K-*ASSAY* ®

Apo E

For the Quantitative Determination of Human Apolipoprotein E in Serum and Plasma

Cat. No. KAI-007

INTENDED USE

For the quantitative determination of human apolipoprotein E in 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

Apolipoprotein E (Apo E), a 34,000 dalton plasma protein, is found primarily in chylomicrons, VLDL, and HDL. Apo E serves as a ligand for lipoprotein receptors. Through its interaction with these receptors, Apo E is involved in the transport and metabolism of cholesterol and other lipids in the body.

Accordingly, an apolipoprotein E assay can be used for studies involving secondary lipid metabolism disorders such as hereditary lipid metabolism disorder, arteriosclerosis, etc.

The **K-*ASSAY*** ® Apo E test is intended for the quantitative determination of human apolipoprotein E by immuno-turbidimetric assay.

PRINCIPLE OF TEST

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

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 3 x 20 mL
Tris(hydroxymethyl)aminomethane

R2: Antiserum Reagent 1 x 20 mL
Anti-human Apolipoprotein E goat antiserum

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 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.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA, sodium citrate, or sodium heparin) test samples must be collected in the manner routinely used for clinical laboratory tests. Use fresh samples if possible. If samples will be frozen, seal tubes from air and avoid repeated freeze/thawing. Long-term storage is not recommended.

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 E Calibrator, Cat. No. KAI-025C (Containing known levels of Apo E).

Purified Water

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 3 µL
↓
• ← R1 (Buffer Reagent) 300 µL
↓ 37 °C, 5 min.
• ← R2 (Antiserum Reagent) 100 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	(Apo E)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ( )
R-1 VOLUME	(300) ( ) (NO)
R-2 VOLUME	(100) ( ) (NO)
WAVELENGTH	(700) (340)
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 a multi-point calibration curve be made using the **K-*ASSAY*** ® Apo E Calibrator. It is recommended that the user determine calibration frequency as this will depend on the instrument and number/type of other assays being run. Initially, calibration should be performed each day.

LIMITATIONS OF PROCEDURE

If Apo E concentration of 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 control serum with a known value is assayed, the result is within ±10% of the assigned value.

Precision

When a sample that has an apolipoprotein E concentration of around 4 mg/dL is assayed 20 times (within-run), the absorbance C.V. is below 5%.

Assay Range

1 - 15 mg/dL

INTERFERENCE

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

REFERENCES

1. Koga, T. *et al*. Japanese Journal of Clinical Chemistry (Rinsho Kagaku). 19:19-27 (1990).
2. Tuguhiko, N. The Japanese Journal of Clinical Pathology (Rinsho Byouri). 38:125-134 (1990).
3. Yasuyuki, O. The Japanese Journal of Clinical Pathology (Rinsho Byouri). 38:1134-1140 (1990).
4. Labeur, C. *et al*., Clinical Chemistry. 36:591-597 (1990).
5. Stein, E.A. Lipid and Lipoprotein Risk Factor. 87-100 (1991).
6. Matsushima, T. Kensa to Gijutsu (in Japanese). 21:155-158 (1993).

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
	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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