HDL CHOLESTEROL (Direct)
ENZYMATIC COLORIMETRIC-DIRECT METHOD

IVD CE

PRODUCT CODE
CS020

INTENDED USE
For the quantitative determination of HDL Cholesterol in serum

CLINICAL SIGNIFICANCE
The fraction of the cholesterol bound to high density lipoprotein is an indicator of the risk of coronary heart disease. High levels of HDL-cholesterol appear to act as protective factor, while low values are one of the major risk factors.
Determination of HDL-cholesterol along with the comprehensive study of the lipid profile of the patient, allows assessing the risk of coronary heart disease.
Law level of HDL-cholesterol are found in cases of unbalanced diet, sedentary lifestyle, alcoholism or smoking.

PRINCIPLE
The assay combines two specific steps: in the first step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the second step remaining cholesterol from the HDL fraction is determined by well-established specific enzymatic reactions in the presence of specific surfactants for HDL.

First Step

LDL,VLDL and Chylomicrons —(CHE+CHO)→ Cholstenone+H_2O_2

$2H_2O_2$ —(Catalase)→ $2H_2O + O_2$

Second Step

HDL —(CHE+CHO)→ Cholstenone+H_2O_2

$2H_2O_2$ +chromogen —(POD)→ Quinonimine+$4H_2O$

REAGENT COMPOSITION

Reagent 1

N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 6.6	100 mmol/L
N-(2-hydroxy-3sulfopropyl)-3,5-dimethoxyaniline (HDAOS)	0.7 mmol/L
Cholesterol esterase	≥800 U/L
Cholesterol oxidase	≥500 U/L
Catalase	≥300 U/L
Ascorbic oxidase	≥300 0U/L

Reagent 2

N,N-bis(2hydroxyethyl)-2- aminoethanesulphonic acid pH 7,0	1.1 mmol/L
4-Aminoantipyrine	100 mmol/L
Peroxidase	≥3500 U/L

HDL CAL
Calibrator, lyophilized human serum

REAGENT PREPARATION
Reagent 1 and Reagent 2 are ready to use.
HDL CAL: Reconstitute with 1 mL of distilled water, cap the vial and mix gently to dissolve the content.

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 contaminations are prevented during their use. Do not freeze the reagents.

- **HDL CAL:** Once reconstitute 2 weeks at 2-8°C or 3 months at –20°C.
Do not use reagents over the expiration date
Signs of reagent deterioration: Presence of particles and turbidity.

SPECIMEN
Serum, heparinized plasma or EDTA plasma. If any samples show precipitates, centrifuge before using.
Stability of the sample: 6 days at 2-8°C and 1 year when stored at -70°C.

PRECAUTION
To avoid contamination, use clean laboratory wares.
HDL CAL, Components from human origin 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.

PROCEDURE
ASSAY

Wavelength	:	550-650 nm
Cuvette	:	1 cm light path
Temperature	:	37°C
Measurement	:	Against reagent blank

Adjust the instrument to zero with distilled water

	Blank	Calibrator	Sample
Reagent 1	300 µL	300 µL	300 µL
Calibrator	--	3 µL	--
Sample	--	--	3 µL

- Mix and incubate for 5 min at 37°C
- Read the absorbance (A1) of the samples and calibrator.
- Add:

	Blank	Calibrator	Sample
Reagent 2	100 µL	100 µL	100 µL

- Mix and incubate for 5 min at 37°C
-. Read the absorbance (A2) of the samples and calibrator against the blank.
- Calculate the increase of the absorbance ΔA = A2-A1

CALCULATION

$$\text{HDL Conc. (mg/dL)} = \frac{\Delta A \text{ sample} - (A2\text{-}A1) \text{ Blank}}{\Delta A \text{ calibrator} - (A2\text{-}A1) \text{ Blank}} \times \text{Calibrator Conc.}$$

Conversion factor: mg/dL X 0.0259 = mmol/L

LINEARITY
Up to 150 mg/dL HDL
If the result obtains greater than the linearity limit, dilute sample 1+1 with normal saline and repeat the test, multiply the result by 2

IINTERFERENCES
No interferences were observed to bilirubin up to 30 mg/dL, haemoglobin up to 500 mg/dL, rheumatoid factors up to 1000 IU/mL or lipemia up to 1200 mg/dL.
Lipaemic samples with a triglyceride concentration >1200 mg/dL should be diluted 1/10 with NaCl 9 g/L and multiply the result by 10.

NORMAL RANGE

	Men	Women
Low risk	>50 mg/dL	>60 mg/dL
Normal risk	35-50 mg/dL	45-60 mg/dL
High risk	<35 mg/dL	<45 mg/dL

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

QUALITY CONTROL
All human serum based control sera with HDL values determined by this method can be employed.

NOTES
1- The reagent 2 presents yellowish coloration due to the peroxidase, but it does not affect its functionality.
2- Keep reagents and samples out of direct sunlight.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBILOGRAPHY

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3. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; 46:3:351 – 364
4. Jacobs, D. et al. In Laboratory and Test Handbook; Jacobs, D.S; Kasten, B.L., De Mott, W.R., Wolfson, W.L., Eds; Lexi - Comp Inc: Hudson (Cleveland), 1990; P. 219.

Bio Research For Medical Diagnostics
Muslim Al Attar Street,P.O.Box:1235,
Amman-11953,Jordan
Tel:+962 64892525, Fax: +962 64892526,
www.bioresearch.com.jo

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany