DIRECT HDL CHOLESTEROL (D-HDL-C)

Diagnostic reagent for determination of HDL (High Density Lipoprotein) concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). Do not freeze.

Ref No	Pack
MH-092	80 mL

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

INTENDED USE

The test is used for quantitative determination of HDL cholesterol concentration in human serum and plasma.

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

High-density lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the peripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized.

Accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD. In this diagnostic test kit, a method for direct measurement of HDL-C, without sample pretreatment, is presented. Direct measurement gives improved accuracy and reproducibility when compared to precipitation methods.

After adding of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.

The cholesterol amount of HDL-Cholesterol can be tested enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups. This is around %40.

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. HDL-C in human serum is resolved with special detergent, and makes color reactions with Cholesterol esterase (CEH), Cholesterol oxidase (CHOD), Peroxidase (POD). Because Non-HDL-Lipoproteins such as chylomicron (CM), low density lipoprotein (LDL), very low density lipoprotein (VLDL) are inhibited by detergents on their surface, the cholesterol in them do not react with the enzyme. Remain HDL Cholesterol is determined by color intensity over trinder reaction.

TEST PARAMETERS

Method : Colorimetric, End Point Reaction
Wavelength : Main: 578 - 600 nm
Bottom: 700 – 750 nm
Linearity : 200 mg/dL

REAGENT COMPONENTS

Reagent 1:

Dextran Sulfate	≤ 10 gr/dL
Magnesium Chloride Hegzahydrate	≤ 5 gr/dL
Preservative	
Brij 35	≤ 10 gr/dL

Reagent 2:

Detergent	≤ 2 %
PEG - Cholesterol Esterase	≤ 5 KU/L
PEG - Cholesterol Oxidase	≤ 5 KU/L
4 AAP	≤ 1 gr/dL
Peroxidase	≤ 8000 U/L

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 30 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

Fresh Serum or EDTA and heparinized plasma on an empty stomach are the recommended specimens. Samples are collected according to the standard procedures.

Separate the serum or plasma as soon as possible after collection (within 3 hours).
Serum is stable for:
12 hours at +20/+25ºC,
7 days at +2/+8ºC.

Unit Conversion:
mmol/L x 38.67 = mg/dL
mg/dL x 0.02586 = mmol/L

REFERENCE INTERVAL (NORMAL VALUES) [7]

Adult Males :< 35 mg/dL (0.90 mmol/L) High Risk
>55mg/dL (1.45mmol/L) No Risk
Adult Females :< 45 mg/dL (1.15 mmol/L) High Risk
>65 mg/dL (1.68mmol/L) No Risk

National Cholesterol Education Program (NCEP) guidelines:

< 40 mg/dL : Low HDL (Major risk factor for CHD)

≥ 60 mg/dL : High HDL ("Negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercises, hormones, sex and age.

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 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 2.7 mg/dL.

Limit of Quantitation (LoQ) [LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%]**:**[8] 3 mg/dL

LoD and LoQ values have been verified by using CLSI EP17-A protocol.

High Linearity: The method is linear up to 200 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:[9]

Repeatability (Within Run)

Mean Concentration	SD*	CV%	n
71,9 mg/dL	0,57		0,8
40			
131,1 mg/dL	0,78		0,6
40			

Repeatability (Day-to-Day Run)

Mean Concentration	SD*	CV%	n
47,91 mg/dL	0,73		1,53
84			
133,25 mg/dL	2,72		2,04
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:[12, 13, 14]
No significant interactions were observed for hemoglobin, conjugated bilirubin, lipemia up to the interferent concentration given in the table.

Interferant and Concentration	HDL Target (mg/dL)	N	%Observed Recovery
Hemoglobin 1260 mg/dL	25,8	3	91
Bilirubin 54 mg/dL	46,3	3	103
Lipemia 1062 mg/dL	53,6	3	111

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