DIRECT LDL CHOLESTEROL (D-LDL-C)

Diagnostic reagent for determination of LDL (Low Density Lipoprotein) concentration.
Liquid. Dual reagents *(Ratio: R1/R2: 3/1)*. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). Do not freeze.

Ref No	Pack
MH-102	80 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of LDL (Low Density Lipoprotein)-cholesterol in human serum and plasma.

TEST SUMMARY AND PROCEDURE [1, 2, 3, 4, 5]

The main function of LDL is to take these molecules from the cells and tissues that produce cholesterol and triglycerides and transport them to the cells and tissues that need them. The blood level of LDL is associated with atherosclerosis, and therefore coronary artery disease, stroke and peripheral vascular disease.

The assay consists of distinct reaction steps:

1. LDL complexes with polyanion, detergent 1 in Reagent 1 can only dissolve in non-LDL lipoprotein particles (CM, HDL, VLDL). The released cholesterol will be consumed by the enzymatic reagent, and without the chromogenic coupler, a colorless reaction will occur.

$$Cholesterol\ ester \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acid$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholestenone + H_2O_2$$

2. Cholesterol released from D-LDL reacts with the chromogenic coupler through detergent 2 in Reagent 2 and forms color.

$$Cholesterol\ ester \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acid$$

$$Cholesterole + O_2 \xrightarrow{Cholesterole\ Oxidase} Cholestenone + H_2O_2$$

$$H_2O_2 + 4 - Aminoantipyrine + TOOS^a \xrightarrow{Peroxidase} Quinoneimine + 4H_2O\ a\ TOOS = N - ethyl - N - (2 - hydroxy - 3 - sulfopropyl) - 3 - methylaniline$$

TEST PARAMETERS

Method : Colorimetric
Wavelenght : Main 572-600 nm /Sub 700-750 nm
Linearity : 600 mg/dL

REAGENT COMPONENTS

Reagent 1:
Polyanion detergent 1
Cholesterol esterase : ≤ 200.000 U/L
Cholesterol oxidase : ≤ 200.000 U/L
Peroxidase : ≤ 200.000 U/L
4-aminoantipyrine
TOOS

Reagent 2:
Detergent 2
TOOS
Tris Buffer

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE [6]

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 45 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.
Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.

SAMPLE

Samples are collected according to the standard procedure.

Serum or plasma should be separated as soon as possible (within 3 hours) after collection.[7]

Direct LDL is stable for: [8]
7 days at +2/+8°C,
12 months at -20°C.

Unit Conversion:
mmol/L x 38.61 = mg/dL

REFERENCE INTERVAL (NORMAL VALUES) [9]

Optimal	:<100 mg/dL (<2.59 mmol/L)
Near optimal,	
Above optimal	: 100 – 129mg/dL (2.59 – 3.34 mmol/L)
Borderline high	: 130 - 159 mg/dL (3.37 – 4,12 mmol/L)
High	: 160 – 189 mg/dL (4,14 – 4,89 mmol/L)
Very high	: ≥ 190 mg/dL (≥4,92 mmol/L)

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

Reference interval has been verified by using CLSI EP28-A3c protocol.

QUALITY CONTROL AND CALIBRATION

Commercially available control material with established values determined by this method may be used. We recommend:

ARCON N (Level I Control) Lyophilized
Ref.No: VT-001

ARCON P (Level II Control) Lyophilized
Ref.No: VT-002

The assay requires the use of an Arcal Auto Calibrator / Arcal Lipids (HDL-LDL Calibrator) Lyophilized. We recommend:

Arcal Lipids (HDL-LDL Calibrator) Lyophilized
Ref.No: VT-004

Calibration Stability: It strongly depends on the application characteristics of in-use auto analyser and capacity of cooling. Calibration stability is 30 days.

If controls are not within acceptable limits, calibration is required and each laboratory should establish its own Quality Control diagrams and corrective and preventive action procedures.

Quality control is recommended every morning. Calibration is not recommended if quality control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD): The limit of the test detection is
4.5 mg/dL.

Limit of Quantitation (LoQ) [LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%]:[10] 5 mg/dL.
LoD and LoQ values have been verified by using CLSI EP17-A protocol.

High Linearity: The method is linear up to 600 mg/dL.

For values above high linearity, dilute sample with 0.9% saline, repeat the test and multiply the result by the dilution factor.
Linearity may considerably vary depending on the instrument used.

Precision Studies:[11]

Repeatibility (Within Run) (Within-Run)

Mean Concentratio	SD*	CV%	n
41,51 mg/dL	0,92	2,22	40
109,37 mg/dL	1,87	1,71	40

Repeatibility (Day to Day) (Day-to-Day Run)

Mean Concentration	SD	CV%	n
40,81 mg/dL	1,25	3,06	84
101,61 mg/dL	3,52	3,46	84

*SD: Standard Deviation
CV: Variation Coefficient

±10% CV% deviations between devices can be observed

Precision Studies data have been verified by using CLSI EP05-A3 protocol.

Interference:[14, 15, 16, 17]
No significant interactions were observed for hemoglobin, conjugated bilirubin, lipemia up to the interferent concentration given in the table.

Interferant and Concentration	LDL Target (mg/dL)	N	% Observed Recovery
Hemoglobin 900 mg/dL	112	3	93
Bilirubin 22,5 mg/dL	119,4	3	90
Lipemia 702 mg/dL	78,4	3	110

The acceptable interference limit is set 10% below the highest interference concentration within ± 10% recovery of the target.
Interferences may affect the results due to medication or endogenous substances.

These performance characteristics have been obtained by using an analyzer. Results may vary if a different instrument or a manual procedure is used.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.

H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.

P264 :Wash your hands properly after using.

P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.

P333+P313 :Seek medical help if it irritates your skin or develops rash.

P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests