

HDL-c direct FS*

Order Information

Cat. No.	Kit size	Instrument	Σ
1 3561 99 10 972	R1 3 x 18.3 mL	BX-3010	375 (3 x 125)
		BX-4000	288 (3 x 96)
	R2 3 x 7.1 mL	BX-3010	375 (3 x 125)
		BX-4000	288 (3 x 96)

Intended Use

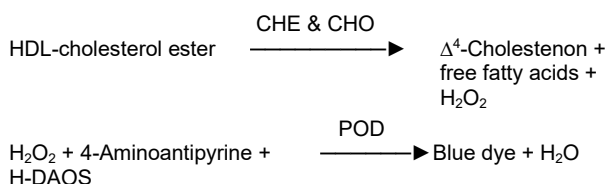
Diagnostic reagent for quantitative in vitro determination of HDL-C (high density lipoprotein cholesterol) in human serum or heparin plasma on automated Sysmex BX-Series.

Summary

Cholesterol, synthesized by body cells and absorbed with food, is a component of cell membranes and a precursor for steroid hormones and bile acids. Cholesterol is transported in plasma via lipoproteins, complexes between lipids and apolipoproteins. Four lipoprotein classes exist: High density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. These classes show distinct relationship to coronary atherosclerosis. LDL is involved in the cholesterol transport to the peripheral cells, contributing to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. One of the important functions of HDL involves the physiological removal of cholesterol from peripheral tissues and cells, and transport to the liver. The concept that HDL could protect against CHD primarily originated from epidemiological studies of the healthy population, in particular the Framingham study. In addition to a number of antioxidant effects, HDL also serves as a powerful mediator of the cellular inflammatory and antithrombotic responses. HDL-particles are macromolecule complexes synthesized by liver and intestine and formed from surface components. HDL-particles are released into plasma during lipolysis of lipoproteins rich in triglycerides. Particles consist of an amphipathic lipid monolayer of phospholipids and cholesterol with embedded amphipathic proteins surrounding a core of hydrophobic lipids, mostly cholesteryl esters and triglycerides. HDL-C monitoring is highly relevant in cardiovascular risk assessment. Elevated HDL-C levels usually correlate with decreased cardiovascular risk; whereas reduced concentrations of HDL-C, especially in combination with elevated triglycerides are associated with high risk of atherosclerotic heart disease, even at or below recommended LDL-C goals. Preferred screening tests for dyslipidemia or lipid disorders are total cholesterol (TC) and HDL-C but the majority of screening guidelines nowadays recommend a full lipid profile including TC, LDL-C, HDL-C and triglycerides. [1-8]

Method

Previous HDL-cholesterol determinations were performed by time-consuming precipitation methods or ultracentrifugation (reference method in combination with cholesterol measurement by Abell- Kendall). However, the direct determination of HDL-cholesterol is used in routine [9]. HDL-c direct FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Block polymer detergents protect LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [10].



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

Reagents

Components and Concentrations

R1:	Buffer	pH 6.85	20 mmol/L
	Peroxidase (POD)		≥ 2000 U/L
	N-(2-hydroxy-3-sulfoethyl)-3,5-dimethoxyaniline sodium salt (H-DAOS)		≥ 0.7 mmol/L
R2:	Buffer	pH 8.15	20 mmol/L
	Cholesterol esterase (CHE)		≥ 400 U/L
	Cholesterol oxidase (CHO)		≥ 700 U/L
	Peroxidase (POD)		≥ 15000 U/L
	4-Aminoantipyrine		≥ 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.

The in-use stability of the reagent is 24 months.

Warnings and Precautions

- Components contained in HDL-c direct FS are classified according to EC regulation 1272/2008 (CLP) as follows:



⚠ 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.

- Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
- Acetaminophen and metamizole medication leads to falsely low results in patient samples.
- In very rare cases, samples of patients with gammopathy might give falsified results [11].
- In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
- Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
- Please refer to the safety data sheets (SDS) 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.
- For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

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

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 lithium heparin plasma

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Stability [12]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
3 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 a commercially available assay, which is standardized against the designated CDC reference method (ultracentrifugation method). Use DiaSys TruLab L Level 1 and Level 2 for internal quality control. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. 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

Measuring range up to 200 mg/dL (5.17 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**	3 mg/dL (0.078 mmol/L)
Onboard stability	18 weeks
Calibration stability	14 weeks

