Liquid Reagents – ready to use

CHOLESTEROL LDL DIRECT

ENZYMATIC SELECTIVE PROTECTION
2 Reagents

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

REF	Kit Size	Content
F05231B	1 x 12.5 L	1 x 10 L R1 + 1 x 2.5 L R2
F05126B	1 x 1.25 L	1 x 1 L R1 + 1 x 250 mL R2
F05365	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
F05366	5 x 25 mL	4 x 25 mLR1 + 1 x 25 mL R2
F05367	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
F19911	5 x 50 mL	4 x 50 mL R1 + 2 x 25 mL R2
F14911	1 x 50 mL	4 x 40 mL R1 + 1 x 10 mL R2
F0443917	5 x 62.5 mL	4 x 62.,5 mL R1 + 1 x 62.5 mL R2
F0417917	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
FA0816	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
FT1016	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
FK0716	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
FB0916	2 x 100 mL	2 x 80 mL R1 + 2 x 20 mL R2

Additionally offered:

F03711SV	1 x 1 mL	LDL-Cholesterol Calibrator	
D13585SV	1 x 2 mL	Lipid Calibrator	Diacal Lipids
D99486	3 x 3 mL	Lipid Control normal	Diacon Lipids
D99486SV	1 x 3 mL	Lipid Control normal	Diacon Lipids
D11487	3 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D11487SV	1 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

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 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.

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

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

   H_2O_2 + F-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:

	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

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

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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 Dialab 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.

We recommend the Dialab **LDL-Cholesterol Calibrator** or the lipid calibration plasma **Diacal Lipids**.

Values in the the LDL-Cholesterol Calibrator are traceable to the CDC reference method Beta-Quantification, and values in Diacal Lipids are traceable to NIST-SRM®-1951 Level 2.

QUALITY CONTROL

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

We recommend the Dialab lipid control sera **Diacon Lipids** and **Diacon Lipids High** and the Dialab multi control sera **Diacon N** (with values in the normal range) and **Diacon P** (with values in the pathological range).

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

AUTOMATION

Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. Reagent 2 contains sodium azide (0.95 g/L). Do not swallow! Avoid contact with skin and mucous membranes.
2. Artificial lipid mixtures (e.g. Intralipid®) may interfere with the test. Serum samples from patients treated with such solutions should not be used.
3. Determination of samples from patients with a rare type of Hyperlipoproteinemia (Hyperlipoproteinemia Type III) may lead to false results.
4. In very rare cases, samples of patients with gammopathy might give falsified results [7].
5. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
6. 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.
7. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
8. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examination and other findings.
9. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements

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