

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
08057966190	LDL-Cholesterol Gen.3 (600 tests)	System-ID 2082 001 cobas c 303, cobas c 503
Materials required (but not provided):		
12172623122	Calibrator f.a.s. Lipids (3 × 1 mL)	Code 20424
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001

English**System information****LDLC3: ACN 20820****Intended use**

In vitro test for the quantitative determination of LDL-cholesterol in human serum and plasma on Roche/Hitachi **cobas c** systems.

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's formula for calculation of LDL-C presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples (VLDL-cholesterol = Trig./5 mg/dL, VLDL-cholesterol = Trig./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 micellar 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.

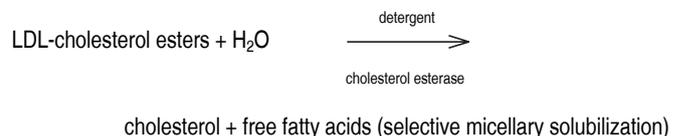
The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum and plasma samples.

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}

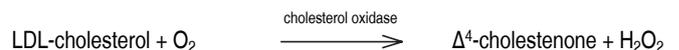
Test principle

Homogeneous enzymatic colorimetric assay

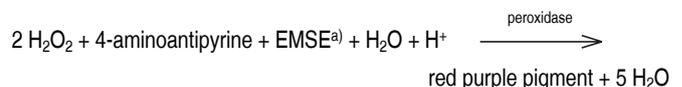
Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by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



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



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



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
- R3** 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

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

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.

H319 Causes serious eye irritation.

H411 Toxic to aquatic life with long lasting effects.

Prevention:

P261 Avoid breathing mist or vapours.

P273 Avoid release to the environment.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P337 + P313 If eye irritation persists: Get medical advice/attention.

P391 Collect spillage.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 26 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, K₂- and K₃-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.

See the limitations and interferences section for details about possible sample interferences.

Stability:^{14,15} 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**Test definition**

Reporting time	10 min		
Wavelength (sub/main)	700/600 nm		
Reagent pipetting		Diluent (H ₂ O)	
R1	78 µL	–	
R3	26 µL	–	

	Sample volumes	Sample dilution	
		Sample	Diluent (NaCl)
Normal	1 µL	–	–
Decreased	2 µL	20 µL	60 µL
Increased	1 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - 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.¹⁶

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (mg/dL, g/L).

Conversion factors: $\text{mmol/L} \times 38.66 = \text{mg/dL}$
 $\text{mmol/L} \times 0.3866 = \text{g/L}$

Limitations – interference

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

Icterus:¹⁷ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 $\mu\text{mol/L}$ or 60 mg/dL).

Hemolysis:¹⁷ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 $\mu\text{mol/L}$ or 1000 mg/dL).

Lipemia (Intralipid):¹⁷ 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.^{18,19}

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 metemazole. Venipuncture immediately after or during the administration of metemazole may lead to falsely low results.

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 28.4 mmol/L (500 mg/dL).

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.²⁰

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.²¹

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet for information. For further instructions refer to the operator's manual.

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 $n \geq 60$ 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 values²²

Levels in terms of risk for coronary heart disease.

mmol/L*

Adult levels:

Optimal	< 2.59 mmol/L
Near optimal/above optimal	2.59-3.34 mmol/L
Borderline high	3.37-4.12 mmol/L
High	4.14-4.89 mmol/L
Very high	≥ 4.92 mmol/L

* calculated by unit conversion factor

mg/dL

Adult levels:

Optimal	< 100 mg/dL
Near optimal/above optimal	100-129 mg/dL
Borderline high	130-159 mg/dL
High	160-189 mg/dL
Very high	≥ 190 mg/dL

Risk classification of patients and treatment therapies are described in international guidelines.²³

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 analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability ($n = 84$) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c 503** analyzer.