Interfering substance	Interferences ≤ 9% up to	Analyte concentration
Ascorbic acid	60 mg/dL	31.7 mg/dL (0.819 mmol/L)
	60 mg/dL	78.6 mg/dL (2.03 mmol/L)
Bilirubin (conjugated)	40 mg/dL	34.0 mg/dL (0.880 mmol/L)
	50 mg/dL	75.8 mg/dL (1.96 mmol/L)
Bilirubin (unconjugated)	60 mg/dL	34.9 mg/dL (0.903 mmol/L)
	60 mg/dL	83.2 mg/dL (2.15 mmol/L)
Hemoglobin	800 mg/dL	35.8 mg/dL (0.925 mmol/L)
	1000 mg/dL	77.9 mg/dL (2.01 mmol/L)
N-acetylcysteine (NAC)	1700 mg/L	31.1 mg/dL (0.805 mmol/L)
	1700 mg/L	80.3 mg/dL (2.08 mmol/L)
Lipemia (triglycerides)	1000 mg/dL	33.3 mg/dL (0.860 mmol/L)
	1600 mg/dL	68.1 mg/dL (1.76 mmol/L)

For further information on interfering substances refer to Young DS [13,14].

Precision			
Within run (n=20)	Sample 1	Sample 2	Sample 3
Mean [mg/dL]	20.0	39.5	199
Mean [mmol/L]	0.516	1.02	5.14
CV [%]	1.42	0.772	1.61
Between day (n=20)	Sample 1	Sample 2	Sample 3
Mean [mg/dL]	19.4	39.2	195
Mean [mmol/L]	0.502	1.01	5.05
CV [%]	1.53	1.88	2.12

Method comparison (n= 132)	
Test x	DiaSys HDL-c direct FS (BioMajesty® JCA-BM6010C)
Test y	DiaSys HDL-c direct FS (BX-3010)
Slope	1.01
Intercept	-0.708 mg/dL (-0.018 mmol/L)
Coefficient of correlation	0.999

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Conversion Factor

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

Reference Range [15]

National Cholesterol Education Program (NCEP) guidelines:

Low HDL-cholesterol (major risk factor for CHD):

< 40 mg/dL (< 1.04 mmol/L)

High HDL-cholesterol ("negative" risk factor for CHD):

≥ 60 mg/dL (≥ 1.55 mmol/L)

A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and progestational agents, genetic factors.

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

Literature

1. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APHA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. J Am Coll Cardiol. 2019;73(24):e285–e350.
2. Gordon DJ, Probstfield JL, Garrison RJ, et al. High density lipoprotein cholesterol and cardiovascular disease. Four Prospective American Studies. Circulation. 1989;79:8-15.
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6. Chapman MJ, Ginsberg HN, Amarenco P, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. European heart journal volume. 2011;32:1345-61.
7. Rifai N, Warnick GR. Lipids, Lipoproteins, Apolipoproteins, and Other Cardiovascular Risk Factors. In: Burtis CA, Ashwood ER and Burns DE, editors. Tietz Textbook of Clinical Chemistry. 4th ed. Missouri: Elsevier Saunders company; 2006. page 903-981.
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9. Langlois MR, Blaton VH. Historical milestones in measurement of HDLcholesterol: Impact on clinical and laboratory practice. *Clin Chimica Acta*. 2006;369:168-178.
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12. Guder WG, da Fonseca-Wollheim F, Heil W, et al. *The Quality of Diagnostic Samples*. 3rd ed. Darmstadt: GIT Verlag; 2010. p. 22-3.
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14. Young DS. *Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products* [Internet]. AACC Press and John Wiley and Sons, Inc; 2020 [cited 2020 May]. Available from: <https://clinfx.wiley.com/aaccweb/aacc/>
15. 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.

Additions and/or changes in the document are highlighted in grey. For deletions, please refer to the customer information for the corresponding edition number of the package inserts.



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 Alte Strasse 9 65558 Holzheim
 Germany
www.diasys-diagnostics.com

