K-*ASSAY* ®

Apo AI

For the Quantitative Determination of Human Apolipoprotein AI in Serum

Cat. No. KAI-002

INTENDED USE

For the quantitative determination of human Apolipoprotein AI (Apo AI) in serum by immunoturbidimetric assay. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Lipids are present in the plasma in a complex form, low density lipoproteins (LDL), very low density lipoproteins (VLDL), high density lipoproteins (HDL), and intermediate lipoproteins. These complexes are composed of lipid and carrier proteins, the apolipoproteins. There are several apolipoproteins: Apo AI, AII, B, CI, CII, CIII, and E.

Apolipoprotein AI is present in the highest concentration of any of the apolipoproteins. Apo AI makes up approximately 60% of the high density lipoprotein.[1] It provides the structural component for HDL formation.[2] Apo A activates lecithin cholesterol acyltransferase (LCAT) which catalyzes the esterification of cholesterol.[3] The cholesterol esters can then be transported to the liver where they are removed from the blood stream, catabolized, and excreted.[4]

Numerous studies have indicated that Apolipoprotein AI may be a useful tool in the assessment of coronary heart disease risk. Patients with coronary disease consistently have lower levels of Apo AI.[2,4,5]

Apo AI has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.[2] The **K-*ASSAY*** ® Apo AI assay uses an immuno-turbidimetric format.

PRINCIPLE OF TEST

The **K-*ASSAY*** ® Apo AI assay quantifies Apolipoprotein AI based on immunoturbidimetric assay. The reagent uses a goat polyclonal antibody specific for human Apolipoprotein AI.

The antibody binds to the Apo AI in the serum forming light scattering immune complexes, which increase the turbidity of the sample. Since the increase in turbidity is proportional to the amount of Apo AI in the sample, the Apolipoprotein AI concentration can be determined by measuring this increase in turbidity. The increase in turbidity is measured at 800 nm. Apolipoprotein AI in the sample is quantitatively determined. The **K-*ASSAY*** ® Apo AI assay can be run using a two-reagent clinical chemistry analyzer. Six calibrators are prepared using the **K-*ASSAY*** ® Apo AI/B Calibrator. These calibrators are used for quantifying the levels of Apo AI present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 3 x 20 mL
Tris(hydroxymethyl)aminomethane
Sodium chloride
Sodium Azide (< 0.1%)

R2: Antiserum Reagent 1 x 20 mL
Anti-human Apolipoprotein AI goat antiserum (70%)
Tris(hydroxymethyl)aminomethane
Sodium chloride
Sodium Azide (< 0.1%)

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 for up to 18 months from the date of manufacture, as indicated by the expiration date on 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.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 800 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

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. 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. Blood should be collected from a fasting patient and the serum collected as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass). Samples not tested within 72 hours should be frozen at -20°C. Avoid multiple freeze-thaws.

Use plastic tubes for storing the samples, do not use glass.

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 3 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 1 x 20 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*** ® Apo AI/B Calibrator, Cat. No. KAI-008C (Containing known levels of Apo AI).

Two-Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 800 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Quality Control Materials

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 3 µL
↓
• ← R1 (Buffer Reagent) 300 µL
↓ 37 °C, 5 min.
• ← R2 (Antiserum Reagent) 100 µL
↓ 37 °C, 5 min.
2-point endpoint, 800 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(Apo AI)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ( )
R-1 VOLUME	(300) ( ) (NO)
R-2 VOLUME	(100) ( ) (NO)
WAVELENGTH	( ) (800)
CALIB. METHOD	(NONLINEAR) (4) (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 Apo AI levels be determined using a multi-point calibration curve prepared using the **K-*ASSAY*** ® Apo AI/B Calibrator. 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 each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if

the value determined for the controls falls outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measurable range for Apo AI is between 20 - 300 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations (> 1,000 mg/dL) should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the Apo AI concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

Precision

The precision for the **K-*ASSAY*** ® Apo AI assay was determined using packaged reagents, pooled human serum, and a Hitachi 704 analyzer.

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 83.1	Mean = 142.6	Mean = 189.0
SD = 1.6	SD = 2.3	SD = 2.9
CV = 1.87%	CV = 1.60%	CV = 1.51%

Concentrations in mg/dL

Precision Assay: Between Runs

Sample I	Sample II	Sample III
N = 10	N = 10	N = 10
Mean = 85.9	Mean = 136.5	Mean = 185.9
SD = 1.1	SD = 1.437	SD = 2.9
CV = 1.28%	CV = 1.05%	CV = 1.57%

Concentrations in mg/dL

Accuracy / Correlation

A comparison of the **K-*ASSAY*** ® Apo AI assay and a Sigma Apo AI Test Kit was performed using a Hitachi 704. The test results provided the following data:

y = 0.980x + 4.776
r = 0.970
n = 55
x = Sigma Apo AI Test Kit
y = **K-*ASSAY*** ® Apo AI Assay

x min	= 91	y min	= 89
max	= 200	max	= 213
mean	= 146	mean	= 147

Linearity

Linearity tests were performed with normal human serum spiked with high concentration fractions of Apo AI. Testing was linear from 20 - 300 mg/dL of Apo AI.

INTERFERENCE

Bilirubin C	No interference up to 20 mg/dL
Bilirubin F	No interference up to 20 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Intralipid	No interference up to 2,000 mg/dL

EXPECTED VALUES

The expected value as reported is between 115 - 224 mg/dL. Each laboratory should establish its own expected values using this kit.

This test system has been evaluated through a WHO/IFCC/CDC collaborative effort and assay values are traceable to the WHO International Reference Material for Apo AI, SP1-01. The evaluation was performed on a Hitachi 717 analyzer using the **K-*ASSAY*** ® Apo AI/B Calibrator, Cat. No. KAI-008C.

REFERENCES

1. Rifai, N., "Lipoproteins and Apolipoproteins: Composition, Metabolism, and Association with Coronary Heart Disease." Arch. Pathol. Lab. Med. 110:694, 1986.
2. Naito, HK., "Clinical Significance of Apolipoprotein Measurements." J. Clin. Immunoassay 9:11, 1986.
3. Glomset, J.A. "The Plasma Lecithin Cholesterol Acyltransferase Reaction." J. Lipid Research. 9:155, 1968.
4. Gordon, T. *et al*. "High Density Lipoprotein as a Protective Factor against Coronary Artery Disease." The Framingham study." Am. J. Med. 62:707, 1977.
5. Marcovina, S. *et al*. "International Federation of Clinical Chemistry Standardization Project for Measurements of Apolipoproteins AI and B. IV. Comparability of Apolipoprotein AI Values by Use of International Reference Material. Clin. Chem. 39/5: 773-781, 1993.
6. Killingsworth, L.M. and Savory, J., "Nephelometric Studies on the Precipitin Reactions," J. Clin. Chem., 19: 403-407, 1973.
7. Sternberg, J.C. " A Rate Nephelometer for Measuring Specific Proteins by Immunoprecipitin Reactions," Clin. Chem., 23:1456-64, 1977.

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

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

ORDERING / PRICING / TECHINCAL INFORMATION

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