07006047001V4.0

LDLC3

cobas®

LDL-Cholesterol Gen.3

Order information

REF	CONTENT		Analyzer(s) on which kit(s) can be used
07005806 190	LDL-Cholesterol Gen.3 (2 × 50 tests)		**cobas c** 111
12172623 122	Calibrator f.a.s. Lipids (3 × 1 mL)	Code 424	
10781827 122	Precinorm L (4 × 3 mL)	Code 304	
11778552 122	Precipath HDL/LDL-C (4 × 3 mL)	Code 319	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	
04774230 190	Diluent NaCl 9 %	Code 951	

English

System information

LDLC3: ACN 552

Intended use

In vitro test for the quantitative determination of LDL-cholesterol in human serum and plasma on the **cobas c** 111 system.

Summary

Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and, in particular, coronary sclerosis.[1,2] The LDLs are derived from VLDLs (Very Low Density Lipoproteins) rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time coupled with an increase in the biological modification rate results in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture.

Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis, HPLC and precipitation methods.[3,4] In the precipitation methods apolipoprotein B-containing LDL-cholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDL and HDL-cholesterol) in the supernate after precipitation with polyvinyl sulfate and dextran sulfate.[5] Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are, however, time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption and centrifugation.[6]

The calculation of the LDL-cholesterol concentration according to Friedewald's formula is based on two cholesterol determinations (total cholesterol and HDL-cholesterol) and one triglyceride determination.[7]

Friedewald formula for calculation of LDL-C presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples (VLDL-cholesterol = Trigl./5 mg/dL, VLDL-cholesterol = Trigl./2.2 mmol/L). The bias in calculating LDL-C using this assumption is only acceptable in samples with a triglyceride concentration < 2.0 mmol/L (177 mg/dL).[8,9] Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to artificially low LDL-cholesterol values. Non-fasting samples cannot be used for the calculation of LDL-C because they contain a high concentration of chylomicrons and in many cases the limit of acceptable triglyceride concentration is exceeded.

For these reasons, a simple and reliable method for routine measurement of LDL-cholesterol without any preparatory steps was developed. This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase - cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order:
HDL < chylomicrons < VLDL < LDL.

The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum and plasma samples.

Non-fasting sample results are slightly lower than fasting results. Comparable non-fasting results were observed with the beta quantification method.[10] This direct assay meets the 1995 NCEP goals of < 4 % total CV, bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error.[11,12,13,14]

Test principle

Homogeneous enzymatic colorimetric assay

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{cholesterol esterase}]{\text{detergent}} \text{cholesterol} + \text{free fatty acids (selective micellary solubilization)}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{LDL-cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$2\,H_2O_2 + \text{4-aminoantipyrine} + \text{EMSE}^{a)} + H_2O + H^+ \xrightarrow{\text{peroxidase}} \text{red purple pigment} + 5\,H_2O$$

a) N-ethyl-N-(3-methylphenyl)-N-succinylethylenediamine

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents - working solutions

R1 Bis-tris[b)] buffer: 20.1 mmol/L, pH 7.0; 4-aminoantipyrine: 0.98 mmol/L; ascorbate oxidase (AOD, Acremonium spec.): ≥ 66.7 µkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 166.7 µkat/L; BSA: 4.0 g/L; preservative

SR MOPS[c] buffer: 20.1 mmol/L, pH 7.0; EMSE: 2.16 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 33.3 µkat/L; cholesterol oxidase (recombinant from E. coli): ≥ 31.7 µkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 333.3 µkat/L; BSA: 4.0 g/L; detergents; preservative

b) bis(2-hydroxyethyl)-amino-tris-(hydroxymethyl)methane

c) 3-morpholinopropane-1-sulfonic acid

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.
Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

LDLC3

Shelf life at 2-8 °C:	See expiration date on reagent.
On-board in use and refrigerated on the analyzer:	3 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on reagent.
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin, K_2- and K_3-EDTA plasma.

