

LDL Cholesterol Reagent

PRODUCT SUMMARY

Stability	:	Until expiry at 2-8°C
Linear Range	:	Up to 500 mg/dL (13 mmol/L)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use

IVD

INTENDED USE

For the quantitative determination of Low Density Lipoprotein [LDL] Cholesterol in human serum. For in vitro diagnostic use.

SUMMARY AND EXPLANATION

Lipoproteins serve to solubilize and transport cholesterol and other lipids in the bloodstream. Different lipoprotein classes have been shown to have various effects on the progression of coronary heart disease (CHD). Low density lipoproteins are associated with increased risk and are seen as a key factor in the pathogenesis of atherosclerosis and coronary artery disease. The measurement of LDL cholesterol (LDL-C) is a powerful predictor of CHD that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease.¹⁻⁸

PRINCIPLE

Lipoproteins other than LDL (i.e. HDL, VLDL and Chylomicrons) are first removed via selective reaction with cholesterol esterase and cholesterol oxidase that is coupled to a non-colored endpoint via catalase reduction of the peroxide byproduct. In the second step, catalase is inhibited and the remaining LDL cholesterol is specifically reacted with cholesterol esterase and cholesterol oxidase. In the presence of peroxidase the peroxide byproduct now reacts with 4-aminoantipyrine and TOOS* to form a colored quinone dye, which is measured spectrophotometrically at 600 nm.

REAGENTS PROVIDED

Active Ingredients

	Concentration
Reagent 1	
buffer	50 mmol/L
cholesterol esterase (yeast)	600 U/L
cholesterol oxidase (bacteria)	500 U/L
catalase (bacteria)	600,000 U/L
ascorbate oxidase (bacteria)	3,000 U/L
TOOS*	2.0 mmol/L
surfactants	0.30 %
stabilizers	

Reagent 2

buffer	50 mmol/L
peroxidase (horseradish)	4,000 U/L
4-aminoantipyrine	4.00 mmol/L
surfactants	1.0 %
sodium azide	0.05 %

PRECAUTIONS

- For in vitro diagnostic use.
- Do not ingest. Avoid contact with skin, eyes and clothing.
- Reagent R2 contains sodium azide. Sodium azide may react with lead joints in copper drain lines to form explosive compounds. Drains should be well flushed with water when discarding the reagent.
- All specimens, controls and calibrator used in this test should be considered potentially infectious. Universal Precautions, as they apply to your facility, should be used for handling and disposing of materials during and after testing.

REAGENT PREPARATION

Reagents are ready to use as supplied. Reconstitute the calibrator with 3.0 mL deionized water. Mix well. Allow to stand at room temperature for 30 minutes.

STORAGE AND STABILITY

The reagent is stable until the expiration date stated on the label when stored at 2° - 8°C.

SYMBOLS IN PRODUCT LABELLING

IVD	For in vitro diagnostic use		Temperature Limitation
LOT	Batch code/Lot number		Use by/Expiration Date
REF	Catalogue number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Consult instructions for use		Manufactured by
REAG 1	Reagent 1 (R1)	REAG 2	Reagent 2 (R2)

DETERIORATION

- R1 should be a clear, colorless to light green solution. If cloudiness develops, the reagent may have deteriorated and should not be used.
- R2 is a clear, light yellow solution.

INTERFERING SUBSTANCES

- No bilirubin interference observed up to 30 mg/dL (513 µmol/L).
- No hemoglobin interference observed up to 490 mg/dL.
- No ascorbic acid interference observed up to 50 mg/dL (2.8 mmol/L).
- No lipemia interference observed up to 1,620 mg/dL (269 mmol/L) triglyceride concentration.
- No lipoprotein (a), i.e. Lp(a), interference observed up to at least 110 mg/dL.
- Anticoagulants containing citrate should not be used.
- Young has reviewed drug effects on serum LDL-C levels.¹¹

SPECIMEN COLLECTION

- Collect whole blood by venipuncture and allow to clot.
- Centrifuge and remove the serum for analysis.
- If not analyzed promptly, samples may be stored refrigerated (2° - 8°C) for up to 5 days or frozen (-20°C) for up to 2 weeks.
- If plasma must be used, the recommended anticoagulant is di-sodium EDTA or heparin.

SAMPLE STORAGE

- The sample may be stored at 2° - 8°C. Serum LDL cholesterol appears stable at least 5 days.⁵
- Specimens may be frozen for up to 2 weeks.

AUTOMATED PROCEDURE PROCEDURE PARAMETERS

Wavelength	600 nm
Secondary Wavelength	700 nm
Reaction Temperature	37°C
Reaction Type	Endpoint
Reaction Times	5 min + 5 min
Sample Volume	4 µL
Reagent 1 Volume	300 µL (R1)
Reagent 2 Volume	100 µL (R2)

The above parameters should be used when programming discrete, photometric, and centrifugal chemistry analyzers for the LDL-C Procedure. Consult your instrument manual for further instructions. Specific programming applications for most popular automated analyzers are available from Thermo Customer Service.

