LDL Cholesterol

CYPRESS DIAGNOSTICS

CE

IVD

Enzymatic. Colorimetric.
Liquid

Store at 2-8 °C

Configuration

REF	HBL012
VOL	120 + 40 mL
Reagent 1	1 x 120 mL
Reagent 2	1 x 40 mL

Intended use

The Cypress Diagnostics kit LDL Cholesterol is an in vitro diagnostic medical device intended to be used for the quantitative measurement of LDL cholesterol in human serum or plasma (EDTA, citrate). The device is not automated. The measurement of LDL cholesterol is intended to be used to aid in the diagnosis and treatment of patients at risk of developing coronary heart disease. This kit is intended to be used by healthcare professionals in a laboratory-based testing environment.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

The LDL cholesterol particle consists of lipoproteins that transport cholesterol to the cells. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis.[1-7] Elevated LDL cholesterol is the primary target of cholesterol lowering therapy.[8] Accurate measurement of LDL cholesterol is very important in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

The LDL cholesterol assay does not require any sample pre-treatment or fractionation step and measures LDL cholesterol levels directly through the use of specially formulated surfactants.[9]

The assay consists of two steps:
In the first step, high density lipoproteins (HDL) cholesterol is eliminated under specific conditions. LDL, very low density lipoproteins (VLDL) and chylomicron (CM) bind with polyvinyl sulfonic acid (PVS), and polyethylene-glycol methyl ester (PEGME) that make them inaccessible for reaction with cholesterol oxidase (CHOD) and cholesterol esterase (CHE), whereas HDL reacts with these enzymes.[9]

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

$$\text{HDL} \xrightarrow{\text{CHOD/CHE}} \text{Fatty acids} + H_2O_2$$

In the 2nd step, LDL cholesterol is released from the PVS/PEGME complex with a specific detergent and reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction. The intensity of the color formed is proportional to the LDL cholesterol concentration in the sample.

$$(\text{LDL} + \text{VLDL} + \text{CM}) \bullet \text{PVS/PEGME} \xrightarrow{Detergent} \text{LDL} + (\text{VLDL} + \text{CM}) \bullet \text{PVS/PEGM}$$

$$\text{LDL} \xrightarrow{\text{CHOD/CHE}} \text{Fatty acids} + H_2O_2$$

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

Reagent composition

Reagent 1	**Enzymes** MES buffer pH 6,5 (< 500 mmol/L) Polyvinylsulfonic acid (PVS) (< 50 g/L) Polyethyleneglycolmethylester (PEGME) (< 50 g/L) 4-Aminoantipyrine (< 50 mmol/L) Cholesterol esterase (CHE) (< 100 U/L) Cholesterol oxidase (CHOD) (< 100 U/L) Peroxidase (POD) (< 100 U/L) $MgCl_2$ (< 50 mmol/L) EDTA (< 10 mmol/L) Detergent (< 2%)
Reagent 2	**Detergent** MES buffer pH 6,5 (< 500 mmol/L) TODB (< 50 mmol/L) EDTA (< 10 mmol/L) Detergent (< 2%)

Precautions

- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation

R1 and R2 are ready to use.

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2-8 °C. The reagents are light sensitive. Do not leave bottles open. Do not freeze the reagents.
R1 and R2: once opened they are stable for 60 days at 2-8 °C. The reagents should be a clear solution. If turbidity or precipitation has occurred, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 600 nm (560-600)
  Linear measuring range: 0 - 2 AU
- HDL/LDL Calibrator, HBC11 1x1 mL
- Cuvettes, matching the analyzer used (1,0 cm light path)
- Thermostatic bath at 37 °C (± 0,5 °C)
- General laboratory equipment

Samples

Sample type: human serum or plasma (EDTA, citrate). Fasting and non-fasting samples can be used. [11] Do not use plasma containing heparin as anticoagulant.

Procedure

Make sure the reagents and samples are at room temperature.
1. Wavelength 600 nm (560-600); Temperature 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

For Blank	10 µL Distilled water + 750 µL R1
For Sample/Calibrator	10 µL Sample/Calibrator + 750 µL R1
Mix, incubate 5 min. at 37 °C. Read the absorbance (A1) of the calibrator and the samples against blank. Add R2.	
For Blank	250 µL R2
For Sample/Calibrator	250 µL R2
Mix, incubate 5 min. at 37 °C. Read the absorbance (A2) against the blank.	

Calculation

Calculate the ΔA = (A2 - A1).

$$\text{LDL (mg/dL)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Calibrator} - \Delta A_{Blank}} \times \text{calibrator conc. (mg/dL)}$$

Conversion Factor: mmol/L = 0,0259 x mg/dL

Quality control

Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use the HDL/LDL Control kit (HBC10). If other controls (not manufactured by Cypress Diagnostics) are used, they have to be validated by the user as they can *vary*. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values[10]

Optimal	< 100 mg/dL (< 2,6 mmol/L)
Near/above optimal	100 - 129 mg/dL (2,6 - 3,3 mmol/L)
Borderline high	130 - 159 mg/dL (3,4 - 4,1 mmol/L)
High	160 - 189 mg/dL (4,1 - 4,9 mmol/L)
Very high	≥ 190 mg/dL (≥ 4,9 mmol/L)

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

Performance characteristics

Measuring range: from 1,64 mg/dL (detection limit) to 250 mg/dL (linearity limit). If the obtained results are greater than 250 mg/dL, dilute the sample 1:2 with NaCl 9 g/L, repeat the determination, and multiply the result by factor 2.

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Precision:

	intra-assay (n=80)			inter-assay (n=80)		
Mean (mg/dL)	97,14	147,37	211,47	97,14	147,37	211,47
SD	1,00	1,19	1,38	1,55	2,23	2,98
CV (%)	1,03	0,81	0,65	1,60	1,51	1,41

Accuracy: **Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.**
The results of the performance characteristics depend on the analyzer used.

Interferences

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides: 1000 mg/dL, Ascorbic acid: 10 mM, Bilirubin: 40 mg/dL, Bilirubin conjugated: 30 mg/dL and Hemoglobin: 1000 mg/dL.

Notes

1. **For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.**
2. **In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.**

Bibliography

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9. **Hongbing Xiao Method and composition for determining low density lipoprotein cholesterol, Chinese Patent CN1379234A, 2002.**
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Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

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