Fasting and non-fasting samples can be used.[6]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[15,16]	7 days at 2-8 °C
	12 months at -20 °C
	12 months at -70 °C

It is reported that EDTA stabilizes lipoproteins.[13]

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	16/37
Unit	mmol/L
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2 µL	7 µL
SR	50 µL	
Total volume	209 µL	

Calibration

Calibrators	C.f.a.s. Lipids Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration frequency	2-point calibration ▪ after reagent lot change ▪ as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.[17]

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factor: mmol/L × 38.66 = mg/dL

Limitations – interference

Criterion: Recovery within ± 0.40 mmol/L of initial values of samples ≤ 4.0 mmol/L and within ± 10 % for samples > 4.0 mmol/L.

Icterus:[18] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[18] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[18] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from HDL-C (≤ 3.03 mmol/L or ≤ 117 mg/dL), VLDL-C (≤ 3.63 mmol/L or ≤ 140 mg/dL), or chylomicrons (≤ 22.6 mmol/L or ≤ 2000 mg/dL triglycerides).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

Nicotinic acid (Niacin), statins (Simvastatin) and fibrates (Clofibrate) tested at therapeutic concentration ranges did not interfere.

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-acetylcysteine at the therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low LDL-C results. Venipuncture should be performed prior to the administration of metamizole. Venipuncture immediately after or during the administration of metamizole may lead to falsely low results.

Ascorbic acid up to 28.4 mmol/L (500 mg/dL) does not interfere.

Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

EDTA plasma may cause decreased values compared to serum.[21]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[22]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.10-14.2 mmol/L (3.87-549 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.10 mmol/L (3.87 mg/dL)
Limit of Detection	= 0.10 mmol/L (3.87 mg/dL)
Limit of Quantitation	= 0.10 mmol/L (3.87 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation for LDL-C is 0.10 mmol/L determined in accordance with the guidelines in CLSI document EP17-A2, based on a minimum of 48 determinations, and a total error goal of 10 % calculated using RMS error model.

Expected values[23]

Levels in terms of risk for coronary heart disease.

Adult levels:

Optimal	< 2.59 mmol/L (< 100 mg/dL)
Near optimal/above optimal	2.59-3.34 mmol/L (100-129 mg/dL)
Borderline high	3.37-4.12 mmol/L (130-159 mg/dL)
High	4.14-4.89 mmol/L (160-189 mg/dL)
Very high	≥ 4.92 mmol/L (≥ 190 mg/dL)

Risk classification of patients and treatment therapies are described in international guidelines.[24]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the **cobas c** 111 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (4 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean mmol/L (mg/dL)*	SD mmol/L (mg/dL)	CV %
Precinorm L	2.63 (102)	0.04 (2)	1.6
Precipath HDL/LDL-C	4.98 (193)	0.04 (2)	0.9
Human serum 1	0.291 (11.3)	0.009 (0.4)	3.0
Human serum 2	2.86 (111)	0.03 (1)	0.9
Human serum 3	3.50 (135)	0.04 (2)	1.1
Human serum 4	8.10 (313)	0.12 (5)	1.4
Human serum 5	13.6 (526)	0.2 (8)	1.4

Intermediate precision	*Mean mmol/L (mg/dL)*	SD mmol/L (mg/dL)	CV %
Precinorm L	2.63 (102)	0.06 (2)	2.4
Precipath HDL/LDL-C	4.98 (193)	0.08 (3)	1.6
Human serum 1	0.291 (11.3)	0.009 (0.4)	3.2

Intermediate precision	*Mean mmol/L (mg/dL)*	SD mmol/L (mg/dL)	CV %
Human serum 2	2.86 (111)	0.03 (1)	1.2
Human serum 3	3.50 (135)	0.04 (2)	1.2
Human serum 4	8.10 (313)	0.16 (6)	1.9
Human serum 5	13.6 (526)	0.2 (8)	1.6

Method comparison

LDL-cholesterol values for human serum samples obtained on a **cobas c** 111 analyzer (y) were compared to those determined using the corresponding reagent on a COBAS INTEGRA 400 plus analyzer (x).

