HDL DIRECT

Cat. No.	Pack Name	Packaging (Content)
BLT00028	HDL 80	R1: 2 x 30 ml, R2: 2 x 10 ml

EN

CE IVD

INTENDED USE
This reagent is intended for *in vitro* quantitative determination of HDL Cholesterol in human serum.

CLINICAL SIGNIFICANCE
High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in liver as complexes of apolipoprotein and phospholipid and are capable of picking up cholesterol and carrying it from arteries 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.[1-8]
Accurate measurement of HDL C is of vital importance when assessing patient's risk for CHD.

PRINCIPLE
The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents.[9]
LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).
The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

$$\text{HDL + LDL + VLDL + CM} \xrightarrow[\text{PEGME}]{\text{PVS}} \text{HDL + (LDL + VLDL + CM) • PVS / PEGME}$$

$$\text{HDL} \xrightarrow{\text{CHOD, CHER}} \text{Fatty Acid + } H_2O_2$$

$$2H_2O_2 \text{ +4-AA+TODB} \xrightarrow{\text{Peroxidase}} \text{Quinone + 5 } H_2O$$

REAGENT COMPOSITION
R1

MES buffer (pH 6.5)	6.5 mmol/l
N, N-Bis(4-sulfobutyl)-3-methylaniline)	3 mmol/l
Polyvinyl sulfonic acid	50 mg/l
Polyethylene-glycol-methyl ester	30 ml/l
$MgCl_2$	2 mmol/l

R2

MES buffer (pH 6.5)	50 mmol/l
Cholesterol esterase	5 kU/l
Cholesterol oxidase	20 kU/l
Peroxidase	5 kU/l
4-aminoantipyrine	0.9 g/l
Detergent	0.5 %

REAGENT PREPARATION
Reagents R1 and R2 are liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C. Once opened both reagents R1 & R2 are stable for 60 days at 2–8°C, when protected from contamination.
Reagents are light-sensitive. Do not let bottles remain open. Keep containers tightly closed.

SPECIMEN COLLECTION AND HANDLING
Use serum or heparin plasma.
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Stability in serum/plasma: 24 hours at 20–25°C
7 days at 4–8°C
12 weeks at -20°C
Discard contaminated specimens.

CALIBRATION
Calibration with HDL/LDL CAL, Cat. No. XSYS0061 is recommended.

QUALITY CONTROL
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
mg/dl = 0.026 mmol/l

EXPECTED VALUES [11]
Adults male: 35.3 - 79.5 mg/dl
Adults female: 42.0 - 88.0 mg/dl
It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Limit of quantification: 1.90 mg/dl
Linearity: 193 mg/dl
Measuring range: 1.90 – 193 mg/dl

PRECISION

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	29.154	0.423	1.48
Sample 2	70.538	1.462	2.05

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	26.65	0.615	2.32
Sample 2	65.77	1.000	1.54

COMPARISON
A comparison between HDL DIRECT (y) and a commercially available test (x) using 40 samples gave following results:
y = 1.056x + 0.154 mg/dl
r = 0.998

INTERFERENCES
Following substances do not interfere:
haemoglobin up to 10 g/l, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl.

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Reagents of the kit are not classified like dangerous.

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE
Wavelength: 600/700 nm
Cuvette: 1 cm

Pipette in Tube	Reagent blank	Sample / Calibrator
Reagent 1	375 µl	375 µl
D.D water	5 µl	-
Sample / Calibrator	-	5 µl
Mix and incubate at 37°C for 5 min.		
Add Reagent 2	125 µl	125 µl
Mix and incubate at 37°C for 5 min.		

Read final absorbances at the specified wavelength against reagent blank.

CALCULATION
$$\text{HDL-C} = \frac{\text{(Abs. of Sample - Abs. of Sample Blank)}}{\text{(Abs. of Cal. - Abs. of Cal. Blank)}} \times \text{Concentration of Calibrator}$$

ASSAY PARAMETERS

Mode	1-Point End
Wavelength (Primary)	600 nm
Wavelength (Secondary)	700 nm
Sample Volume	5 µl
Reagent 1 Volume	375 µl
Reagent 2 Volume	125 µl
Incubation time	5 min.
Incubation temperature	37°C
Normal low	42
Normal high	79.5
Linearity low	1.9
Linearity high	193
Blank with	Reagent
Absorbance limit (max.)	0.3
Units	mg/dl

Program parameters for specific clinical analyzers are available on request.

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SYMBOLS USED ON LABELS

REF Catalogue Number	Manufacturer	See Instruction for Use
LOT Lot Number	CE Mark - Device comply with the Directive 98/79/EC	Storage Temperature
Expiry Date	IVD In Vitro Diagnostics	CONT Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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