H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

LDL CHOLESTEROL (DIRECT) CE

STABLE LIQUID REAGENT

Cat. No.:	43161,43161S	43162,43162S
	60 ml	500 ml
	(1x45 ml+1x15 ml)	(1x375 ml +1x125 ml)

Quantitative determination of LDL cholesterol measurement in the serum

Principle

First step.

Cholesterol in HDL, VLDL and chylomicron (CM) in the specimen are catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R1 solution into the specimen. LDL cholesterol are not catalyzed at first step. The generated hydrogen peroxide is consumed by catalase.

$$LDL,\ VLDL\ and\ chylomicron \xrightarrow{Cholesterol-esterase} Free\ cholesterol + Fatty\ acid$$

$$Free\ cholesterol + O_2 \xrightarrow{Cholesterol-oxidase} cholestenone + H_2O_2$$

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O_2 + O_2$$

Second step.

Subsequently, LDL cholesterol in the specimen is catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R2 solution into the specimen. N-ethyl-N-(3-sulfopropyl)-m-anisidine (ESPAS) and 4-aminoantipyrine (4-AA) are condensed by the generated hydrogen peroxide and Peroxidase to generate a quinone dye. The absorbance of the generated quinone dye is analyzed to obtain the LDL cholesterol concentration in the specimen with reference of a standard solution.

$$LDL\text{-}C \xrightarrow{Cholesterol-esterase} Free\ cholesterol + Fatty\ acid$$

$$Free\ cholesterol + O_2 \xrightarrow{Cholesterol-oxidase} cholestenone + H_2O_2$$

$$2\,H_2O_2 + 4\text{-}aminoantipyrine + ESPAS \xrightarrow{Peroxidase} Quinone\ dye$$

Reference values

Desirable value:	**< 130 mg/dl**	**< 3.36 mmol/l**
Increased risk for coronary heart disease:	130-159 mg/dl	3.36-4.1 mmol/l
High risk for coronary heart disease:	≥160 mg/dl	(≥4.13 mmol/l)

It is recommended that each laboratory should assign its own normal range.

Reagents

R1

Cholesterol Esterase
Cholesterol Oxidase
Catalase
4-aminoantipyrine

R2

Peroxidase
ESPAS

3. LDL cholesterol standard

Ready for use.For details please check the insert.
Available only in Cat. No.: 43161S and 43162S

Onboard stability is up to 27 days without calibration

Samples

Use serum as a specimen. It is recommended to measure LDL-C immediately after collection.

PROCEDURE

Reagents 1 and 2 are ready for use.

Assay conditions

Main wavelength:	600 nm
Sub wavelength:	800 nm
Light path:	1 cm
Temperature:	37 °C
Method:	endpoint (increasing)

Pipette into cuvette

	Sample	Standard	Reagent blank
Specimen	2.4 µl	2.4 µl	2.4 µl
Reagent 1	225 µl	225 µl	225 µl
Mix and incubate for 5 min and then measure the absorbance difference between at 600 nm and 800 nm against distilled water as the reference with light path length 1 cm			
Absorbance	Abs A1	Abs S1	Abs B1
Reagent R2	75 µl	75 µl	75 µl
Mix and incubate for 5 min and then measure the absorbance difference between at 600 nm and 800 nm against distilled water as the reference with light path length 1 cm			
Absorbance	Abs A2	Abs S2	Abs B2

Calibration

S1: Distilled water

S2: Sentinel HC calibrator
Wako HC calibrator
Randox HC calibrator

Calibration frequency

Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation

Sample abs = Abs A2 - K×Abs A1
Blank abs = Abs B2 – K×Abs B1
Standard = Abs S2 – K×Abs S1

$$\text{LDL - C (mg/dl)} = \frac{Sample\ abs - Blank\ abs}{S\tan dard\ abs - Blank\ abs} \times \text{std. conc (mg/dl)}$$

$$K = \frac{Sample\ volume\ (\mu l) + R1\ volume\ (\mu l)}{Sample\ volume\ (\mu l) + R1 volume\ (\mu l) + R2\ volume\ (\mu l)} = \frac{227,4}{302,4}$$

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits

PERFORMANCES DATA

Linearity

The test is linear up to 11,6 mmol/l (450 mg/dl).
The reagent is linear between the concentration of 3,0-450 mg/dl (0.07-11,6 mmol/l).

Sensitivity

The absorbance when measuring water is less than 0,05. The absorbance when measuring standard (100 mg/dl) is in the range of 0,05-0,17

Specificity

When measuring control serum, the data spread of assay expected value is within 10%
When the sample's triglyceride concentration exceeds up to 1000 mg/dl, dilute the sample with physiological saline and repeat the measurement.
There is minimal interference of measurements with up to 20 mg/dl bilirubin, 50 mg/dl ascorbic acid, 500 mg/dl hemoglobin, 1200 formazine turbidity unit, 200 mg/dl EDTA (Na salt), and 80 U/ml heparin.

Precision

When measuring the absorbance of each test at 3 times, CV of the absorbance is less than 5%. Intra-run precision were CV's of 1,20% and 0,56% with concentrations of 57,7 and 147,4 mg/dl of serum controls, respectively.

Correlation

A comparative study has been performed between our reagent and other commercial LDL-cholesterol reagent.The parameters of linear regression are as follows:
y=1.025x+1,41 (mg/dl), r=0,9976, N=62

Note

The sample should be handled with proper care to avoid infection.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

National Cholesterol Education Program : Executive summary of third report of the National Cholesterol Education Program expert panel on detection, evaluattion and treatment of high blood cholesterol in adults (J AMA,285:2486-2497,2001)