

Apo B

For the Quantitative Determination of Human Apolipoprotein B in Serum

Cat. No. KAI-004

INTENDED USE

For the quantitative determination of human Apolipoprotein B (Apo B) 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 A1, AII, B, C1, CII, CIII, and E.

Apolipoprotein B is the major low density lipoprotein (LDL). Apo B is an integral component of the four major atherogenic lipoproteins: very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and lipoprotein(a).¹ Apo B plays a major role in the recognition of cellular receptors for the catabolism of LDL.²

Apo B measurements are useful in the diagnosis of atherosclerosis. Numerous studies have indicated that apolipoprotein B may be useful in assessing coronary heart disease risk. Patients with coronary disease consistently have higher levels of Apo B than control values.^{1,2,6,7}

Apo B has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.² The **K-ASSAY®** Apo B assay uses an immunoturbidimetric format and measures B100 and B48.

PRINCIPLE OF TEST

The **K-ASSAY®** Apo B assay quantifies apolipoprotein B based on immunoturbidimetric assay. The antiserum used in the kit is a goat polyclonal antibody specific for human apolipoprotein B. The Apo B antibody interacts with the Apo B in the serum forming immune complexes. The immune complexes cause an increase in light scattering, which can be measured at 600 nm. Since the increase in turbidity is proportional to the amount of Apo B in the sample, the apolipoprotein B concentration can be determined by measuring this increase in turbidity. Apolipoprotein B in the sample is quantitatively determined.

The **K-ASSAY®** Apo B assay can be run using a two-reagent clinical chemistry autoanalyzer. Six calibrators are prepared using the **K-ASSAY®** Apo A1/B Calibrator. These calibrators are used for quantifying the level of Apo B present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)
R1: Buffer Reagent 3 x 20 mL
Tris(hydroxymethyl)aminomethane
Sodium azide (< 0.1%)

R2: Antiserum Reagent 1 x 20 mL
Anti-human Apolipoprotein B goat antiserum (50%)
Tris(hydroxymethyl)aminomethane
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 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.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 600 nm. Refer to the instrument manual from the manufacturer regarding the following:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and / or equipment to be used
- Operational precautions, limitations, and hazards
- Service and maintenance information

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. 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 sample, 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 A1/B Calibrator, Cat. No. KAI-008C (Containing human serum with known levels of Apo B).

Two-Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 600 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Normal and Abnormal Controls of known concentration

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°, 5 min.
↓	
• ← R2 (Antiserum Reagent)	100 µL
↓	37°, 5 min.
↓	
2-point endpoint, 600 nm	

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(Apo B)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ()
R1 VOLUME	(300) () (NO)
R2 VOLUME	(100) () (NO)
WAVELENGTH	() (600)
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	(-32000) (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 B levels be determined using a multi-point calibration curve prepared using the **K-ASSAY®** Apo A1/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 B is between 25 to 250 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 B 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 B assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 36.0	Mean = 70.2	Mean = 120.2
SD = 0.7	SD = 1.5	SD = 1.7
CV = 2.06%	CV = 2.12%	CV = 1.44%

Concentration in mg/dL

Precision Assay: Between Runs

Sample I	Sample II	Sample III
N = 10	N = 10	N = 10
Mean = 58.6	Mean = 83.3	Mean = 124.5
SD = 1.3	SD = 1.9	SD = 1.4
CV = 2.25%	CV = 2.33%	CV = 1.09%

Concentration in mg/dL

Accuracy / Correlation

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

$y = 1.442x + 0.00$
 $r = 0.885$
 $n = 50$
 $x =$ Sigma Apo B Test Kit
 $y =$ **K-ASSAY**® Apo B assay

x min = 26	y min = 42
max = 106	max = 175
mean = 65	mean = 107

Linearity

Linearity tests were performed with normal human serum spiked with high concentration fractions of Apo B. Testing was linear from 25 to 250 mg/dL of Apo B.

INTERFERENCE

Bilirubin F and C:	No interference up to 20 mg/dL
Hemoglobin:	No interference up to 500 mg/dL
Lipemia:	No interference up to 20% volume of Intralipid® 10%

EXPECTED VALUES











The expected value as reported is between 60 to 130 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 B, SP3-07. This evaluation was performed on a Hitachi 717 analyzer using the **K-ASSAY**® Apo A/B Calibrator, Cat. No. KAI-008C.

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LABELING SYMBOLS

	Lot Number
	Reagent
	Expiration or "Use By" Date
	Catalog Number
	For <i>In Vitro</i> Diagnostic Use
	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE



ECIREP

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