# DIRECT LDL-CHOLESTEROL (LDL)

**RX SERIES**

## INTENDED USE

An LDL-cholesterol test system is a device intended for the quantitative *in vitro* determination of LDL-cholesterol concentration in human serum and plasma. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and the RX **imola**.

| **Cat. No.** | | |
|---|---|---|
| CH 3841 | R1. Enzyme Reagent 1 | 3 x 51 ml |
| | R2. Enzyme Reagent 2 | 3 x 20 ml |

**GTIN:** 05055273201307

## CLINICAL SIGNIFICANCE [1, 2]

Low Density Lipoproteins (LDL) are synthesised in the liver by the action of various Lipolytic enzymes on triglyceride rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal 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.

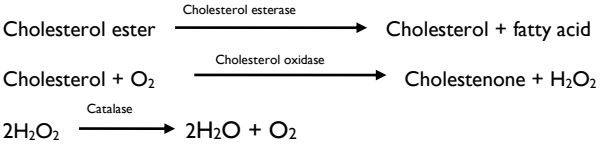
Accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

In this diagnostic test kit an elimination method for the measurement of LDL-Cholesterol, without sample pretreatment, is presented which correlates well with precipitation methods.

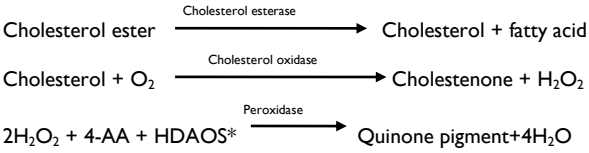
## PRINCIPLE [3, 4]

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase.




2. Specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by detergents in Reagent 2.




The intensity of the quinoneimine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm.

In the second reaction catalase is inhibited by sodium azide in Enzyme Reagent 2.

*Key: 4 - AA = 4 - Aminoantipyrine
HDAOS = N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxylaniline, sodium salt

## SAMPLE COLLECTION AND PREPARATION [5]

Sample to be collected from a fasting individual. Samples may be taken from a non-fasting individual, but subsequent results should be interpreted with care. Serum, Heparinized Plasma or EDTA Plasma are the recommended samples. EDTA Plasma causes decreased results. Serum stable for 6 days at +2 to +8°C. Do not freeze the samples. If any samples show precipitates, centrifuge before using.

## REAGENT COMPOSITION

| Contents | Initial Concentration of Solution |
|---|---|
| **R1. Enzyme Reagent 1** | |
| PIPES Buffer | 50 mmol/l, pH 7.0 |
| Piperazine-1, 4-bis (2-ethanesulfonic acid) | |
| HDAOS | 2.0 mmol/l |
| N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxylaniline, sodium salt | |
| Cholesterol Esterase | ≥600U/l |
| [E.C.3.1.1.13. Microorganism, 37°C] | |
| Cholesterol Oxidase | ≥500U/l |
| [E.C.1.1.3.6. *Streptomyces* sp, 37°C] | |
| Catalase | ≥600KU/l |
| [E.C.1.11.1.6. Microbial] | |
| Ascorbate Oxidase | ≥3000U/l |
| [E.C.1.10.3.3. *Acremonium* sp.] | |
| Surfactant | 0.3% (w/v) |
| **R2. Enzyme Reagent 2** | |
| PIPES Buffer | 50 mmol/l, pH 7.0 |
| Piperazine-1, 4-bis (2-ethanesulfonic acid) | |
| 4-Amino antipyrine | 4 mmol/l |
| Peroxidase | ≥4KU/l |
| [E.C.1.11.1.7, Horse Radish, 25°C] | |
| Surfactant | 1.00% (w/v) |
| Sodium Azide | 0.05% (w/v) |

## SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Enzyme Reagent 2 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety Data Sheets are available on request.

**The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.**

## STABILITY AND PREPARATION OF REAGENTS

**R1. Enzyme Reagent 1**

Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

**R2. Enzyme Reagent 2**

Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

## MATERIALS PROVIDED

Direct LDL-C Reagent

## MATERIALS REQUIRED BUT NOT PROVIDED

Direct HDL-C/LDL-C Calibrator, CH 2673.
Randox Lipid Controls:-

| | |
|---|---|
| Level 1 | LE 2661 or LE 2668 |
| Level 2 | LE 2662 or LE 2669 |
| Level 3 | LE 2663 or LE 2670 |

RX **series** Saline (Cat No SA 3854)

## PROCEDURE NOTES

To avoid the potential for reagent carryover, it is recommended that the testing order of the reagents is confirmed. Please consult the reagent carryover document available on www.randox.com under support and documentation - Reagent product inserts or by contacting Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

## CALIBRATION

0.9% NaCl solution as zero calibrator and Randox Direct HDL-C/LDL-C Calibrator are recommended for calibration. Values are assigned according to the requirements of the "LDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN.

## QUALITY CONTROL

Randox Lipid Control Sera, Level 1, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

## INTERFERENCES

The assay is unaffected by icteric samples (Bilirubin <25 mg/dl), haemolytic samples (Hb <1.0 g/dl) and lipaemic samples (triglycerides <600 mg/dl). Lipaemic samples with a triglyceride concentration > 600 mg/dl should be diluted 1+9 with 0.9% (w/v) NaCl before assay. The corresponding result should be multiplied by 10.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[6]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

## EXPECTED VALUES[7,8]

| mg/dl | mmol/l | |
|---|---|---|
| < 100 | <2.59 | Optimal |
| 100 - 129 | 2.59 - 3.35 | Near or above optimal |
| 130 - 159 | 3.36 - 4.12 | Borderline High |
| 160 - 189 | 4.13 - 4.89 | High |
| >190 | >4.90 | Very High |

National Cholesterol Education Program (NCEP) Guidelines
As LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges.

## SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using an RX **daytona** analyser.

## LINEARITY

The method is linear up to 22.2 mmol/l (860 mg/dl).
In the event of a rerun, the linearity is extended to 222 mmol/l (8600 mg/dl).

## SENSITIVITY

The minimum detectable concentration of LDL cholesterol with an acceptable level of precision was determined as 0.189 mmol/l (7.30 mg/dl).

## PRECISION

**Intra assay**

| | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 2.74 | 3.66 | 4.42 |
| SD | 0.040 | 0.042 | 0.132 |
| CV(%) | 1.47 | 1.14 | 2.99 |
| n | 20 | 20 | 20 |

**Inter assay**

| | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 2.52 | 3.96 | 5.34 |
| SD | 0.063 | 0.088 | 0.084 |
| CV(%) | 2.50 | 2.21 | 1.58 |
| n | 20 | 20 | 20 |

## CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.04 X + 0.01
and a correlation coefficient of R = 0.99.

40 patient samples were analysed spanning the range 0.83 to 5.41 mmol/l.

## REFERENCES

1. Naito H.K., *et al*, *Clin Chem*, **41**: 132-133, 1995.
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US Patent No. 6,194,164 B1

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

Revised 28 Jun 23 bm

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