* Fluid Stable

Chemistry Parameters 1				Sysmex BX-3010 Chemistry Analyzer Analytical Parameters																						
Method No.	*	Method Name	HDL-CD	Reagent Name	Reagent (μL)	Water (μL)																				
Print Name	HDL-CD	MethodColor		R1	HDL-CD	120																				
Sample Type	Serum			R2	HDL-CD	30																				
Unit	mg/dL			Diluent	Disable																					
Assay Type	End			Sample Ppt. Wash	Disable																					
Measuring points		Start	End	Stirring Speed R1	Middle	R2 Middle																				
		1	22 - 23																							
		2	45 - 46																							
Wave Length	Prim. 600	Sec. 700		<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th>No.</th> <th>Normal Range Name</th> <th>Min</th> <th>Max</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Male-G1</td> <td>*</td> <td>*</td> </tr> <tr> <td>2</td> <td>Male-G2</td> <td>*</td> <td>*</td> </tr> <tr> <td>3</td> <td>Male-G3</td> <td>*</td> <td>*</td> </tr> <tr> <td>4</td> <td>Female-G1</td> <td>*</td> <td>*</td> </tr> </tbody> </table>			No.	Normal Range Name	Min	Max	1	Male-G1	*	*	2	Male-G2	*	*	3	Male-G3	*	*	4	Female-G1	*	*
No.	Normal Range Name	Min	Max																							
1	Male-G1	*	*																							
2	Male-G2	*	*																							
3	Male-G3	*	*																							
4	Female-G1	*	*																							
Normal	Sample Volume (μL)	Diluted Sample (μL)	Diluent (μL)	Technical Range																						
	Low Normal High			(Conc)	3	200																				
<input type="checkbox"/> Diluent	0.0 < 1.5 < 0.0			(mAbs/10)	*	*																				
<input type="checkbox"/> Rerun (High/Prozone)				Previous Result Comparison (%)	*	* %																				
<input type="checkbox"/> Diluent	0.0 < 1.5 < 0.0			Abnormal Range	*	*																				
<input type="checkbox"/> Rerun (Low)				Panic Range	*	*																				
<input type="checkbox"/> Diluent	0.0 < 1.5 < 0.0			Decimal Point	2	Profile SI Disable																				

*Entered by user

Chemistry Parameters 2				Sysmex BX-3010 Chemistry Analyzer Analytical Parameters		
Method No.	*	Method Name	HDL-CD	Sample	Serum	
Limit Checks <input checked="" type="checkbox"/> Duplicate Limit 100 mAbs/10 <input checked="" type="checkbox"/> Sensitivity Limit 2000 mAbs/10 <input checked="" type="checkbox"/> Linearity Limit % <input type="checkbox"/> Prozone Limit % SL1-S - SL1-F SL2-S - SL2-F Sensitivity mAbs/10 <input checked="" type="checkbox"/> 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: <input checked="" type="checkbox"/> Duplicate Limit 50 mAbs/10		
				Instrument Factor a 1.00 b 0.00		

Calibration Registration

**Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters**

Method No.

Method Name

Sample Type

Replication

Check Interval

Test without calibration

Calibration Type

Reagent Lot

Calibrator Name

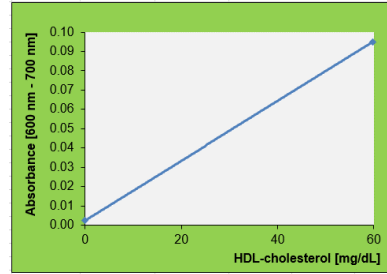
	Conc.	WORK	MASTER	Calibr. Lot No.	<input type="checkbox"/> All
C1	0	Automatic entry	Automatic entry	*	
C2	*	Automatic entry	Automatic entry	*	
C3	*				
C4	*				
C5	*				
C6	*				
C7	*				

K C1 Blank
 Reagent Blank for C1

Reagent Lot No.

(R1)
 (R2)

Last



The calibration curve is lot dependent.

Reagent blank mAbs/10 Last

Blank mAbs/10 Last

Calibration Curve Conc.

