HDL-C Immuno FS*

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

Order information

Cat. No.	Kit size		Number of tests	
1 3521 99 10 972	R1	3 x 18.3 mL	BX-3010	3 x 125 tests
			BX-4000	3 x 96 tests
	R2	3 x 7.1 mL	BX-3010	3 x 125 tests
			BX-4000	3 x 96 tests

Method

Previous HDL-cholesterol determinations were performed by time consuming precipitation methods [1]. HDL-C Immuno FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins are used to form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [2].

Principle

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human β-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL

HDL-cholesterol + H_2O + O_2 $\xrightarrow{\text{CHE \& CHO}}$ Cholest-4-en-3-one + fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ Blue complex + H_2O

Reagents

Components and Concentrations

R1:	Good's buffer	pH 7.0	25 mmol/L
	4-Aminoantipyrine		0.75 mmol/L
	Peroxidase	(POD)	2 kU/L
	Ascorbate oxidase		2.25 kU/L
	Anti-human β-lipoprotein antibody (sheep)		
R2:	Good's buffer	pH 7.0	30 mmol/L
	Cholesterol esterase	(CHE)	4 kU/L
	Cholesterol oxidase	(CHO)	20 kU/L
	N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt	(F-DAOS)	0.8 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C protected from light and contamination is avoided. Do not freeze the reagents!

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. H412 Harmful to aquatic life with long lasting effects. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection. P302+P352 If o skin: Wash with plenty of water/soap. P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
2. The reagents contain animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. 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.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

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

Specimen

Serum or heparin plasma

Stability [3]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
3 months	at	–20°C

Freeze only once. Discard contaminated specimens.

Calibrators and Controls

For calibration, DiaSys TruCal Lipid has to be used. The assigned values of the calibrator have been made traceable to the reference material NIST-SRM®-1951 Level 2. For internal quality control, DiaSys TruLab L control should be assayed. 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 130 mg/dL (3.4 mmol/L) HDL-C (in case of higher concentrations re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function).	
Limit of detection**	0.5 mg/dL (0.013 mmol/L) HDL-C
On-board stability	3 weeks
Calibration stability	3 weeks

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

Interfering substance	Interferences < 10 %	Analyte concentration
Ascorbate	up to 30 mg/dL	55.0 mg/dL (1.42 mmol/L)
Hemoglobin	up to 500 mg/dL	55.0 mg/dL (1.42 mmol/L)
Bilirubin, conjugated	up to 60 mg/dL	54.9 mg/dL (1.42 mmol/L)
Bilirubin, unconjugated	up to 60 mg/dL	35.1 mg/dL (0.907 mmol/L)
Lipemia (triglycerides)	up to 1800 mg/dL	37.4 mg/dL (0.967 mmol/L)
For further information on interfering substances refer to Young DS [7].		

Precision BX-4000			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	34.7	54.3	94.3
Mean [mmol/L]	0.898	1.40	2.44
Coefficient of variation [%]	0.990	0.908	0.746
Between run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	31.7	54.4	90.8
Mean [mmol/L]	0.819	1.41	2.35
Coefficient of variation [%]	2.53	1.01	1.83

Method comparison (n=105)	
Test x	DiaSys HDL-C Immuno FS (Biomajesty 6010C)
Test y	DiaSys HDL-C Immuno FS (BX-4000)
Slope	1.07
Intercept	–2.81 mg/dL (–0.073 mmol/L)
Coefficient of correlation	0.995

Conversion factor

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

Reference Range [4]

National Cholesterol Education Program (NCEP) guidelines:

Low HDL-cholesterol (major risk factor for coronary heart disease (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 reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Wiebe DA, Warnick GR. Measurement of high-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p. 127-44.
2. Nauck M, Maerz W, Wieland H. New immunoseparation-based homogenous assay for HDL-cholesterol compared with three homogenous and two heterogeneous methods for HDL-cholesterol. Clin Chem 1998; 44: 1443-51.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 22-3.
4. 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. 02-5215; September 2002.
5. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998; 19: 1434-503.
6. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
7. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

Manufacturer

IVD CE
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

HDL-C Immuno FS

Chemistry Code 100 45

Chemistry Parameters 1

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No.	*	Method Name	HDL	Reagent Name	Reagent (µL)	Water (µL)
Print Name	HDL	MethodColor		R1 HDL	120	
Sample Type	Serum			R2 HDL	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	*	*

	Normal 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) 0.5 – 130; (mAbs/10) * – *

Previous Result Comparison (%): * – * %

Abnormal Range (Conc): * – *

Panic Range (Conc): * – *

Decimal Point: 1 Profile SI: Disable

***Entered by user**

Chemistry Parameters 2

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No. * Method Name HDL Sample Serum

Limit Checks

- ✓ Duplicate Limit: 100 mAbs/10
- ✓ Sensitivity Limit: 2000 mAbs/10
- ✓ Linearity Limit: %
- (mAbs/10)/min
- ☐ Prozone Limit: Higher %
- SL1-S – SL1-F
- SL2-S – SL2-F
- Sensitivity: mAbs/10
- ✓ Absorbance Limit
  Abs. in reaction: Increase
  Limit: 25000 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

HDL-C Immuno FS

Chemistry Code 100 45

Calibration Registration

Sysmex BX-3010 Chemistry Analyzer
Analytical Parameters

Method No. *

Method Name HDL

Sample Type Serum

Replication Duplicate

Check Interval 21

Test without calibration

Disable

Calibration Type Linear

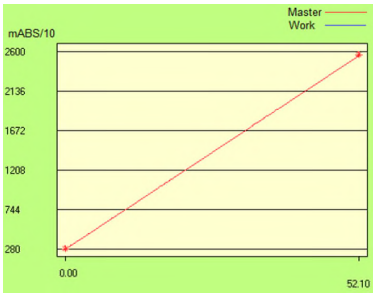
Reagent Lot New Add

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**

HDL-C Immuno FS

Chemistry Code 100 45

Chemistry Parameters

Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters

Method	*	Name	HDL		Reagent Name	Reagent (µL)	Water (µL)
Print Name	HDL			R1	HDL	160	
Sample	Serum			R2 ✓ Enable	HDL	40	
Unit	mg/dL						
Assay Type	End			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) 0.5 – 130
(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 HDL 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 Increase
  - Limit 25000 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

HDL-C Immuno FS

Chemistry Code 100 45

Registration Calibration

Sysmex BX-4000 Chemistry Analyzer
Analytical Parameters

Method: * Name: HDL

Sample: Serum

Sampling: Duplicate

Check Interval: 21 days

Auto: Change Lot Full Calibration

Auto Interval: hours

Type: Linear Lot: New

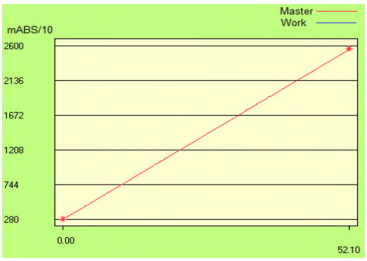
Material Name: TruCal Lipid

	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: * Last:
R2: *



The calibration curve is lot dependent

Reagent blank: mAbs/10 Last:

Blank: Automatic entry mAbs/10 Last:

Type: Conc.:

Absorbance: mAbs/10 Recalculation