BIOLABO
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LDL-CHOLESTEROL Direct Method

Reagent for quantitative determination of LDL-Cholesterol in human serum or plasma

REF 90416	**R1** 1 x 30 mL	**R2** 1 x 10 mL	100-125 Tests
REF 90816	**R1** 2 x 30 mL	**R2** 2 x 10 mL	200-250 Tests

Enclosed in each Kit REF 95506 **R1**: 1 x 2 mL **R2** 1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (3)

Low density lipoproteins (LDL), are synthesized in the liver by the action of various lipolytic enzymes on triglycerides rich very low density lipoproteins (VLDL). Many epidemiological and clinical studies have shown that an increased LDL-Cholesterol is associated with increased risk of atherosclerosis and coronary artery disease (CAD). Some studies showed that a reduction in LDL-Cholesterol is correlated with regression in atherosclerotic lesion.

PRINCIPLE

Direct method using selective detergents without specimen pre-treatment.
During the first phase, only non-LDL lipoproteins are solubilised by detergent 1. Such generated Cholesterol, subjected to Cholesterol oxidase (CO) and Cholesterol esterase (CE) actions, produces a colourless compound.

During the second phase, detergent 2 solubilises LDL-Cholesterol. The chromogenic coupler allows for colour formation that is proportional to the concentration of LDL-Cholesterol. The absorbance is measured at 546 nm (520-580).

REAGENTS

Vial R1 **REAGENT ENZYMES**

MES Buffer pH 6.3	Cholesterol oxidase
Ascorbic acid oxidase	Cholesterol esterase
4-amino-antipyrine	Detergent 1
Peroxydase	Preservative

Vial R2 **SPECIFIC DETERGENT**

MES Buffer pH 6.3	DSBmT
Detergent 2	Preservative

MES: morpholino-ethane-sulfonic acid
DSBmT: N,N-bis (4-sulphobutyl)-m-toluidine-disodium

REF 95506 **BIOLABO HDL / LDL: CK MB CALIBRATOR**
Vial R1 (lyophilisate): 1 x 2 mL Vial R2 (diluent): 1 x 5 mL
See enclosed Batch specific Package Insert

REAGENTS PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store at 2-8°C well recap in the original vial and away from light

- When used and stored as described in the insert, unopened reagents and calibrator are stable until expiry date stated on the label of the vial.
- Once opened, when free from contamination, reagents are stable at least for 3 months at 2-8°C (transfer the requested quantity, well recap and store at 2-8°C).
- Once reconstituted, REF 95506, see package insert (batch specific)
- Discard any reagent if cloudy or if the absorbance at 546 nm is > 0.050.

This kit should be refrigerated during transport.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

SPECIMEN COLLECTION AND HANDLING (2) (4)

Specimens should be collected after 12-14 h fasting. Do not use oxalate, fluoride, citrate or heparin.

Plasma: collected on EDTA. Centrifuge and remove plasma from blood cells as soon as possible (within 3 hours).

Serum: Centrifuge and remove serum from blood cells as soon as possible (within 3 hours).

LDL-Cholesterol is stable in the specimen for:

- 1 to 3 days at 2-8°C.
- 1 month at - 20°C.

INTERFERENCES (6) (7)

Tested concentrations (mg/dL) without significant interferences (± 10%):

Direct bilirubin:	20
Total bilirubin:	20
Haemoglobin:	500
Ascorbic acid:	50
Triglycerides (TG):	1293
Gamma-globulins:	5000

Above the upper limit, dilute specimen with saline solution before assaying and multiply the result according to dilution factor.

