LDL CHOLESTEROL

Direct Reagent Kit

Liquid Reagent

INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of Direct LDL Cholesterol in human serum and plasma.

TEST PRINCIPLE & REACTION

The reagent is based on the following reactions :

1. Elimination of non LDL- Cholesterol

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Catalase}} O_2 + H_2O$$

2. Specific measurment of LDL-Cholesterol after release of LDL-Cholesterol by detergents in Reagent 2.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{TOOS} \xrightarrow{\text{Catalase}} \text{Quinone} + H_2O$$

The intensity of the quinone pigment produced is proportional to the cholesterol concentration when measured at 578 nm.

KIT CONTENTS:

Reagent 1 : R1 Reagent

Reagent 2: R2 Reagent

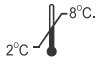
Reagent 3: Direct LDL Calibrator (Separately Provided)

Product Insert : 01 No.

PREPARATION OF REAGENT & STABILITY:

The Reagent 1 & Reagent 2 are ready to use.

Calibrator : Reconstitute with distilled water (Volume mentioned on calibrator vial label). Let it stand for 30 minutes at room temperature. Dissolve the content of the vial swirling gently to avoid the for mation of foam.



STORAGE AND STABILITY:

Reconstituted calibrator is stable only for7 days at 2 - 8°C.

SPECIMEN COLLECTION AND STORAGE:

Fresh Serum (Free of Hemolysis).

PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assy bring all the reagents to room temperature.
4. After use store the kit contents immediately as 2-8°C.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual):

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

Addition sequence	B	C	T
(R1) Reagent	375 µl	375 µl	375 µl
Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix and Incubate for 5 min. at 37°C.			
(R2) Reagent	125 µl	125 µl	125 µl

Mix and incubate for 5 min. at 37°C. Measure the absorbance of calibrator & Test against reagent blank at 578 nm.

CALCULATIONS:

$$\text{LDL-C Conc.} = \frac{\text{Abs of Test}}{\text{Abs of Calibrator}} \times \text{Calibrator Conc.}$$

NORMAL VALUES*:

Serum/Plasma

< 130 mg/dl	:	Desirable
< 130- 159 mg/dl	:	Border Line High risk for CHD
> 160 mg/dl	:	High risk for CHD

Each Laboratory should establish it's own normal range representing its patientpopulation.

LINEARITY :

This procedure in linear upto 1000 mg/dl. If the values exceed this limit, dilute the sample with normal saline (NaCl 0.9 %) and repeat the assy. Multiply result by dilution factor.

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.

APPLICATIONS:

Input parameters for semi- auto / auto analyzers are given below:

INPUT PARAMETERS	VALUES
Reaction type	End point
Wave length	578 nm (578 - 620 nm)
Temperature	37°C
Incubation	5 min. + 3 min.
Reagent volume	R1 375 µl + R2 125 µl
Sample volume	5 µl
Zero setting	Deionised water
Light path	1.0 cm
Unit	mg/dl
Linearity	1000 mg/dl
Calibrator Conc.	As Indicated on the Vial Label

QUALITY CONTROL:

For accuracy, it is necessary to run known controls with every assay.

REFERENCES:

1. Crouse J.R *et* al., Studies of Low density Lipoprotein molecular weight in human being with coronary artery disease. J.Lipid Res 26:5666 (1985)
2. Barr, T. *et al.* Protein-Lipid Relationships in Human Plasma. Am J Med 1951;11:480
3. Gordon, *et al* High Density Lipoprotein as a protective Factor Against Coronary heart disease. Am J Med 1977;62:707.

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