LDL-c direct FS*

Order Information

Cat. No.	Kit size	Instrument	Σ
1 4131 99 10 972	R1 3 x 12.3 mL	BX-3010	240 (3 x 80)
		BX-4000	183 (3 x 61)
	R2 3 x 5.1 mL	BX-3010	240 (3 x 80)
		BX-4000	183 (3 x 61)

Intended Use

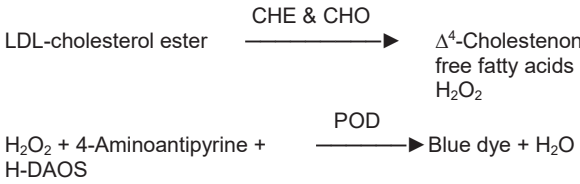
Diagnostic reagent for quantitative in vitro determination of LDL-C (low density lipoprotein cholesterol) in human serum or heparin plasma on automated Sysmex BX-Series.

Summary

Cholesterol is usually obtained from the intestinal absorption of dietary and biliary cholesterol but can also be synthesized de novo in various tissues, predominantly in liver and intestine. An adult on a low-cholesterol diet typically synthesizes about 800 mg of cholesterol per day. Cholesterol is essential for all cells and is used extensively as a major structural component of cell membranes and as substrate for the synthesis of bile acids, vitamin D, and sex hormones (estradiol, progesterone, androsterone and testosterone). Cholesterol is insoluble in water and, therefore, must be transported bound to proteins. Lipoproteins are complex particles with a central core containing cholesterol esters and triglycerides (TG) surrounded by free cholesterol, phospholipids, and apolipoproteins, which facilitate lipoprotein formation and function. Plasma lipoproteins can be divided into different classes based on size, lipid composition, and apolipoproteins; the four major classes are: Chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Low-density lipoproteins are derived from VLDL and IDL (Intermediate Density Lipoprotein) in plasma and contain a large amount of cholesterol and cholesterol esters. The principal role of LDL is to deliver these two forms of cholesterol to peripheral tissues. At least two-thirds of circulating cholesterol reside in LDL. Evidence from epidemiologic, genetic, and clinical intervention studies has shown that LDL is causal in the process of developing atherosclerotic cardiovascular disease (ASCVD). High LDL-C is one of the major risk factors that contribute to the formation of atherosclerotic plaques within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Results of recent clinical studies on lowering LDL-C indicate continued benefits at low concentrations. A direct linear relationship between the pharmacological lowering of LDL-C and the relative risk reduction in cardiovascular events has been observed for three different drug classes: statins, ezetimibe and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. The standard lipid panel represents a well-established platform to assess risk, but this panel alone may be insufficient and/or misleading. By now, the majority of screening guidelines recommend the measurement of a full lipid profile including total cholesterol (TC), LDL-C, HDL-cholesterol (HDL-C) and TG. [1-6]

Method

Different methods exist to determine LDL-C. The reference method is the ultracentrifugation, which is tedious and technically demanding, therefore, not suitable for routine. A common approach to determine LDL-C in clinical laboratory is the Friedewald calculation, which estimates LDL-C from measurements of TC, triglycerides (TG), and HDL-C but the method only approximates LDL-C and is subject to well-established limitations. At the end of the last century, homogeneous LDL-C methods for fully automated determination were introduced. Those methods enable direct determination of LDL-cholesterol and show other advantages compared to previously used methods. LDL-c direct FS is a homogeneous method without centrifugation steps for direct measurement of LDL-cholesterol. Block polymer detergents protect HDL, VLDL and chylomicrons in a way that only LDL-cholesterol is selectively determined by an enzymatic cholesterol measurement. [7]



The intensity of the formed dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents

Components and Concentrations

R1:	Buffer	pH 6.65	20 mmol/L
	Peroxidase (POD)		≥ 2000 U/L
	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (H-DAOS)		≥ 0.7 mmol/L
R2:	Buffer	pH 8.15	20 mmol/L
	Cholesterol esterase (CHE)		≥ 2000 U/L
	Cholesterol oxidase (CHO)		≥ 2000 U/L
	Peroxidase (POD)		≥ 15000 U/L
	4-Aminoantipyrine (4-AA)		≥ 1.5 mmol/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