Specimens with TG > 1293 mg/dL should not be diluted. Increase the volume of reagent R1 and R2, in accordance with the ratios and take into account the dilution factor to calculate the result.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. BIOLABO LDL-Cholesterol calibrator REF 95806 or any calibrator of human origin traceable to reference material or method.
3. HDL LDL CK-MB Calibrator REF 95506
4. or any traceable calibrator (human origin).
5. HDL LDL CK-MB controls (human origin)
   REF 95516 HDL LDL CK-MB Control level 1
   REF 95526 HDL LDL CK-MB Control level 2
6. or any normal and pathological control sera of human origin

CALIBRATION

- Do not use aqueous calibrator
- Use BIOLABO LDL-Cholesterol calibrator REF 95806
- Or HDL LDL CK-MB Calibrator REF 95506 traceable to SRM 1951b (Standard Reference Material®) which was evaluated at CDC (Center for Disease Control)
- Or a calibrator of human origin traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagents.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- REF 95516 HDL LDL CK-MB Control level 1
- REF 95526 HDL LDL CK-MB Control level 2
- Or any assayed control sera of human origin referring to the same method (Selective detergent).
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (5)

LDL-Cholesterol	mg/dL	[mmol/L]
Recommended values	< 130	[< 3.36]
Low risk	130-159	[3.36 - 4.11]
High risk	≥ 160	[≥ 4.13]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES CHARACTERISTICS (5)

Within run N = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	98.1	146.5	209.8
S.D. mg/dL	0.72	0.97	1.31
C.V. %	0.73	0.66	0.62

Between run N = 40	*Low level*	*Medium level*	*High level*
Mean mg/dL	98.1	142.7	207.3
S.D. mg/dL	2.23	2.78	3.59
C.V. %	2.27	1.95	1.73

Detection limit: approximately 7 mg/dL.
Sensitivity for 100 mg/dL: approximately 0.212 abs. at 546 nm.

Result of study with designated comparison method–DCM (n = 54):

BIOLABO Mean: 123 mg/dL — DCM Mean: 125 mg/dL
BIOLABO S.D.: 30.7 mg/dL — DCM S.D.: 30.9 mg/dL
BIOLABO = 0.955 (DCM) + 3.02 mg/dL — r = 0.958

LINEARITY

The reaction is linear from 7 to 900 mg/dL (0.18 to 23 mmol/L).
Above, dilute the specimen (1 + 1) with saline solution and re-assay applying dilution factor 2 to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Do not use aqueous Calibrator

Let stand reagents and specimens at room temperature.

Set up the instrument to read micro-volumes.	**Blank**	**Calibrator**	**Assay**
Reagent R1	300 µL	300 µL	300 µL
Calibrator		3 µL	
Specimen			3 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A1 at 546 nm against reagent blank			
Add:	**Blank**	**Calibrator**	**Assay**
Reagent R2	100 µL	100 µL	100 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A2 at 546 nm against reagent blank			

Notes:
1. Depending on the instrument specifications, one can modify the above volumes taking into account the dilution ratio (i.e. R1 240 µL, R2 80 µL, specimen 2.4 µL or 3 µL). Refer to § **LINEARITY**.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Automatic Analyser with bichromatic reading (546-660nm) is recommended.

With manual procedure, calculate **ΔAbs. = (A2 – 0,75 A1)** for assay and Calibrator.

Calculate the result as follows:

$$\text{LDL-C} = \frac{\Delta\text{Abs. Assay}}{\Delta\text{Abs. Calibrator}} \times \text{Calibrator concentration}$$

mg/dL x 0.02586 = mmol/L

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 819-850.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 684-689*
(3) *Gotto, A.M., Lipoprotein metabolism and the ethiology of hyperlipidemia, Hospital Practice, 23 ; Suppl. 1, 4 (1988)*
(4) *National Institutes on Health publication No 95-3044,p.8, p.48, (1995).*
(5) *Bachorik P. S. et al., National Cholesterol Education Program Recommendations for Measurement of Low Density Lipoprotein Cholesterol ; Executive summary,Clinical Chemistry, (1995), Vol.41, n°10. P.1414-1420.*
(6) *National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Protocol No 7, Vol. 4,, No 8, (June. 1984).*
(7) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-379-386*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with