

Alpha-1 Microglobulin

For the Quantitative Determination of Human Alpha-1 Microglobulin in Urine, Serum, And Plasma

Cat. No. KAI-056

INTENDED USE

For the quantitative determination of human alpha-1 microglobulin in human urine, serum and plasma by immunoturbidimetric assay. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Alpha-1 microglobulin is a low molecular weight glycoprotein of 24 - 31 kDa that was initially isolated from the urine of patients with renal tubular disorders in 1975. It is mainly synthesized in the liver and is widely distributed in various body fluids. The measurement of alpha-1 microglobulin in serum and urine has been considered to be useful for the diagnosis of functional renal disorders and the assessment of the progress and the prognosis of diseases. The **K-ASSAY®** Alpha-1 Microglobulin assay is intended for the quantitative determination of human alpha-1 microglobulin in urine, serum, and plasma by immunoturbidimetric assay.

PRINCIPLE OF TEST

When an antigen-antibody reaction occurs between alpha-1 microglobulin in a sample and alpha-1 microglobulin antibody that has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of alpha-1 microglobulin in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 1 x 50 mL
Glycine buffer solution (170 mM)

R2: Latex Suspension 1 x 50 mL
0.25 % w/v suspension of latex particles sensitized with rabbit anti-human alpha-1 microglobulin antibody

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.

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 until the expiration date indicated on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard. Opened reagents can be used for 1 month if stored at 2-8°C. **It is recommended that R-2 be gently inverted once a week after it is opened.**

SPECIMEN COLLECTION AND PREPARATION

The sample may be urine, serum or plasma (depending on calibrator used).

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2 and H2-A2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Testing should be performed immediately. If this is not possible, the sample should be placed in a tightly sealed container and stored at -20°C. Avoid repeat freeze/thaw cycles.

Suitable for two-reagent clinical chemistry analyzers that can measure a rate reaction at an absorbance of 570 nm primary and 800 nm secondary. 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

Reagent 1 (R-1) Buffer Reagent 1 x 50 mL
Reagent 2 (R-2) Latex Suspension 1 x 50 mL

Materials Required But Not Supplied

Calibrators:

Urine samples:

K-ASSAY® Urine Alpha-1 Microglobulin Calibrator, Cat. No. KAI-067C

Serum or Plasma samples:

K-ASSAY® Serum / Plasma Alpha-1 Microglobulin Calibrator, Cat. No. KAI-068C

Clinical chemistry analyzer capable of accurately reading at 570 and 800 nm, accurately dispensing the required volumes, and 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:

Urine Sample 7 µL
↓
• ←R1 (Buffer Reagent) 125 µL
↓ 37 °C, 5 min.
• ←R2 (Latex Suspension) 125 µL
↓ 37 °C, 5 min.
2-point endpoint, 570 / 800 nm (primary / sub)

Serum / Plasma Sample 3 µL
↓
• ←R1 (Buffer Reagent) 180 µL
↓ 37 °C, 5 min.
• ←R2 (Latex Suspension) 180 µL
↓ 37 °C, 5 min.
2-point endpoint, 570 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

Urine Samples

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(A1MG)
ASSAY CODE	(2 POINT END): (28) - (50)
SAMPLE VOLUME	(7) (7)
R1 VOLUME	(125) (100) (NO)
R2 VOLUME	(125) (100) (NO)
WAVELENGTH	(800) (570)
CALIB. METHOD	(NONLINEAR) (4) (5)
STD.(1) CONC.-POS.	(0.00) - (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.	() - ()
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY UNIT	(0)
ABS. LIMIT (INC/DEC)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (LOWER)
EXPECTED VALUE	(-99999) - (99999)
PANIC VALUE	(-99999) - (99999)
INSTRUMENT FACTOR	(1.00)

Use isotonic saline as STD (1)

*2-5: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a multi-point calibration curve be made using either the **K-ASSAY®** Urine Alpha-1 Microglobulin Calibrator or **K-ASSAY®** Serum / Plasma Alpha-1 Microglobulin Calibrator. Please be sure to use the proper instrument application for the calibrator you are using. It is recommended that the user determine calibration frequency as this will depend on the instrument and type/number of other assays being run. Initially, calibration should be performed each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included with every assay performed. The value determined for the controls should fall within the stated limits of the values assigned to the controls. The validity of the assay is in question if the values for the controls generated by the assay's calibration curve do not fall within this range. Recalibrate if the values determined for the controls fall outside the stated range.