MATERIALS PROVIDED

LDL Cholesterol Reagent 1
LDL Cholesterol Reagent 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Thermo Lipid Controls, Cat. No. 1919-030, or equivalent.
- Automated clinical chemistry analyzer able to accommodate two-reagent assays.
- Thermo HDL-C/LDL-C Calibrator, Cat. No. 1913-003.

CALIBRATION

The Thermo HDL-C/LDL-C Calibrator (Cat. No. 1913-003) is required for calibration. Refer to the Calibrator package insert for directions. Calibrators for this test should have values assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL).

QUALITY CONTROL

Reliability of test results should be monitored by including at least two levels of control sera, with known LDL cholesterol concentrations, in each assay run. The National Cholesterol Education Program (NCEP) recommends controls that span the medical

decision points and are traceable to the NRS/CHOL. The recovery of control results within the established acceptable range should be used only to monitor assay accuracy and precision.

LINEARITY

The procedure is linear to 500 mg/dL (13 mmol/L).

LIMITATIONS

1. A sample with more than 500 mg/dL (13 mmol/L) LDL-C should be diluted with 0.9% saline and reassayed, multiplying the result by the dilution factor.
2. Specimens with more than 1,620 mg/dL (269 mmol/L) triglycerides should be diluted with 0.9% saline before assaying, and the results multiplied by the dilution factor.
3. A sample with less than 5 mg/dL (0.13 mmol/L) LDL-C should be reported as Less Than 5 mg/dL (0.13 mmol/L).

EXPECTED VALUES

In 1987, the National Cholesterol Education Program discouraged the use of "Normal Range" guidelines for LDL cholesterol in favor of the following cutpoints for patient classification for the prevention and management of coronary heart disease.⁹

LDL Cholesterol:

Desirable: <130 mg/dL (3.4 mmol/L)
 Borderline High Risk: 130 - 159 mg/dL (3.4 - 4.1 mmol/L)
 High Risk: > 160 mg/dL (> 4.2 mmol/L)

These ranges should serve only as guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

A total of 153 sera with LDL cholesterol concentrations ranging from 46 to 222 mg/dL (1.2 - 5.8 mmol/L) were tested with this method (y) and compared to results of another LDL method (x). The correlation was $r = 0.984$ and linear regression analysis yields: $y = 1.00x - 4.2$ mg/dL (0.11 mmol/L). These specimens had triglyceride concentrations ranging from 42 to 1,622 mg/dL (1.1 - 42 mmol/L).

In this study, 32 specimens had triglyceride concentrations > 400 mg/dL (> 10 mmol/L). Analysis of that group separately yields: $y = 0.97(x) + 0.5$ and $r = 0.982$. Also, 72 specimens from patients known to be fasting were tested with this method (y) and compared to results from the Friedewald calculation.¹⁰ The correlation coefficient was $r = 0.970$ and the linear regression analysis yields: $y = 0.90x + 25$ mg/dL (0.65 mmol/L).

PRECISION

Within run and run-to-run precision studies yielded the following:

WITHIN RUN:	Level 1	Level 2
No. of data points	25	25
Mean (mg/dL / mmol/L)	130.4 / 3.4	259 / 6.7
SD (mg/dL / mmol/L)	1.7 / 0.04	4.0 / 0.10
CV%	1.3	1.6
TOTAL:	Level 1	Level 2
No. of data points	25	25
Mean (mg/dL / mmol/L)	128.9 / 3.4	258 / 6.7
SD (mg/dL / mmol/L)	4.3 / 0.11	8.2 / 0.21
CV%	3.3	3.2

P. O. L. PERFORMANCE

In addition, the assay was tested at 3 physician office laboratory (POL) sites. Sites A & B tested 50 samples, and site C tested 51. Site A used the COBAS Mira Plus[®], site B used the COBAS Mira[®] and site C used the Dimension ES chemistry analyzer. All three sites compared results to the Friedewald calculation. Comparisons yielded the following:

Accuracy vs. Reference Methods

LAB I.D.	r	n	y = mx + b	RANGE (mg/dL / mmol/L)
A	0.904	50	$y = 1.08x + 2.5$	51 - 218 / 1.3 - 5.7
B	0.968	50	$y = 0.93x + 10.9$	58 - 238 / 1.5 - 6.2
C	0.967	51	$y = 0.92x + 10.9$	62 - 211 / 1.6 - 5.5

While this LDL-C assay has not yet been evaluated for POL use via the Cholesterol Reference Method Laboratory Network (CRMLN) protocol, it has successfully completed the CRLMN certification program for LDL cholesterol using the Hitachi 717[®] chemistry analyzer.

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*TOOS = N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

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840518 (R1)

REF

Reorder Information

Catalogue No.	REAG 1	REAG 2
TR53201	1 x 30 mL	1 x 10 mL
TR53202	2 x 30 mL	2 x 10 mL
TH53201	2 x 48 mL	2 x 16 mL
TR53203	2 x 120 mL	2 x 40 mL

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