Biomaghreb

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LDL-CHOLESTEROL DIRECT

IN VITRO DIAGNOSTIC USE

IVD

REF **24015**	**(200 Tests)**	**R1:** 2 x 30 ml	**R2:** 2 x 10 ml	**R3:** 1 x 1 ml (Lyoph)
REF **24022**	**(100 Tests)**	**R1:** 1 x 30 ml	**R2:** 1 x 10 ml	**R3:** 1 x 1 ml (Lyoph)

CLINICAL SIGNIFICANCE

Low density lipoproteins (LDL), are synthesized in the liver by the action of various lipolytic enzymes on triglycerides rich very low density lipoproteins (VLDL). Many epidemiological and clinical studies have shown that an increased LDL-Cholesterol is associates with increased risk of atherosclerosis and coronary artery disease (CAD). Some studies showed that the reduction in LDL-Cholesreol is correlated with regression in atherosclerotic lesion.

PRINCIPLE

Direct method with selective detergents, without pre-treatment of the specimen.
During the first phase only non-LDL lipoproteins are solubilised by detergent 1.
The cholesterol thus generated, subjected to the action of Cholesterol Oxidase (CO) and Cholesterol Esterase (CE) produces a colorless compound.
During the second phase, detergent 2 solubilises LDL cholesterol.
The chromogenic couple develops a color reaction proportional to the concentration of LDL-cholesterol. The reading is taken at 546nm (520-580).

REAGENT COMPOSITION

Reagent 1 Enzymes	GOOD pH 7.0 (20°C) Cholesterol esterase (CHE) Cholesterol oxydase (CHOD) Catalase N- (2-hydroxy-3sulfopropyl) -3,5- dimethoxyaniline (TODS)	50 mmol/L 380 U/L 380 U/L 400U/mL 0.45 mmol/L
Reagent 2 Enzymes	GOOD PH 7.0 4- Aminoantipyrine (4-AA) Peroxydase (POD)	50 mmol/L 1.00mmol/L 1000 U/L
Reagent 3	Calibrator HDLc/LDLc freeze-dried human serum	

SAFETY CAUTIONS

Biomaghreb reagents are intended for use by qualified personnel for in vitro use (do not pipette by mouth).

- Refer to the current SDS available on request or at www.biomaghreb.com;
- Verify the integrity of the reagents before use; and
- Disposal of waste: comply with the legislation in force.

For safety reasons, treat any specimen or reagent of biological origin as potentially infectious.
Respect the legislation in force.

REAGENT PREPARATION

Reagents R1 and R2 are ready for use.
-Reconstitute the vial of the HDLc/LDLc calibrator with 1 ml of distilled water, then homogenize the contents of the bottle gently.
-Wait 30 minutes before use.
For safety reasons, treat the calibrator as potentially infectious.

SAMPLE PREPARATION

The patient must be taken after at least 12h -14h fasting
-**Plasma :** collected on EDTA or sodium heparinate or lithium ; citrate must not be used.
Separate plasma from blood cells by centrifugation within 3 hours after collection.
-**Serum:** Centrifugally separate the Serum from the blood cells within 3 hours after collection.
Sera and plasma should not be left at room temperature for more than 14 hours.
LDL-cholesterol is stable in the specimen:

- 7 days at 2-8°C ;
- 1 month at -20°C.

PRESERVATION AND STABILITY

Store at 2-8°C, in the original bottle, tightly stoppered and protected from light.

- Before opening : if stored under the recommended conditions, the reagents are stable until the expiry date indicated on the label.
- After opening and in the absence of contamination: Reagents R1 and R2 are stable for 8 weeks at 2-8°C.
- After reconstitution: The calibrator is stable 2 weeks at 2-8°C and 3 months at -20°C.

LIMITS

Do not use the reagents if they are cloudy or after the expiry date

ADDITIONAL EQUIPMENT

- Basic equipment of the medical analysis laboratory ;
- Spectrophotometer or Clinical Biochemistry Analyzer.

LINEARITY

The reaction is linear from 3,7 mg/dl up to 1000 mg/dl.
Above this concentration, dilute the sample 1+1 with a
9 g/l NaCl solution and repeat the determination.
Multiply the result by 2.
The linearity limit depends on the ratio of specimen/reagent volumes.

PROCEDURE

Wavelength……...600-700 nm
Tank: ……1 cm thick
Temperature.....37°C
spectrophotometer zero : distilled water

	Blank	**Calibrator**	**Dosage**
Reagent R1	300 µl	300 µl	300 µl
Calibrator	- -	4 µl	- -
Sample	- -	- -	4 µl
Stir well, leave to stand for 5 minutes at 37°C. Record absorbances A1 at 600 nm against the reagent blank.			
Add	**Blank**	**Calibrator**	**Dosage**
Reagen R2	100 µl	100 µl	100 µl
Mix well, let stand 5 minutes at 37°C. Record absorbances A2 against the reagent blank			

CALCULATION

Calculating the increase in absorbance A = A2-A1

$$\frac{\text{A sample}}{\text{A calibrator}} \times \text{Calibrator Concentration}$$

= mg/dl of LDL direct

with mg/dl x 0,02586 = mmol/l

REFERENCE VALUES

Low risk	< 100 mg/dl
Moderate risk	130-160 mg/dl
High risk	> 160 mg/dl

-Concentrations tested (mg/dl) without significant interference (+10%):

Bilirubine conjuguée : 30 mg/dl
Total Bilirubin: 30 mg/dl
Hemoglobin : 500 mg/dl
Ascorbic acid : 50 mg/dl

REFERENCES

- Kaplan A et al. Lipoprotein.Clin Chem the C.V. Mosby Co.St Louis. Okada M. et al. Low-density lipoprotein cholesterol can be; chemically measured J. Lab. Clin.Mad., 1998; 132, 195-201;
-Young Ds. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995;
-Young DS.Effects of disease on Clinical lab.Tests, 4th ed AACC 2001. 5- Burtis A et al. Clinical Guide to laboratory tests, 3rd ed AACC 1995.


Manufacturer


Use by


In Vitro Diagnostic


Temperature Limitation


Catalogue number


See insert


Store away from light


Sufficient for < n > essais


Batch number