Hemoglobin: No interference
- Urea: No interference
- Uric Acid: No interference
- Urobilinogen: No interference
- Bence-Jones Proteins: No interference
- Kappa Light Chain: No interference
- Lambda Light Chain: No interference
- Administered Diuretics
  - Furosemide: No interference ≤ 400 µg/mL
  - Trichlormethiazide: No interference ≤ 20 µg/mL
- Analgesic Medications
  - Acetaminophen: No interference ≤ 0.2 mg/mL
  - Ibuprofen: No interference ≤ 2.0 mg/mL
- Oral Diabetes Medications
  - Glibenclamide: No interference ≤ 15 µg/mL
  - Metformin Hydrochloride: No interference ≤ 4.0 µg/mL

EXPECTED VALUES

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

- < 2 mg albumin / dL urine
- (or < 25 mg albumin / L urine or 0.02 g albumin / L urine), or
- ≤ 30 mg / 24 hours (or 0.03 g / day).

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


LABELING SYMBOLS

LOT Lot Number
REAG Reagent
EXP Expiration or “Use By” Date
CAT Catalog Number
IVIVC For In Vivo Diagnostic Use
2-8°C Temperature Limitation. Store between 2 and 8 degrees C
PB Potential Human Biohazard
MFR Manufacturer
INSP Consult Package Insert for Instructions for Use
REP Authorized Representative in the European Community
EU AUTHORIZED REPRESENTATIVE

EC Inscription

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