



DIRECT LDL CHOLESTEROL LR liquid reagent

REF **C220140** 195 Test
CE **C220140A** 195 Test
IVD For in vitro medical device

Use

Kit for enzymatic measurement of low density lipoprotein (cholesterol LDL) in serum or plasma. Elimination method

Summary

High LDL levels are associated with an increased risk of coronary heart disease.

Principle

This LDL - Cholesterol assay is a homogeneous, direct method for measuring levels of LDL without the need for sample pre-treatment.

The elimination method consists of two specific steps. In the first step, chylomicron, VLDL and HDL fractions are eliminated under specific conditions so cholesterol is derived only from LDL. In fact these fractions are oxidized to cholestenone and hydrogen peroxide, that is subsequently degraded by catalase. In the second step, after various enzymatic reactions and in the presence of specific surfactants, remaining LDL cholesterol can be measured specifically as a colour formation (quinone pigment), which intensity is proportional to the concentration of LDL cholesterol contained in the sample.

Absorbance measurements are taken at 600 nm.

Reagents

R1	Pipes buffer pH 7.0	100.0 mmol/l
	cholesterol esterase	800 U/l
	cholesterol oxidase	500 U/l
	catalase	300 kU/l
	TOOS	0.6 mmol/l
R2	Goods buffer pH 7.0	50.0 mmol
	peroxidase	4 KU/l
	4-aminoantipyrine	4.0 mmol/l

Preparation of Reagents

Reagents are liquid and ready to use. Keep out the reagents from refrigerator only for the use and recap them immediately.

Storage and stability

- Store the kit at 2-8°C
- After opening, the vials R1, R2 are stable until the expiration date if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.

Precaution in Use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with care, according to good laboratory practice. Caution: the reagent R2 contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contacting with skin, eyes and mucous membranes. In case of contact with eyes rinse immediately with plenty of water and seek medical advice.

Waste Management

Please refer to the local legal requirements.

Specimen collection and preparation

- Serum or heparinized plasma or EDTA plasma.
- The LDL-Cholesterol in the serum is stable up to 6 days if stand at 2-8°C.

Note

- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedure

Wavelength λ : 600 nm
 Working temperature 37°C
 Optical path 1 cm
 Reaction "end point"
 Bring the reagents at 15 -25°C before using them.

- Bireagent Procedure "substrate starter"

	BLANK	STD	SAMPLE
Reagent R1	300 μ l	300 μ l	300 μ l
Distilled Water	4 μ l	--	--
Sample	--	--	4 μ l
Standard	--	4 μ l	--

Mix, incubate at 37°C for 5' and then add:

	BLANK	STD	SAMPLE
Reagent R2	100 μ l	100 μ l	100 μ l

Mix, then incubate at 37°C for 5'. Measure the absorbance values of sample adding (EC) and standard (ESTD) against the reagent blank.

Calculation

$$LDL [mg/dl] o [mmol/l] = \frac{EC}{ESTD} \times Conc. STD$$

Conversion Factor

$$LDL [mg/dl] \times 0.0259 = LDL [mmol/l]$$

Reference Values

Serum - plasma < 128 mg/dl

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Linearity

Reaction is linear up to a concentration of 1000 mg/dl. Samples with values exceeding 1000 mg/dl must be diluted with saline solution. Multiply, then, the result for diluting factor.

"Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results :

MEANS [mg/dl] N = 108.6 H = 177.6
 S. D. N = 0.44 H = 1.93
 C.V.% N = 0.62 H = 1.09

"Inter-Assay" precision (between-Run)

Determined on 20 samples for each control (N-H) (Normal - High). Results :

MEANS [mg/dl] N = 153 H = 207
 S. D. N = 3.39 H = 3.63
 C.V.% N = 2.21 H = 1.75

Analytical Sensitivity

The test sensitivity in terms of detection limit is 7.0 mg/dl.

Interferences

No interference was observed by presence of:

Bilirubin \leq 30 mg/dl
 Hemoglobin \leq 500 mg/dl
 Triglycerides \leq 1200 mg/dl

Lipaemic samples whit a triglyceride concentration > 1200 mg/dl should be diluted 1+9 with 0.9% (w/v) NaCl before assay, the corresponding results should be multiplied by 10.

For a comprehensive review of interfering substances

Correlation

A study based comparing this method with a similar method on 20 samples has given a correlating factor $r = 0.99$

$$y = 0.9634 x + 5.35$$

Quality Controls

It's necessary, each time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

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Sachiko Izawa et al.: A new direct method for measuring HDL-cholesterol which does not produce any biased values. J. Med. and Pharm. Sci., 1385-1388, 37 (1997).

Warnick, G.R., Wood, P.D., "National Cholesterol Education Program Recommendations for measurement of high-density lipoprotein cholesterol: executive summary". Clin. Chem. 41: 1427-1433 (1995).

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Producer

Direct LDL Cholesterol LR
MOD. 7.3.5 Rev.0 del 2005-07

Gesana Production s.r.l