Absorbance mAbs/10

*Entered by user

Chemistry Parameters			Sysmex BX-4000 Chemistry Analyzer Analytical Parameters																						
Method	* <input type="text"/>	Name	<input type="text" value="HDL-CD"/>	Reagent Name	<input type="text"/>																				
Print Name	<input type="text" value="HDL-CD"/>	R1	<input type="text" value="HDL-CD"/>	Reagent (µL)	<input type="text" value="160"/>																				
Sample	<input type="text" value="Serum"/>	R2	<input checked="" type="checkbox"/> Enable	<input type="text" value="HDL-CD"/>	<input type="text" value="40"/>																				
Unit	<input type="text" value="mg/dL"/>																								
Assay Type	<input type="text" value="End"/>	Diluent	<input type="checkbox"/> Enable	<input type="text"/>	<input type="text"/>																				
Measuring points		Start		End	Decimal Points																				
		1	<input type="text" value="33"/>	-	<input type="text" value="34"/>																				
<input type="checkbox"/> Enable		2	<input type="text" value="67"/>	-	<input type="text" value="68"/>																				
Wave Length	Prim. <input type="text" value="600"/>	Sec	<input type="checkbox"/> Disable	<input type="text" value="700"/>																					
					Normal Range																				
					<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th>No.</th> <th>Normal Range Name</th> <th>Min</th> <th>Max</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Male-G1</td> <td>*</td> <td>*</td> </tr> <tr> <td>2</td> <td>Male-G2</td> <td>*</td> <td>*</td> </tr> <tr> <td>3</td> <td>Male-G3</td> <td>*</td> <td>*</td> </tr> <tr> <td>4</td> <td>Female-G1</td> <td>*</td> <td>*</td> </tr> </tbody> </table>	No.	Normal Range Name	Min	Max	1	Male-G1	*	*	2	Male-G2	*	*	3	Male-G3	*	*	4	Female-G1	*	*
No.	Normal Range Name	Min	Max																						
1	Male-G1	*	*																						
2	Male-G2	*	*																						
3	Male-G3	*	*																						
4	Female-G1	*	*																						
Normal	Sampling	Sample (µL)	Diluent (µL)	Technical Range																					
<input type="checkbox"/> Dilution	<input type="text" value="2"/>	<input type="text"/>	<input type="text" value="0"/>	(Conc)	<input type="text" value="3.0"/> - <input type="text" value="200"/>																				
				(mAbs/10)	<input type="text" value="*"/> - <input type="text" value="*"/>																				
<input type="checkbox"/> Rerun (High/Prozone)																									
<input type="checkbox"/> Dilution	<input type="text" value="2"/>	<input type="text"/>	<input type="text" value="0"/>																						
<input type="checkbox"/> Rerun (Low)																									
<input type="checkbox"/> Dilution	<input type="text" value="2"/>	<input type="text"/>	<input type="text" value="0"/>																						
				SPT Wash	<input type="checkbox"/> Enable																				
				Reagent Name	<input type="text"/>																				
				Stirring Speed	R1 <input type="text" value="Middle"/> R2 <input type="text" value="Middle"/>																				

*Entered by user

Chemistry Parameters			Sysmex BX-4000 Chemistry Analyzer Analytical Parameters		
Method No.	* <input type="text"/>	Name	<input type="text" value="HDL-CD"/>	Sample	<input type="text" value="Serum"/>
Limit Checks					
<input checked="" type="checkbox"/> Duplicate Limit	<input type="text" value="100"/>			mAbs/10	
<input checked="" type="checkbox"/> Sensitivity Limit	<input type="text" value="2000"/>			mAbs/10	
<input checked="" type="checkbox"/> Linearity Limit	<input type="text"/>	%	<input type="text"/>	(mAbs/10)/min	
<input type="checkbox"/> Prozone Limit	<input type="text"/>	%	<input type="text" value="Upper"/>		
	SL1-S	<input type="text"/>	-	SL1-F	<input type="text"/>
	SL2-S	<input type="text"/>	-	SL2-F	<input type="text"/>
	Sensitivity	<input type="text"/>		mAbs/10	
<input checked="" type="checkbox"/> Absorbance Limit					
	Reaction	<input type="text"/>			
	Limit	<input type="text"/>		mAbs/10	
					Blank measurement
					Blank measurement:
					<input type="text" value="Disable reagent blank and S1 blank"/>
					Measurement of Reagent Blank during Run:
					<input type="text" value="None"/>
					Reagent blank measurement at calibration:
					<input type="text" value="Reagent blank (No sample)"/>
					The number of measurement:
					<input type="text" value="Duplicate"/>
					Reagent blank limit checks:
	<input checked="" type="checkbox"/> Duplicate Limit	<input type="text" value="50"/>		mAbs/10	
					Instrument Factor
					a <input type="text" value="1.00"/> b <input type="text" value="0.00"/>

Registration Calibration

**Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters**

Method Name

R Lot No. R1 Last
R2

Sample

Sampling

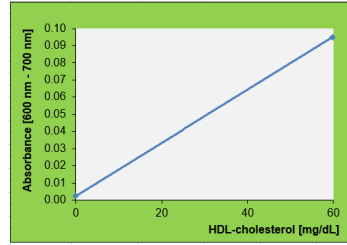
Check Interval days

Auto

Auto Interval hours

Type Lot

Material Name



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

Reagent blank mAbs/10 Last

Blank mAbs/10 Last

Type Conc.

Absorbance mAbs/10

	Conc.	WORK	MASTER	Lot No. (S) <input type="checkbox"/> All
S1	<input type="text" value="0"/>	Automatic entry	Automatic entry	
S2	<input type="text" value="*"/>	Automatic entry	Automatic entry	
S3	<input type="text" value="*"/>			
S4	<input type="text" value="*"/>			
S5	<input type="text" value="*"/>			
S6	<input type="text" value="*"/>			
S7	<input type="text" value="*"/>			

K S1 Blank Reagent Blank for S1

***Entered by user**