Sample size (n) = 167

Passing/Bablok[25]	Linear regression
y = 0.993x - 0.022 mmol/L	y = 0.979x + 0.031 mmol/L
τ = 0.932	r = 0.999

The sample concentrations were between 0.184 and 14.0 mmol/L (7.11 and 541 mg/dL).

References

1 Rifai N, Warnick GR, McNamara JR, et al. Measurement of Low-Density-Lipoprotein Cholesterol in Serum: a Status Report. Clin Chem 1992;38:150-160.

2 Naito HK, Strong JP, Scott MG, et al. Atherogenesis: current topics on etiology and risk factors. Clin Chem 1995;41:132-133.

3 Wieland H, Seidel D. Quantitative Lipoprotein Electrophoresis. In: Handbook of Electrophoresis, Vol III, ed. Lewis A, Boca Raton: CRC Press 1983;83-102.

4 Bachorik PS. Measurement of Low-Density Lipoprotein Cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. AACC Press 2000;12:245-263.

5 Armstrong V, Seidel D. Evaluation of a Commercial Kit for the Determination of LDL-Cholesterol in Serum Based on Precipitation of LDL with Dextran Sulfate. Ärztl Lab 1985;31:325-330.

6 Pisani T, Gebski CP, Leary ET, et al. Accurate Direct Determination of Low-density Lipoprotein Cholesterol Using an Immunoseparation Reagent and Enzymatic Cholesterol Assay. Arch Pathol Lab Med 1995 Dec;119(12):1127-1135.

7 Friedewald WF, Levy RI, Frederickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin Chem. 1972;18(6):499-502.

8 van der Heul-Nieuwenhuijsen L, Stek S, Tax M, et al. Measuring LDL-cholesterol: are we doing it wrong? Ned Tijdschr Klin Chem Labgeneesk 2012;37:221-222.

9 Tighe DA, Ockene IS, Reed G, et al. Calculated low density lipoprotein cholesterol levels frequently underestimate directly measured low density lipoprotein cholesterol determinations in patients with serum triglyceride levels ≤ 4.52 mmol/l: An analysis comparing the LipiDirect® magnetic LDL assay with the Friedewald calculation. Clinica Chimica Acta 365 (2006):236-242.

10 Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein Cholesterol Concentrations in the Plasma of Human Subjects as Measured in the Fed and Fasted States. Clin Chem 1988;34:2456-2459.

11 National Cholesterol Education Program. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). NIH Publication No. 93-3095 1995.

12 Bachorik PS, Ross JW. National cholesterol education program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. Clin Chem 1995;41:1414-1420.

13 Cooper GR, Myers GL, Smith SJ, et al. Standardization of Lipid, Lipoprotein, and Apolipoprotein Measurements. Clin Chem 1988;34(8B):B95-B105.

14 Data on file at Roche Diagnostics.

15 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.

16 Jansen EHLM, Beekhof PK, Schenk E. Long Term Stability of Lipid Metabolism in Frozen Human Serum: Triglycerides, Free Fatty Acids, Total-, HDL- and LDL-cholesterol, Apolipoprotein-A1 and B. J Mol Biomark Diagn 2014;5:4.

17 LDL Cholesterol Method Certification Protocol for Manufacturers. National Reference System for Cholesterol. Cholesterol Reference Method Laboratory Network 1997, October.

18 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

19 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

20 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

21 Rifai N, Dufour RD, Cooper GR. Preanalytical Variation in Lipid, Lipoprotein, and Apolipoprotein Testing. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of Lipoprotein Testing. 2nd ed. Washington, AACC Press; 2000. p. 161-176.

22 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

23 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No 01-3670; May 2001.

24 Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:2889-2934.

25 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com