Warnings and Precautions

1. ⚠ Reagent 1: Warning. Contains: Mixture of 5-chlorine-2-methyl-2H-isothiazol-3-on and 2-methylen-2H-isothiazol-3-on (3:1). H317 May cause an allergic skin reaction. P280 Wear protective gloves/protective clothing/eye protection. P302+P352 If on skin: Wash with plenty of water/soap.
2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains animal and biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
5. Artificial lipid mixtures (e.g. Intralipid®) may interfere with the test. Serum samples from patients treated with such solutions should not be used.
6. Determination of samples from patients with a rare type of Hyperlipoproteinemia (Hyperlipoproteinemia Type III) may lead to false results.
7. In very rare cases, samples of patients with gammopathy might give falsified results [8].
8. Acetaminophen and metamizole medication leads to falsely low results in patient samples.
9. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
10. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use. The bottles are placed directly into the reagent rotor.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Stability [9,10,11]:

1 day	at	20 – 25°C
7 days	at	4 – 8°C
12 months	at	–20°C

Only freeze once. Discard contaminated specimens.

Calibrators and Controls

DiaSys TruCal Lipid is recommended for calibration. Calibrator values have been made traceable to NIST-SRM®-1951 Level 2. Use DiaSys TruLab L Level 1 and Level 2 for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 3 mL

Performance Characteristics

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 490 mg/dL (12.7 mmol/L). In case of higher concentrations re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function.	
Limit of detection**	4 mg/dL (0.103 mmol/L)
Onboard stability	12 weeks
Calibration stability	12 weeks

Interfering substance	Interferences ≤ 9% up to	Analyte concentration
Ascorbic acid	500 mg/dL	70.1 mg/dL (1.81 mmol/L)
	500 mg/dL	161 mg/dL (4.16 mmol/L)
Bilirubin (conjugated)	60 mg/dL	85.8 mg/dL (2.22 mmol/L)
	60 mg/dL	156 mg/dL (4.03 mmol/L)
Bilirubin (unconjugated)	60 mg/dL	77.6 mg/dL (2.01 mmol/L)
	60 mg/dL	164 mg/dL (4.24 mmol/L)
Hemoglobin	1000 mg/dL	77.6 mg/dL (2.01 mmol/L)
	1000 mg/dL	164 mg/dL (4.24 mmol/L)
Lipemia (triglycerides)	1500 mg/dL	77.8 mg/dL (2.01 mmol/L)
	1700 mg/dL	162 mg/dL (4.19 mmol/L)
N-acetylcysteine (NAC)	1600 mg/L	74.2 ng/dL (1.92 mmol/L)
	1600 mg/L	165 ng/dL (4.27 mmol/L)

For further information on interfering substances refer to Young DS [12,13].

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	92.2	146	439
Mean [mmol/L]	2.39	3.78	11.3
CV [%]	1.12	1.12	1.73
Total Precision (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	87.1	141	424
Mean [mmol/L]	2.25	3.65	11.0
CV [%]	2.32	2.03	1.76

Method comparison (n= 118)	
Test x	DiaSys LDL-c direct FS (BioMajesty®JCA-BM6010C)
Test y	DiaSys LDL-c direct FS (BX-3010)
Slope	1.00
Intercept	0.648 mg/dL (0.017 mmol/L)
Coefficient of correlation	0.999

** according to CLSI document EP17-A2

Conversion Factor

LDL-C [mg/dL] x 0.02586 = LDL-C [mmol/L]

Reference Range [14]

Desirable	< 100 mg/dL	2.59 mmol/L
Above optimal	100 – 129 mg/dL	2.59 – 3.34 mmol/L
Borderline high risk	130 – 159 mg/dL	3.37 – 4.12 mmol/L
High risk	160 – 189 mg/dL	4.14 – 4.89 mmol/L
Very high risk	> 190 mg/dL	> 4.92 mmol/L

Patient risk classification, management and treatment therapies are described in the 2018 AHA/ACC Guideline on the Management of Blood Cholesterol [15].

