K-*ASSAY* ®

Lp(a)

For the Quantitative Determination of Human Lipoprotein(a)

Cat. No. KAI-017 and KAI-044

INTENDED USE

For the quantitative determination of human lipoprotein(a) [Lp(a)] in human serum by immunoturbidimetric assay. The test may provide in conjunction with other lipoprotein tests, the risk assessment of coronary artery disease for specific populations. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Lipoprotein(a) or Lp(a) is a lipoprotein particle structurally similar to LDL, but distinctive due to the covalent linkage of apolipoprotein(a) [Apo(a)] to apolipoprotein B (a protein marker for LDL) by a disulfide bond.

Apo(a) is a protein that has similarity to plasminogen, but does not have fibrinolytic activity. Recently much attention has been placed on the physiologic role of the Apo(a) portion of the Lp(a) molecule. The competition of Lp(a) with plasminogen for binding in the coagulation/fibrinolysis system and the presence of cholesterol esters with the Lp(a) particle have been studied.

The Lp(a) values of African American populations have been documented in the literature to have a mean and median Lp(a) values approximately twice that of Caucasian American populations.[4-11] The frequency distribution for Caucasian American populations is non-bell-shaped distribution with the majority of the values at the lower levels. The Lp(a) distribution for African American populations is a bell-shaped curve. Even though African Americans have higher levels of Lp(a), they do not have increased atherosclerotic progression or mortality.[6] Elevated plasma concentrations of Lp(a) are associated with coronary atherosclerosis in Caucasians. African-Americans have a higher median plasma Lp(a) concentration, but they do not have a greater incidence of coronary atherosclerosis. Studies by Moliterno et al. suggest that the plasma concentration of Lp(a) is not an independent risk factor for coronary artery disease in African Americans. A study by Schreiner et al. suggests that Lp(a) may be associated with carotid atherosclerosis in both blacks and whites, but note that the association may be affected by the presence of other cardiovascular risk factors, particularly in females.[13]

The K-*ASSAY* ® Lp(a) test is intended for the quantitative determination of human Lp(a) by immunoturbidimetric assay.

PRINCIPLE OF TEST

When a serum sample is mixed with anti-human Lp(a) antiserum, agglutination is caused by the antigen-antibody reaction. The turbidity is measured at 340 and 700 nm and the Lp(a) in the sample is quantitatively determined.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent
   Tris(hydroxymethyl)aminomethane (100 mM)

R2: Antiserum Reagent
   Anti-human Lp(a) goat antiserum (40%)

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.

a **WARNING:** This product can expose you to chemicals including thiourea which is known to the State of California to cause cancer/birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov. The R-1 reagent contains 0.38% of thiourea (CAS No. 62-56-6).

REAGENT PREPARATION

The reagents are ready to use. They do not need to be reconstituted.

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 one 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

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot. Serum should be stored refrigerated (2-8°C) and can be used within two weeks or should be stored at -80°C for up to 1 year.

For storage of samples for more than a few days, use of plastic tubes is recommended instead of glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a two-point or multi-point calibration method. 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-017, Lp(a) (L)

Reagent 1 (R-1) Buffer Reagent	3 x 21 mL
Reagent 2 (R-2) Antiserum Reagent	3 x 3 mL

or

KAI-044, Lp(a)

Reagent 1 (R-1) Buffer Reagent	1 x 21 mL
Reagent 2 (R-2) Antiserum Reagent	1 x 3 mL

Materials Required But Not Supplied

Calibrators: K-*ASSAY* ® Lp(a) Calibrator, Cat. # KAI-018C

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 717):

Sample   17 μL
↓
• ← R1 (Buffer Reagent)   350 μL
↓   37 °C, 5 min.
• ← R2 (Antiserum Reagent)   50 μL
↓   37 °C, 5 min.
2-point endpoint, 340 / 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(LPA)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(17) (17)
R-1 VOLUME	(350) ( ) (NO)
R-2 VOLUME	(50) ( ) (NO)
WAVELENGTH	(700) (340)
CALIB. METHOD	(NONLINEAR) (1) (6)
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)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-320000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

*1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a multi-point calibration curve be made using the K-*ASSAY* ® Lp(a) Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and number / type of other assays being performed. 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.

LIMITATIONS OF PROCEDURE

The measurable range for Lp(a) is 5 to 150 mg/dL. If the Lp(a) value of a sample is greater than highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

Specificity

When a sample with a known value is assayed, the result is within ±10% of the assigned value.

Precision

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 10.5	Mean = 22.9	Mean = 44.9
SD = 0.317	SD = 0.490	SD = 0.587
CV = 3.02%	CV = 2.14%	CV = 1.31%

Precision Assay: Between Runs

Sample IV	Sample V	Sample VI
N = 10	N = 10	N = 10
Mean = 9.7	Mean = 18.6	Mean = 45.1
SD = 0.35	SD = 0.40	SD = 0.81
CV = 3.65%	CV = 2.15%	CV = 1.80%

Accuracy / Correlation

A comparison of the K-*ASSAY* ® Lp(a) and another company's Lp(a) ITA was performed with the following results:

$y = 1.0084x + 1.1518$
$r = 0.9967$
$n = 85$
x = another company's Lp(a) assay
y = **K-*ASSAY*®** Lp(a) Assay

x min	= 3.00	y min	= 4.60
max	= 93.80	max	= 97.00
mean	= 18.64	mean	= 19.94

Assay Range

5 - 150 mg/dL (or value of highest calibration point)

INTERFERENCE

Bilirubin C	No interference up to 25 mg/dL
Bilirubin F	No interference up to 25 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Intralipid	No interference up to 1,000 mg/dL
Plasminogen	No interference up to 150 mg/dL

EXPECTED VALUE

The expected range for Lp(a) concentration has been reported to be between 10 and 30 mg/dL (ELISA). African American ranges are higher.[15]

In our laboratory, the expected value of Lp(a) in normal serum is less than 30 mg/dL. This value was determined by analyzing 240 healthy Asian individuals. Asian Lp(a) values (Chinese) have been shown to be similar to Caucasian plasma Lp(a) concentrations.[14] Each laboratory should establish its own expected values using this kit.

In our Lp(a) expected range study, "Healthy Subjects" were defined as individuals, that upon medical examination, were tested and found normal (within established normal range) for the following tests:

  Hepatic function: AST (GOT), ALT (GPT), γ-GTP

 Renal function: BUN, Creatinine

  Lipid: TG (Triglyceride), T-CHO (Total cholesterol), HDL-C

  Appearance of serum: No hemolysis and icterus.

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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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