LDL DIRECT

(Selective Detergent)

ENZOPAK

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Ref. CC3-LDL.022, 20ml/50 Test
CC3-LDL.22M, 40 ml/100 Test
CC3-LDL.22MU, 80ml/200 Test

INTENDED USE

Quantitative Determination of LDL Cholesterol (Direct).

DIAGNOSTIC SIGNIFICANCE

The LDL Cholesterol 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 with clinical and other laboratory data.

PRINCIPLE

Determination of serum LDL Cholesterol (low-density lipoprotein cholesterol) (Direct) levels without the need for any pre-treatment or centrifugation steps.
The assay takes place in two steps.
Elimination of lipoprotein non-LDL Cholesterol.

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O+O_2$$

Measurement of LDL Cholesterol

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2H_2O_2 + \text{TOOS + 4-AA} \xrightarrow{\text{POD}} 2H_2O+O_2$$

The Intensity of the color formed is proportional to the LDL Cholesterol concentration in the sample.

PRESENTATION

All reagents to be stored at 2-8^{0}C	No. of Bottles		
	20ml/50 T	40ml/100 T	80ml/200 T
• 1 LDL Direct	1	1	1
• 2 LDL Direct	1	1	1
• HDL/LDL Calibrator	1	1	1
• Distilled Water	1	1	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• GOOD pH 7.0 (2^{0}C)	100 mmol/L
• Cholesterol esterase (CHE)	380 U/L
• Cholesterol oxidase (CHOD)	380 U/L
• Catalase	400 U/ml
• N-(2hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (TOOS)	0.45 mmol/L
• 4-Amino antipyrine (4-AA)	1.00 mmol/L
• Peroxidase (POD)	100 U/L
• HDLc/LDLc CAL	Standard, Lyophilized Human serum

PRECAUTION

Do not use reagents over the expiration date.
Sign of reagent deterioration.
Presence of particles and turbidity.

PREPARATION OF WORKING REAGENT

LDL Direct Cholesterol Reagents are ready to use.

HDL/LDL Calibrator Preparation & Stability

Refer the calibrator insert before use.

REAGENT STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contamination are prevented during their use.
R1 and R2: Once opened is stable for 4 weeks at 2-8°C.

SPECIMEN COLLECTION

Serum: After Serum Separation, the test should be performed without delay.
Repeated freezing and thawing should be avoided.
Stability of the sample: 7 days at 2-8°C.

REACTION PARAMETERS

Type of Reaction	: Fix Time
Wavelength	: 578 nm
Flow cell temperature	: 37°C
Delay Time	: 300 Sec
Interval Time	: 300 Sec
No. of Reading	: 2
Sample Volume	: 5 µl
Reagent Volume (R1+R2)	: 375 + 125 µl
Calibrator Concentration	: As mentioned on vial
Light Path	: 1 cm
Zero setting with	: Distilled water

TEST PROCEDURE

Pipette into Test Tube	Blank	Calibrator	Sample
R1 (µL)	375	375	375
Calibrator (µL)	-	5	-
Sample (µL)	-	-	5

Mix and Incubate for 5 min at 37°C.
Add:

R2 (µL)	125	125	125

Mix and read absorbance (A1) after 5 second. Incubate for 5 min at 37°C. Read the absorbance (A2).

TEST RESULT

$$\text{LDL Chol. (mg/dl)} = \frac{\text{(A2-A1) of Unknown}}{\text{(A2-A1) of calibrator}} \text{X Calibrator Value}$$

QUALITY CONTROL

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 tolerance.

LIMITATIONS FOR INTERFERENCE

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 LDL cholesterol determination has been reported by young et al.

NORMAL VALUES

Level of the risk
<154.68 mg/Dl

These values are for orientation purpose; each laboratory should establish its own reference range.

LINEARITY

The method is linear upto a concentration of 610 mg/dl. Specimens with LDL values above 610 mg/dL should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.

PRECISION

	Intra –assay		
Mean (mg/dL)	32.9	50.8	101.4
SD	0.3	0.2	0.7
CV	0.8	0.5	0.7

Inter – assay		
32.8	50.0	100.0
0.4	0.7	1.1
1.3	1.5	1.1

REFERENCES

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