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Clinical Interpretation

The lipid guidelines of the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) 2019 have set targets for the reduction of low-density lipoproteins (LDL) as follows:

Very high-risk patients:

≥ 50% LDL-C reduction from baseline and an absolute LDL-C treatment goal of < 1.4 mmol/L (< 55 mg/dL)

High risk patients:

≥ 50% LDL-C reduction and a LDL-C goal of < 1.8 mmol/L (< 70 mg/dL)

Literature

1. Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2015. Biochemistry. 8th edition. Section 26.3 The Complex Regulation of Cholesterol Biosynthesis Takes Place at Several Levels, Page 779 – 788.
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14. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the 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). JAMA. 2001; 285(19): 2486-2497.
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IVD CE

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* Fluid Stable

LDL-c direct FS

Chemistry Code 100 55

Chemistry Parameters 1

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No.	*	Method Name	LDL-CD	Reagent Name	Reagent (µL)	Water (µL)
Print Name	LDL-CD	MethodColor		R1 LDL-CD	120	
Sample Type	Serum			R2 LDL-CD	30	
Unit	mg/dL			Diluent Disable		
Assay Type	End			Sample Ppt. Wash Disable		

Measuring points

	Start	End
1	22	23
2	45	46

Stirring Speed R1: Middle R2: Middle

Wave Length: Prim. 600 Sec. 700

Normal Range

No.	Normal Range Name	Min	Max
1	Male-G1	*	*
2	Male-G2	*	*
3	Male-G3	*	*
4	Female-G1	*	*

	Sample Volume (µL) Low	Normal	High	Diluted Sample (µL)	Diluent (µL)
☐ Diluent	0.0 <	1.5 <	0.0		
Rerun (High/Prozone)					
☐ Diluent	0.0 <	1.5 <	0.0		
Rerun (Low)					
☐ Diluent	0.0 <	1.5 <	0.0		

Technical Range
(Conc) 4 – 490
(mAbs/10) * – *

Previous Result Comparison (%): * – * %

Abnormal Range (Conc): * – *

Panic Range (Conc): * – *

Decimal Point: 2 Profile SI: Disable

***Entered by user**

Chemistry Parameters 2

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No. * Method Name LDL-CD Sample Serum

Limit Checks

- ✓ Duplicate Limit: 100 mAbs/10
- ✓ Sensitivity Limit: 2000 mAbs/10
- ✓ Linearity Limit: %
- (mAbs/10)/min
- ☐ Prozone Limit: %

SL1-S – SL1-F

SL2-S – SL2-F

Sensitivity: mAbs/10

- ✓ Absorbance Limit
  Abs. in reaction
  Limit: mAbs/10

Blank measurement

Blank measurement: Disable reagent blank and C1 blank

Measurement of Reagent Blank during Run: None

Reagent blank measurement at calibration: Reagent blank (No sample)

The number of measurement: Duplicate

Reagent blank limit checks:
- ✓ Duplicate Limit: 50 mAbs/10

Instrument Factor

a 1.00 b 0.00

LDL-c direct FS

Chemistry Code 100 55

Calibration Registration

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No. *

Method Name LDL-CD

Sample Type Serum

Replication Duplicate

Check Interval 84

Test without calibration

Disable

Calibration Type Linear

Reagent Lot New Add

Calibrator Name TruCal Lipid

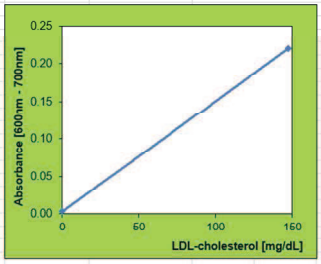
	Conc.	WORK	MASTER	Calibr. Lot No.	☐ All
C1	0	Automatic entry	Automatic entry	*	
C2	*	Automatic entry	Automatic entry	*	
C3	*				
C4	*				
C5	*				
C6	*				
C7	*				

K Automatic entry

☐ C1 Blank
☐ Reagent Blank for C1

Reagent Lot No.
(R1) *
(R2) *

Last



The calibration curve is lot dependent.

