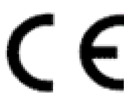
CUSTOMISED SOLUTIONS
FOR YOUR LABORATORY

LDL Cholesterol – Instructions for use



R1: 2x 18 mL R2: 2x4,5 mL **R3330000054**

Diagnostic reagent for quantitative in vitro determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma on photometric systems

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, enzymatic selective protection
Wavelength:	600 / 700 nm (bichromatic)
Temperature:	37 °C
Sample:	Serum, heparin plasma
Linearity:	up to 400 mg/dL (10.3 mmol/L)
Sensitivity:	The lower limit of detection is 1 mg/dL (0.03 mmol/L)

SUMMARY [1, 2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteints, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL cholesterol contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL cholesterol indicates high risk. HDL-cholesterol has a protective effect impending plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL -cholesterol values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-cholesterol and LDL cholesterol.

In the last few years several controlled clinical trials using diet, life style changes and/or different drugs (especially HMG CoA reductase inhibitors [statins] have demonstrated that lowering total cholesterol and LDL cholesterol levels reduce drastically CHD risk.

TEST PRINCIPLE

Dialab Cholesterol LDL Direct is a homogeneous method for LDL-cholesterol measurement without centrifugation steps for the direct measurement of LDL-cholesterol. In a first step, LDL is selectively protected while non-LDL-lipoproteins are precessed enzymatically. In a second step, LDL is released and LDL-cholesterol selectively determined in a colour producing enzymatic reaction.

1. LDL + Reagent 1 $\longrightarrow$ Protected LDL

   HDL, VLDL, Chylomicrons $\xrightarrow{\text{CHE \& CHO}}$ Cholestenone + H_2O_2

   $H_2O_2 \xrightarrow{\text{Catalase}} H_2O$

2. Protected LDL + Reagent 2 $\longrightarrow$ LDL

   LDL-C $\xrightarrow{\text{CHE \& CHO}}$ Cholestenone + H_2O_2

   H_2O_2 + H-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ blue colour

REAGENT COMPOSITION

COMPONENTS		CONCENTRATION	
Reagent 1			
Good's Buffer	pH 6.8	20	mmol/L
Cholesterol esterase	*(CHE)*	≥ 2.5	kU/L
Cholesterol oxidase	*(CHO)*	≥ 2.5	kU/L
N-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline	*(H-DAOS)*	0.5	mmol/L
Catalase		≥ 500	kU/L
Reagent 2			
Good's Buffer	pH 7.0	25	mmol/L
4-Aminoantipyrine		3.4	mmol/L
Peroxidase	*(POD)*	≥ 15	kU/L

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.
Sample Start:
Not possible (Selective protection of LDL-Chol. Lipoprotein fraction in first incubation step with Reagent 1).

REAGENT STABILITY AND STORAGE

Conditions: Protect from light
Close immediately after use
Avoid contamination.
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8°C
Stability up to the indicated expiration date

SAMPLE STABILITY AND STORAGE [3]

Stability:	at 20 – 25 °C	1 day
	at 4 - 8 °C	7 days
	at - 20 °C	3 months

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.
Substrate Start:

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	Blank	Sample or Cal.
Sample or calibrator	-	10 µL
Reagent 1	1000 µL	1000 µL
Mix, incubate 5 min. at 37 °C, read absorbance (A1), then add:		
Reagent 2	250 µL	250 µL
Mix, incubate 5 min. at 37 °C and read absorbance (A2). ΔA = [(A2 – A1) sample or calibrator] – [(A2 – A1) blank]		

CALCULATION

$$\text{LDL-C [mg/dL]} = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Calibrator}} \times \text{Conc. Cal [mg/dL]}$$

UNIT CONVERSION

mg/dL x 0.02586 = mmol/ L

REFERENCE RANGE [4] *

Desiderable	≤ 130 mg/dL (3.4 mmol/L)
Borderline high risk	130 – 160 mg/dl (3.4 – 4.1 mmol/L)
High risk	> 160 mg/dL (> 4.1 mmol/L)

* Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges as necessary.

Clinical Interpretation

The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L) [2].

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine LDL-cholesterol concentrations within a measuring range from 1 – 400 mg/dL (0.03 – 10.3 mmol/L).

When values exceed this range samples should be diluted 1+1 with NaCl solution (9 g/L) and the results multiplied by 2.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 1 mg/dL (0.03 mmol/L).

PRECISION (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	59.8	0.657	1.10
Sample 2	93.7	1.09	1.17
Sample 3	125	1.17	0.94

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	68.0	0.938	1.38
Sample 2	96.8	1.11	1.15
Sample 3	119	2.21	1.85

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	50 mg/dL
Free bilirubin	50 mg/dL
Conjugated bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	600 mg/dL

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between this Cholesterol LDL Direct (y) and a commercially available homogenous test (x) using 50 samples gave following results: y = 0.970 x + 4.70; r= 0.993.

CALIBRATION

The assay requires the use of a LDL Cholesterol Calibrator.

QUALITY CONTROL

All control sera with LDL Cholesterol values determined by this method can be used.

Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. Reagent 2 contains sodium azide (0.95 g/L). Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. Artificial lipid mixtures (e.g. Intralipid®) may interfere with the test. Serum samples from patients treated with such solutions should not be used.
4. Determination of samples from patients with a rare type of Hyperlipoproteinemia (Hyperlipoproteinemia Type III) may lead to false results.
5. In very rare cases, samples of patients with gammopathy might give falsified results [7].
6. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
7. When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be exluded. In the event of such a problem occurring, please refer to the instrument'smanual for channel setting and washing procedure options.
8. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
9. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examination and other findings.
10. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements

REFERENCES

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998;19: 1434-503.

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3. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p.22-3.
4. Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press;1997.p.25-48.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bachorik PS. Measurement of low-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press;1997.p.145-60.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem lab Med 2007; 45(9); 1240-1243.

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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