KAMIYA BIOMEDICAL COMPANY

K-*ASSAY*®

KL-6 (Ver.2)
(Krebs von den Lungen-6)

For the Quantitative Determination of KL-6 in Serum and Plasma

Cat. No. KAI-350

INTENDED USE

The **K-*ASSAY*®** KL-6 (Ver.2) assay is for the quantitative determination of Krebs von den Lungen-6 in human serum and plasma by immunoturbidimetric assay. FOR RESEARCH USE ONLY IN THE U.S. NOT FOR USE IN DIAGNOSTIC PROCEDURES IN THE U.S.

INTRODUCTION AND SUMMARY

Sialylated carbohydrate antigen KL-6 has a molecular weight of over 1 million and is located on MUC1, a transmembrane type glycoprotein expressed by type II epithelial cells.[1,2] Research studies have reported that KL-6 may be an indicator of interstitial pneumonia and pulmonary fibrosis as well as the assessment of treatment. [1,2]

The **K-*ASSAY*®** KL-6 (Ver.2) assay is a latex-enhanced immunoturbidimetric assay, developed to quantitatively determine levels of KL-6 in serum and plasma samples.

PRINCIPLE OF TEST

Latex particles coated with antibody specific to human KL-6 form immune complexes in the presence of KL-6 from the sample. The immune complexes cause an increase in light scattering, which is proportional to the concentration of KL-6 in the sample. The light scattering is measured by reading turbidity at 570 nm. The sample KL-6 concentration is determined versus a calibration curve from KL-6 calibrators of known concentrations.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 1 x 15 mL
Tris(hydroxymethyl)aminomethane (100 mM)

R2: Latex Suspension 1 x 5 mL
Solution of latex particles coated with mouse anti-human KL-6 antibodies

WARNINGS AND PRECAUTIONS

For Research Use Only in the U.S. Not For Use in Diagnostic Procedures in the U.S.

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 < 0.1% w/v 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. Gently invert before use, avoid bubble formation.

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 on the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened reagents can be used until the expiration date on the package and bottle labels if they are kept tightly capped and at 2-8°C when not in use. Discard reagents if they become contaminated.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at approximately 570 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

SPECIMEN COLLECTION AND PREPARATION

Fresh serum or plasma should be used. Please take care not to cause hemolysis during sample collection.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1), Buffer Reagent 1 x 15 mL
Reagent 2 (R-2), Latex Suspension 1 x 5 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** KL-6 Calibrator (Ver.2), Cat. No. KAI-351C.

Two Reagent Chemistry Analyzer Capable of:
Accurate absorbance readings at approx. 570 nm
Accurately dispensing the required volumes
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 2.0 µL
↓
• ←R1 (Buffer Reagent) 120 µL
↓ 37 °C, 5 min.
• ←R2 (Latex Suspension) 40 µL
↓ 37 °C, 5 min.
Endpoint, 570 nm (main) / 800 nm (secondary)

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(KL-6v2)
ASSAY CODE	(2 POINT END)(10) (19)(34)(0)(0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(2.0) (0.0) (0)
REAGENT VOL (R1)	(120) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(40) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(0)(0)(LOWER)
CALIB. TYPE	(SPLINE)
POINT	(5)
SPAN POINT	(5)
SD LIMIT	(999.9)
DUPLICATE LIMIT	(32000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(U/mL)
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)

*1-5: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a calibration curve be made using the **K-*ASSAY*®** KL-6 Calibrator (Ver.2). It is recommended that each laboratory determine calibration frequency, as this would depend on the analyzer in use as well as the types and number of other assays being run.

QUALITY CONTROL

A quality control program is recommended for all laboratories. It is recommended the **K-*ASSAY*®** KL-6 Control (Ver.2) containing two levels of controls be run with each batch of samples to monitor the procedure.

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.

RESULTS / CALCULATIONS

KL-6 levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measurable range for this KL-6 assay is between 50 to 5,000 U/mL. If the KL-6 concentrations are greater than this range, dilute the sample with isotonic saline and re-assay. Multiply the result by the dilution factor to compensate for the dilution.

PERFORMANCE

Precision

Within-run (Intra-assay) CV is less than 10% (n=5).

Accuracy

Control serum recovers within 15% of the assigned value.

Correlation

A comparison of the **K-*ASSAY*®** KL-6 (Ver.2) assay and another company's KL-6 latex-enhanced immuno-turbidimetric assay was performed with the following results:

y = 1.0457x + 82.296
r = 0.9757
n = 96
x = another company's latex-enhanced immunoturbidimetric KL-6 assay
y = **K-*ASSAY*®** KL-6 (Ver.2) Assay

Assay Range

50 - 5,000 U/mL

INTERFERENCE

Testing was performed on a Roche / Hitachi 917 analyzer with the following results.

Bilirubin, Conjugated	No interference up to 20.1 mg/dL
Bilirubin, Unconjugated	No interference up to 19.7 mg/dL
Chyle (Formazine Turbidity)	No interference up to 1,480
Hemoglobin	No interference up to 480 mg/dL
Rheumatoid Factor	No interference up to 500 IU/mL
Sodium Citrate	No interference up to 15 mg/mL
Sodium Fluoride	No interference up to 25 mg/mL
Sodium Heparin	No interference up to 150 U/mL
EDTA-2Na	No interference up to 5 mg/mL

REFERENCES

1. Kohno, N. Respiration 16:391 (1997).
2. Kohno, N. *et al.* Japanese Journal of Clinical and Experimental Medicine 75:217 (1998).

LABELING SYMBOLS

REF	Catalog Number
	Expiration or "Use By" Date
LOT	Lot Number
	Consult Package Insert for Instructions for Use
CE	CE Mark Registered
2°C 8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

Advena Ltd.
Tower Business Centre, 2nd Flr.,
Tower Street, Swatar, BKR 4013 Malta

ORDERING / PRICING / TECHNICAL INFORMATION

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094