Reagent blank mAbs/10 Last

Blank Automatic entry mAbs/10 Last

Calibration Curve Conc.

Absorbance mAbs/10 Recalculation

***Entered by user**

LDL-c direct FS

Chemistry Code 100 55

Chemistry Parameters

Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters

Method: * | Name: LDL-CD

Print Name: LDL-CD

Sample: Serum

Unit: mg/dL

Assay Type: End

	Reagent Name	Reagent (µL)	Water (µL)
R1	LDL-CD	160	
R2 ✓ Enable	LDL-CD	40	
Diluent ☐ Enable			

Measuring points

	Start	End
1	33	34
☐ Enable 2	67	68

Decimal Points: 0

Wave Length: Prim. 600 Sec ☐ Disable 700

Normal Range

No.	Normal Range Name	Min	Max
1	Male-G1	*	*
2	Male-G2	*	*
3	Male-G3	*	*
4	Female-G1	*	*

	Sampling	Sample (µL)	Diluent (µL)
☐ Normal Dilution	2		0
☐ Rerun (High/Prozone) Dilution	2		0
☐ Rerun (Low) Dilution	2		0

Technical Range

(Conc)	4 –	490
(mAbs/10)	–	

SPT Wash ☐ Enable — Reagent Name:

Stirring Speed: R1 Middle R2 Middle

***Entered by user**

Chemistry Parameters

Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters

Method No.: * | Name: LDL-CD | Sample: Serum

Limit Checks

✓ Duplicate Limit: 100 mAbs/10

✓ Sensitivity Limit: 2000 mAbs/10

✓ Linearity Limit: % (mAbs/10)/min

☐ Prozone Limit: % Upper

SL1-S – SL1-F

SL2-S – SL2-F

Sensitivity: mAbs/10

✓ Absorbance Limit

Reaction

Limit: mAbs/10

Blank measurement

Blank measurement:
Disable reagent blank and S1 blank

Measurement of Reagent Blank during Run:
None

Reagent blank measurement at calibration:
Reagent blank (No sample)

The number of measurement:
Duplicate

Reagent blank limit checks:
✓ Duplicate Limit: 50 mAbs/10

Instrument Factor

a 1.00 b 0.00

LDL-c direct FS

Chemistry Code 100 55

Registration Calibration

Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters

Method: * Name: LDL-CD

Sample: Serum

Sampling: Duplicate

Check Interval: 84 days

Auto: Change Lot Full Calibration

Auto Interval: hours

Type: Linear Lot: New

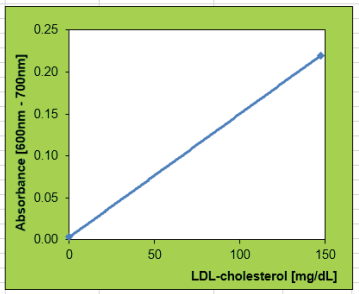
Material Name: TruCal Lipid

	Conc.	WORK	MASTER	Lot No. (S) ☐ All
S1	0	Automatic entry	Automatic entry	
S2	*	Automatic entry	Automatic entry	
S3	*			
S4	*			
S5	*			
S6	*			
S7	*			

K: Automatic entry ☐ S1 Blank ☐ Reagent Blank for S1

***Entered by user**

R Lot No. R1: * R2: * Last:



This exemplary calibration curve developed at Sysmex BX-3010 Chemistry Analyzer is lot dependent.

Reagent blank: mAbs/10 Last:

Blank: Automatic entry mAbs/10 Last:

Type: Conc.:

Absorbance: mAbs/10 Recalculation