CALCULATIONS

Alpha-1 microglobulin levels are determined by the analyzer using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The Alpha-1 microglobulin test is suitable for measuring in the range of:

Urine: 0.2 - 34.0 mg/L

Serum and Plasma: 1.0 - 137.0 mg/L

Reagents should not be used after the expiration date indicated on the kit label. Do not mix reagents with different lot numbers.

If the alpha-1 microglobulin concentration is greater than highest calibrator value, dilute one part sample with four parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

Specificity

When a sample containing a known level of alpha-1 microglobulin was measured, the value obtained for the sample was in the range of the known concentration, $\pm 10\%$.

Precision

When a sample containing a known level of alpha-1 microglobulin is tested 10 times, the CV of the test should typically be under 10%.

Urine (Within Run)

<u>Sample I</u>	<u>Sample II</u>	<u>Sample III</u>
n = 10	n = 10	n = 10
Mean = 0.7 mg/L	Mean = 3.3 mg/L	Mean = 17.4 mg/L
SD = 0.029	SD = 0.012	SD = 0.059
CV = 3.93 %	CV = 0.37 %	CV = 0.34 %

Urine (Between Runs)

<u>Sample I</u>	<u>Sample II</u>	<u>Sample III</u>
n = 15	n = 15	n = 15
Mean = 0.7 mg/L	Mean = 3.3 mg/L	Mean = 17.2 mg/L
SD = 0.05	SD = 0.04	SD = 0.31
CV = 6.3 %	CV = 1.2 %	CV = 1.8 %

Serum (Within Run)

<u>Sample I</u>	<u>Sample II</u>	<u>Sample III</u>
n = 10	n = 10	n = 10
Mean = 7.1 mg/L	Mean = 32.1 mg/L	Mean = 66.1 mg/L
SD = 0.145	SD = 0.263	SD = 0.540
CV = 2.04 %	CV = 0.82 %	CV = 0.82 %

Serum (Between Runs)

<u>Sample I</u>	<u>Sample II</u>	<u>Sample III</u>
n = 15	n = 15	n = 15
Mean = 7.5 mg/L	Mean = 32.3 mg/L	Mean = 67.1 mg/L
SD = 0.15	SD = 0.31	SD = 1.27
CV = 2.0 %	CV = 0.9 %	CV = 1.9 %

Accuracy / Correlation

Correlation between this test and the latex agglutination method of another company is given below:

Urine samples:

$$\begin{aligned}y &= 1.00x - 0.52 \\r &= 1.00 \\n &= 55 \\x &= \text{Company A's latex reagent} \\y &= \mathbf{K-ASSAY}^{\circledR} \text{ Alpha-1 Microglobulin}\end{aligned}$$

Serum samples:

$$\begin{aligned}y &= 1.00x + 2.83 \\r &= 1.00 \\n &= 55 \\x &= \text{Company A's latex reagent} \\y &= \mathbf{K-ASSAY}^{\circledR} \text{ Alpha-1 Microglobulin}\end{aligned}$$

Assay Range

Urine:

0.2 - 34.0 mg/L (or value of highest calibration point)

Serum / Plasma:

1.0 - 137.0 mg/L (or value of highest calibration point)

Lower Limit of Detection

Urine: 0.2 mg/L

Serum and Plasma: 1.0 mg/L

INTERFERENCE

Bilirubin C	No interference up to 30 mg/dL
Bilirubin F	No interference up to 30 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Lipid (Intrafat)	No interference up to 5 %

Dust particles or other particulate matter in the reaction solution may result in extraneous light scattering, which may affect the accuracy of the test.

EXPECTED VALUES











It is recommended that each laboratory establish its own expected range to reflect its patient population.

Urine: 1.0 - 5.0 mg/L

Serum and Plasma: 10.0 - 30.0 mg/L

Expected values were taken from the literature:
Ito, Y., "Alpha-1 Microglobulin (protein HC)." Nippon-rinsho, 47:176, 1989.

LABELING SYMBOLS

	Catalog Number
	Expiration or "Use By" Date
	Lot Number
	Consult Package Insert for Instructions for Use
	For <i>In Vitro</i> Diagnostic Use
	CE Mark Registered
	For Prescription Use Only
	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE



 Authorized Representative in
the European Community

Advena Ltd.

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