K-*ASSAY*®

KL-6
(Krebs von den Lungen-6)

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

Cat. No. KAI-250

INTENDED USE

The **K-*ASSAY*®** KL-6 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] 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 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
100 mM Tris(hydroxymethyl)aminomethane

R2: Antiserum Reagent
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 less than 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.

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 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 2-point calibration method.

PROCEDURE

Materials Supplied

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

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** KL-6 Calibrator, Cat. No. KAI-251C.

Two Reagent Clinical 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 2.5 µL
↓
• ← R1 (Buffer Reagent) 150 µL
↓ 37° 5 min.
• ← R2 (Reacting Reagent) 50 µL
↓ 37° 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-6)
ASSAY CODE / ASSAY POINT	(2 POINT END) (10) (19) (34) (0) (0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(2.5) (0.0) (0)
R-1 VOLUME (R1)	(150) (0)
R-2 VOLUME (R3)	(50) (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 calibrator

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a calibration curve be made using the **K-*ASSAY*®** KL-6 Calibrator. 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 testing laboratories. It is recommended the **K-*ASSAY*®** KL-6 Control 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.

LIMITATIONS OF PROCEDURE

The measurable range for this KL-6 assay is between 70 to 10,000 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 assay and another company's KL-6 latex-enhanced immunoturbidimetric assay was performed with the following results:

y = 1.065x - 2.3
r = 0.990
n = 122
x = another company's latex-enhanced immunoturbidimetric KL-6 assay
y = **K-*ASSAY*®** KL-6 Assay

An additional comparison with another company's KL-6 ECLIA (Electro Chemiluminesence Immuno-Assay) was performed with the following results:

y = 0.973x - 172.6
r = 0.957
n = 73
x = another company's ECLIA KL-6 assay
y = **K-*ASSAY*®** KL-6 Assay

Assay Range

70 - 10,000 U/mL

INTERFERENCE

Bilirubin F	No interference up to 18.9 mg/dL
Bilirubin C	No interference up to 21.3 mg/dL
Chyle (Formazine Turbidity)	No interference up to 1,410
Hemoglobin	No interference up to 500 mg/dL
RF	No interference up to 450 IU/mL
Sodium Citrate	No interference up to 150 mg/mL
Sodium Heparin	No interference up to 1,500 U/mL
EDTA-2Na	No interference up to 15 mg/mL

EXPECTED VALUES

Normal ranges have not been established for KL-6. However, a research study has reported the expected range as 98-313 U/mL.[2] Our internal data suggests a cutoff of 500 U/mL.

Each laboratory should establish its own expected values using this kit.

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

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use

ORDERING / PRICING / TECHNICAL INFORMATION

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