K-ASSAY®

RLP-C
(Remnant Lipoprotein Cholesterol)

For the Quantitative Determination of Remnant Lipoprotein Cholesterol in Serum

Cat. No. KAI-260

INTENDED USE

The **K-ASSAY ®** RLP-C assay is for the quantitative determination of Remnant Lipoprotein Cholesterol in human serum by colorimetric assay. FOR RESEARCH USE ONLY IN THE U.S. NOT FOR USE IN DIAGNOSTIC PROCEDURES IN THE U.S.

INTRODUCTION AND SUMMARY

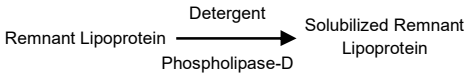
The hydrolyzed products of Chylomicron (CM) and Very Low Density Lipoprotein (VLDL) are collectively called remnant lipoprotein (RLP). Remnant lipoprotein is one of the lipoproteins that develop arteriosclerosis and is also one of the risk factors of the disease. Measurement of RLP-C is a useful method to evaluate the status of RLP.

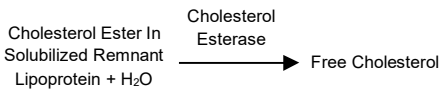
Research studies have reported that RLP-C may be an important risk factor for coronary artery disease, myocardial infarction, and arteriosclerosis, especially when testing samples with otherwise normal total cholesterol levels. Research studies have reported that RLP-C has about twice the association with ischemic heart disease as LDL cholesterol.

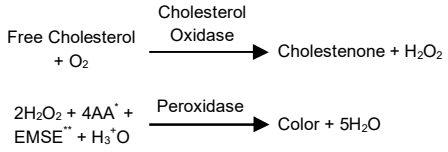
The **K-ASSAY ®** RLP-C assay is a colorimetric assay, developed to quantitatively determine levels of RLP-C in serum samples.

PRINCIPLE OF TEST

Phospholipase-D and a surfactant selectively enable remnant lipoproteins to be soluble. Cholesterol esters derived from remnant lipoproteins are hydrolyzed by cholesterol esterase to form free cholesterol. The free cholesterol, combined with one derived from remnant lipoprotein directly, is oxidized by cholesterol oxidase to give hydrogen peroxide and cholestenone, while the surfactant inhibits the reaction of cholesterol oxidase with cholesterol from the other lipoproteins. The hydrogen peroxide generated reacts with 4-AA and EMSE in the presence of Peroxidase to give red-purple dye. The amount of remnant lipoprotein cholesterol is then determined by colorimetric methodology.[1]







* 4-AA: 4-Aminoantipyrine
** EMSE: N-Ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 1 x 12 mL
EMSE (0.2 - 2 µmol/mL)

R2: Enzyme Reagent 1 x 4 mL
4-Aminoantipyrine (0.3 - 1 µmol/mL)
Cholesterol Oxidase (0.25 - 1.25 IU)
Cholesterol Esterase
Phospholipase

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 < 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 up to 12 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 for 4 weeks if stored at 2-8°C. Discard reagents if they become contaminated.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at approximately 600 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 should be used. Samples should be stored at 2-8°C and assayed within 2 days after 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 12 mL
Reagent 2 (R-2), Enzyme Reagent 1 x 4 mL

Materials Required But Not Supplied

Calibrators: **K-ASSAY®** RLP-C Calibrator,
Cat. No. KAI-261C.

Purified Water

Saline

Two Reagent Chemistry Analyzer Capable of:
Accurate absorbance readings at approx. 600 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 3.2 µL
↓
• ←R1 (Buffer Reagent) 150 µL
↓ 37 °C, 5 min.
• ←R2 (Enzyme Reagent) 50 µL
↓ 37 °C, 5 min.
Endpoint, 600 nm (main) / 700 nm (secondary)

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(RLPC)
ASSAY CODE / ASSAY POINT	(2 POINT END)(10) (16)(34)(0)(0)
WAVELENGTH	(700) (600)
SAMPLE VOLUME	(3.2) (0.0) (0)
REAGENT VOL (R1)	(150) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(50) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(0)(0)(LOWER)
CALIB. TYPE	(LINEAR)
POINT	(2)
SPAN POINT	(2)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(mg/dL)
STD.(1) Conc.-POS.	(0.0) - (1)
STD.(2) Conc.-POS.	(*2) - (2)

Use isotonic saline as STD (1)
*2: Input concentration of calibrator

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a 2-point calibration curve be made using isotonic saline (0 mg/dL) and the **K-ASSAY ®** RLP-C 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 laboratories. It is recommended the **K-*ASSAY*®** RLP-C 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 RLP-C assay is between 1 to 80 mg/mL. If the RLP-C 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=10).

Accuracy

Control serum recovers within 10% of the assigned value.

Correlation

A comparison of the **K-*ASSAY*®** RLP-C assay and another company's RLP-C assay was performed with the following results:

y = 1.004x + 0.271
r = 0.995
n = 133
x = another company's RLP-C assay
y = **K-*ASSAY*®** RLP-C Assay

Assay Range

1 - 80 mg/dL

INTERFERENCE

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-A2 guideline with the following results.

Ascorbic Acid:	No interference up to 50 mg/dL
Bilirubin (Conjugated):	No interference up to 20 mg/dL
Hemoglobin:	No interference up to 500 mg/dL
Lipemic Sample:	No interference up to 3,000 FTU (Formazin Turbidity Units)

REFERENCES

1. Miyauchi, K. *et al.* Clinical Chemistry. 53:2128-2135 (2007).

LABELING SYMBOLS

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

ORDERING / PRICING / TECHNICAL INFORMATION

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