SBio LDL-D CHOLESTEROL KIT

(Direct Enzymatic Method)

(For invitro diagnostic use only)

REF	90920040
Pack Size	40 ml

CE

8°C 2°C Store at 2-8°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	L1 LDL-D Reagent 1	Direct Enzymatic Direct Enzymatic Method
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	L2 LDL-D Reagent 2	
Date of Manufacture	REF Catalogue Number	EC REP Authorised Representative in the European Community	C Calibrator (for 1ml)	This way up

INTENDED USE

LDL-D Cholestrol Kit is used for the determination of LDL Cholesterol in serum..

PRINCIPLE OF THE TEST

Direct determination of serum LDLc (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps. The assay takes place in two steps. First by the elimination of lipoprotein non-LDL Cholesterol and then the measurement of LDLc. The intensity of the color formed is proportional to the LDLc concentration in the sample.

Elimination of non-LDL Cholesterol

Cholesterol Esterase

Cholesterol esters + H_2O ⟶ Cholesterol + Fatty acids

Cholesterol Oxidase

Cholesterol + 0_2 ⟶ 4-Cholestenone + H_2O_2

Peroxidase

$2H_2O_2$ + 4 AAP ⟶ Colourless End Product

Measurement of LDL Cholesterol

Cholesterol Esterase

Cholesterol esters ⟶ Cholesterol + Fatty acids

Cholesterol Oxidase

Cholesterol + O_2 ⟶ 4-Cholestenone + H_2O_2

Peroxidase

$2H_2O_2$+ TOOS ⟶ Quinonimine + $4H_2O$
+ 4-Aminoantipyrine

CLINICAL SIGNIFICANCE

The LDL particles are lipoproteins that transport cholesterol to the cells. Often called "bad cholesterol" because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRESENTATION	40 ml
L1 : LDL-D Reagent 1	30 ml
L2 : LDL-D Reagent 2	10 ml
C : Calibrator (for 1ml)	1 No.

COMPOSITION

Goods Buffer 50mM; 4-AAP 14mM; CHE 4800 U; POD 4800 U; CHOD 3600 U; Magnesium Chloride Hexahydrate 6mM; Non Reactive Stabilizers, Detergents and Preservatives.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents L1 and L2 are ready to use, Cap the bottles immediately after use and avoid contamination.

Calibrator: Reconstitute with 1 ml of D.W. Mix gently to dissolve the contents. Once reconstituted the calibrator is stable for 2 weeks at 2-8°C or 3 months at -20°C. Do not repeatedly thaw and refreeze.

SAMPLE REQUIRED

Serum or Heparinized plasma. Serum should be separated from the clot, as soon as possible. LDL is reported to be stable in the sample for 1 week when stored at 2-8° C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength / filter : 546 nm
Temperature : 37°C
Light path : 1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic Guyette holder, micropipette and appropriate laboratory equipment.

Pipette into clean dry test tubes labelled as Blank (B), Calibrator (C) and Test (T):

Addition Sequence	B (ml)	C (ml)	T (ml)
LDL-D Reagent 1 (L1)	0.375	0.375	0.375
Calibrator	-	0.005	-
Sample	-	-	0.005
Mix and incubate for 5 mins. at 37°C and add			
LDL-D Reagent 2 (L2)	0.125	0.125	0.125

Mix well and incubate for 5 mins. at 37°C and read the absorbance of the Calibrator and the Test against Blank.

CALCULATIONS

$$\text{LDLc in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Calibrator}} \times \text{Conc. of Calibrator}$$

QUALITY CONTROL

The following process is recommended for QC during the assay of LDL-D Cholestrol. Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, re-calibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

The procedure is linear upto 1000 mg/dl. If values exceed this limit dilute the serum 1+ 1 with normal saline and repeat the assay (Results x 2).

Interferences :

No interferences were observed with Ascorbic acid up to 50 mg/dl, Hemoglobin up to 500 mg/dL or Bilirubin up to 30 mg/dL. A list of drugs and other interfering substances with HDL cholesterol determination has been reported byYoung et. al.

Precision:

	Intra-assay			Inter-assay		
Mean (mg/dL)	63.2	107.0	49.0	65.2	112	253
SD	0.64	1.89	0.79	0.3	0.7	1.7
CV	1.01	1.76	1.61	0.45	0.60	0.65

Sensitivity : 1 mg /dL= 0.0012A.

Accuracy :

Results obtained when compared with other commercial reagents (x). The results obtained using 92 samples were the following: Correlation coefficient (r) : 0.998. Regression equation: y = 4.6 + 0.940x. The results of the performance characteristics depend on the analyzer used.

Method comparison:

Comparative studies were done to compare our reagent with another commercial LDL-D Cholesterol Assay. No significant differences were observed. Details of the comparative studies are available on request.

REFERENCE RANGE

Low Risk : < 100 mg/di
Normal Risk : 139 - 160 mg/dl
High Risk : > 160 mg/di

It is recommended that each laboratory establish its own normal range representing its patient population.

NOTE

Components from human origin in the calibrator have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

Do not use the reagents if there are particles or turbidity.

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

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EC REP

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LDL1(SBio)/M20/VER-02