Repeatability	Mean mmol/L	SD mmol/L	CV %
PCCC1 ^{d)}	1.46	0.00724	0.5
PCCC2 ^{e)}	2.94	0.0149	0.5
Human serum 1	0.187	0.00303	1.6
Human serum 2	1.29	0.00699	0.5
Human serum 3	2.46	0.0110	0.4
Human serum 4	7.07	0.0280	0.4

Human serum 5	12.3	0.0546	0.4
<i>Intermediate precision</i>	<i>Mean</i>	<i>SD</i>	<i>CV</i>
	<i>mmol/L</i>	<i>mmol/L</i>	<i>%</i>
PCCC1 ^{d)}	1.46	0.0126	0.9
PCCC2 ^{e)}	2.94	0.0260	0.9
Human serum 1	0.187	0.00320	1.7
Human serum 2	1.30	0.00896	0.7
Human serum 3	2.46	0.0131	0.5
Human serum 4	7.04	0.0403	0.6
Human serum 5	12.2	0.0726	0.6

d) PreciControl ClinChem Multi 1

e) PreciControl ClinChem Multi 2

The data obtained on **cobas c 503** analyzer(s) are representative for **cobas c 303** analyzer(s).

Method comparison

LDL-cholesterol values for human serum samples obtained on a **cobas c 503** analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Sample size (n) = 88

Passing/Bablok ²⁴	Linear regression
$y = 0.996x + 0.00876 \text{ mmol/L}$	$y = 0.994x + 0.0140 \text{ mmol/L}$
$r = 0.992$	$r = 1.000$

The sample concentrations were between 0.190 and 13.8 mmol/L.

LDL-cholesterol values for human serum samples obtained on a **cobas c 303** analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Sample size (n) = 72

Passing/Bablok ²⁴	Linear regression
$y = 1.020x + 0.0207 \text{ mmol/L}$	$y = 1.026x + 0.00233 \text{ mmol/L}$
$r = 0.986$	$r = 1.000$

The sample concentrations were between 0.230 and 13.5 mmol/L.

References

- 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.
- Naito HK, Strong JP, Scott MG, et al. Atherogenesis: current topics on etiology and risk factors. *Clin Chem* 1995;41:132-133.
- Wieland H, Seidel D. Quantitative Lipoprotein Electrophoresis. In: *Handbook of Electrophoresis, Vol III*, ed. Lewis A, Boca Raton: CRC Press 1983;83-102.
- Bachorik PS. Measurement of Low-Density Lipoprotein Cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. *AACC Press* 2000;12:245-263.
- 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.
- 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.
- 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.
- 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.
- 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 $\leq 4.52 \text{ mmol/l}$: An analysis comparing the LipiDirect® magnetic LDL assay with the Friedewald calculation. *Clinica Chimica Acta* 365 (2006):236-242.
- 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.
- 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 1993.
- Bachorik PS, Ross JW. National cholesterol education program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. *Clin Chem* 1995;41:1414-1420.
- Cooper GR, Myers GL, Smith SJ, et al. Standardization of Lipid, Lipoprotein, and Apolipoprotein Measurements. *Clin Chem* 1988;34(8B):B95-B105.
- WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.
- 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.
- LDL Cholesterol Method Certification Protocol for Manufacturers. National Reference System for Cholesterol. Cholesterol Reference Method Laboratory Network 1997, October.
- Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. *Clin Chem* 1986;32:470-475.
- Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". *Eur J Clin Chem Clin Biochem* 1996;34:385-386.
- 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.
- 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.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. *Clin Chem Lab Med* 2007;45(9):1240-1243.
- 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.
- 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.
- 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

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

CONTENT

Contents of kit

0108057966190c503V4.0

LDLC3

LDL-Cholesterol Gen.3

cobas®



Volume after reconstitution or mixing

GTIN

Global Trade Item Number

COBAS, COBAS C and PRECICONTROL are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

© 2022, Roche Diagnostics



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

+800 5505 6606

