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 uriniferous 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).[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] 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.[5]

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 Lyphocheck 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). 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.[6]

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 or 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 (R-1) Buffer Reagent	4 x 20 mL
Reagent 2 (R-2) Antiserum Reagent	2 x 10 mL

or

KAI-057, Microalbumin (L)

Reagent 1 (R-1) Buffer Reagent	3 x 80 mL
Reagent 2 (R-2) Antiserum Reagent	1 x 80 mL

Materials Required But Not Supplied

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

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 340 / 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

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
↓
• ← R1 (Buffer Reagent) 210 μL
↓ 37 °C, 5 min.
• ← R2 (Antiserum Reagent) 70 μL
↓ 37 °C, 5 min.
2-point endpoint, 340 / 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(MALB)
ASSAY CODE	(2 POINT END)(10) (16)(34)(0)(0)
WAVELENGTH	(700) (340)
SAMPLE VOLUME	(7.0) (0.0) (0)
REAGENT VOL (R1)	(210) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(70) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000)(34)(LOWER)
CALIB. TYPE	(SPLINE)
POINT	(6)
SPAN POINT	(6)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(mg/dL)
STD.(1) Conc.-POS.	(*1) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	(*6) - (6)

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

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a 6 point calibration curve using the **K-*ASSAY*®** Microalbumin Calibrator be made. It is recommended that the user determine calibration curve frequency, as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed prior to the first run of 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 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-rerun 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.

	Sample		
	1	**2**	**3**
N	80	80	80
Mean (mg/dL)	0.311	0.987	27.250
Within Run S.D.	0.019	0.021	0.126
Within Run C.V. %	6.007	2.168	0.463
Between Run S.D.	0.012	0.014	0.063
Between Run C.V. %	3.840	1.411	0.229
Total S.D.	0.021	0.024	0.166
Total CV %	6.914	2.407	0.608

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 = Roche Tina-Quant Albumin
y = **K-*ASSAY*®** Microalbumin

x min	= 0.33 mg/dL	y min	= 0.38 mg/dL
max	= 33.04 mg/dL	max	= 30.24 mg/dL
mean	= 6.918 mg/dL	mean	= 6.346 mg/dL

Linearity

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP6-A 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 = 0.9925x + 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 EP17-A 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)

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 ≤ 350 mg/dL
Ascorbic Acid:	No interference ≤ 100 mg/dL
Bilirubin:	No interference ≤ 66 mg/dL
Calcium:	No interference ≤ 160 mg/dL
Creatinine:	No interference ≤ 500 mg/dL
Glucose:	No interference ≤ 2,000 mg/dL
Hemoglobin:	No interference ≤ 300 mg/dL
Urea:	No interference ≤ 4,200 mg/dL
Uric Acid:	No interference ≤ 70 mg/dL
Urobilinogen:	No interference ≤ 20 mg/dL
<u>Bence-Jones Proteins</u>	
Kappa Light Chain:	No interference ≤ 30 mg/dL
Lambda Light Chain:	No interference ≤ 30 mg/dL
<u>Administered Diuretics</u>	
Furosemide:	No interference ≤ 400 µg/mL
Trichlormethiazide:	No interference ≤ 20 µg/mL
<u>Analgesic Medications</u>	
Acetaminophen:	No interference ≤ 0.2 mg/mL
Ibuprofen:	No interference ≤ 2.0 mg/mL
<u>Oral Diabetes Medications</u>	
Gilbenclamide:	No interference ≤ 15 µg/mL
Metformin Hydrochloride:	No interference ≤ 4.0 µg/mL

EXPECTED VALUE

The expected value for urinary albumin as per the literature is :
< 2 mg albumin / dL urine or
< 20 mg albumin / L urine or
< 0.02 g albumin / L urine.[7,8]
or
≤ 30 mg / 24 hours (or ≤0.03 g / day).[8]

For spot AM samples, the expected values are:
< 0.03 mg albumin / mg creatinine.[8]

Microalbuminuria is typically defined as:
30-300 mg albumin / 24 hours.[8]

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

REFERENCES

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3. Morgensen, C.E. and Christensen, C.K., *N. Engl. J. Med.* 311: 89-93 (1984).
4. Viberti, G.C., *et al*., *Lancet.* 1430-1432 (1982).
5. American Diabetes Association, "Standards of Medical Care in Diabetes-2008", *Diabetes Care 2008* 31[Suppl 1]:S29-S30.
6. World Health Organization, *Use of Anticoagulants in Diagnostic Laboratory Investigations* (WHO / DIL / LAB / 99.1 Rev. 2, 2002), p. 46.
7. Burtis, Carl A., *et al., Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition* (Elsevier Saunders, 2006), p. 547.
8. Jacobs, David S., *et al., Laboratory Test Handbook, 4th Edition* (Lexi-Comp Inc, 1996), p. 643.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostics Use
2-8 °C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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