DIAGNOSTICA PRODUCTS

Liquid Reagents – ready to use

# CHOLESTEROL LDL DIRECT

## ENZYMATIC COLORIMETRIC

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.No. | Kit Size | Content |
|---|---|---|
| **DIA010331** | 60 ml | 2x22.5 ml R1 + 1x15 ml R2 |
| **DIA010332** | 4 x 20 ml | 3x20 ml R1 + 1x20 ml R2 |
| **DIA010333** | 4 x 25 ml | 3x25 ml R1 + 1x25 ml R2 |

**Additionally offered:**

| | | |
|---|---|---|
| **DIA040070** | 1 x 3 mL | **LDL- Cholesterol Calibrator** |
| **DIA040081** | 1 x 3 mL | **HDL/LDL- Cholesterol Calibrator** |
| **DIA030012** | 1 x 5 mL | **Diacon N** (Control Normal) |
| **DIA030022** | 1 x 5 mL | **Diacon P**(Control Abnormal) |

## 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

Diagnostica Cholesterol LDL Direct is a homogeneous method for LDL-cholesterol measurement without centrifugation steps.

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 | 50 | mmol/L |
| Cholesterol esterase | *(CHE)* | 6 | kU/L |
| Cholesterol oxidase | *(CHO)* | 5 | kU/L |
| N-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline | *(H-DAOS)* | 2 | mmol/L |
| Catalase | | 600 | kU/L |
| **Reagent 2** | | | |
| Good's Buffer | pH 7.0 | 50 | mmol/L |
| 4-Aminoantipyrine | | 4 | mmol/L |
| Peroxidase | *(POD)* | 4 | kU/L |

## REAGENT PREPARATION

**Substrate Start:**
Reagents are ready for 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
Do not freeze the reagents!
Avoidcontamination.
**Substrate Start:**
Storage: at 2 – 8°C
Stability up to the indicatedexpiration 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** | - | 4 µL |
| **Reagent 1** | 300 µL | 300 µL |
| Mix, incubate 5 min. at 37 °C, read absorbance (A1), then add: | | |
| **Reagent 2** | 100 µL | 100 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 0.0259 = 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.

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**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 | 132.5 | 3.28 | 2.48 |
| Sample 2 | 204.0 | 2.20 | 1.09 |
| **Inter-assay n = 20** | **Mean [mg/dL]** | **SD [mg/dL]** | **CV [%]** |
| Sample 1 | 129.8 | 4.29 | 3.31 |
| Sample 2 | 198.2 | 7.19 | 3.62 |

**PECIFICITY/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 | 1000 mg/dL |

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

**METHOD COMPARISON**
A comparison between Diagnostica Cholesterol LDL Direct (y) and a commercially available homogenous test (x) using 51 samples gave following results: y = 0.9634 x + 5.35; r= 0.99.

## CALIBRATION

The assay requires the use of a LDL Cholesterol Calibrator.
We recommend the Diagnostica **LDL-Cholesterol Calibrator** or **HDL/LDL-Cholesterol Calibrator**
Values in the theLDL-Cholesterol Calibrator and the HDL/LDL-Cholesterol Calibrator are traceable to the CDC reference method Beta-Quantification, and values inDiacal 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 Diagnostica lipid control sera **Diacon Lipids** and **Diacon Lipids High** and the Diagnostica multi control sera **Diacon N** (with values in the normal range) and **DiaconP** (with values in the pathological range).
Each laboratory should establish corrective action in case of deviations in control recovery.

## AUTOMATION

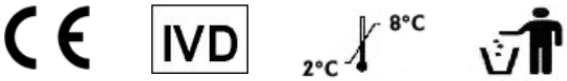
Special applications for automated analyzers can be made on request.

## 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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