N BIO - Direct LDL

(Direct clearance method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - Direct LDL	1 x 40 ml	DLDL01040M
N BIO - Direct LDL	2 x 40 ml	DLDL01080M

GENUINE BIOSYSTEM

INTRODUCTION

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The relative protein and lipid determine the density of these lipoproteins and provide the basis on which to begin their classification. The classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density-lipoprotein (LDL) and high-density lipoprotein (HDL). LDL are synthesized in the liver by the action of various lipolytic enzymes on triglyceride rich VLDL. LDL-cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. Accurate measurement of LDL-cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

METHOD PRINCIPLE

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL and HDL by cholesterolesterase, cholesterol oxidase and subsequently catalase.

$$\text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

2. Specific measurement of LDL-Cholesterol after release of LDL Cholesterol by detergents in R2 Reagent. In the second reaction catalase is inhibited by sodium azide in R2-Reagent.

$$\text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{\text{peroxidase}} \text{quinone pigment} + 4\,H_2O$$

The colour intensity is proportional to the LDL-cholesterol concentration when measured at 600 nm.

KIT CONTENTS

Reagent Name	DLDL01040M	DLDL01080M
R1 LDL Reagent	1 x 30 ml	2 x 30 ml
R2 LDL Reagent	1 x 10 ml	2 x 10 ml
R3 - Calibrator	1 vial	1 vial

R3-Calibrator will be provided, the exact concentration will be printed on the label.

WORKING REAGENT PREPARATION AND STABILITY

The reagents are stable up to the kit expiry date printed on the package when stored at 2-8°C. Protect from light.

Calibrator: Reconstitute with required amount of distilled water which will be mentioned in the bottle label. Let it stand for 30 minutes at room temperature. Dissolve the content of the vial by swirling gently to avoid the formation of foam. The reconstituted calibrator is stable only for 7 days at 2-8°C.

CONCENTRATIONS IN THE TEST

Cholesterol Esterase	≥ 800 U/L
Cholesterol Oxidase	≥ 400 U/L
TOOS	
4-aminoantipyrine	≥5000 U/L
peroxidase	≥ 3.5 KU/L
sodium azide	0.05 %
surfactants	1.4 %

WARNINGS AND NOTES

- Product for in vitro diagnostic use only.
- The reagents are ready to use.
- Do not pipette by mouth.
- The reagents contain 0.05% sodium azide as a preservative.
- Avoid contact with skin and mucous membranes.

SPECIMEN

Serum, heparinized or EDTA plasma.
Blood should be collected only if the patient has been fasting for 12 - 14 hours.
Serum and plasma can be stored up to 6 days at 2 - 8°C. Sample are stable for 1 year when stored at for -70°C. Samples may be frozen once. If any samples show precipitates, centrifuge before using. Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Wavelength	578 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Calibrator (C)	Test (T)
R1 LDL Reagent	450 µl	450 µl	450 µl
Distilled Water	5 µl	-	-
R3 - Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix well and incubate for 5 mins at 37° C, than add			
R2 LDL Reagent	150 µl	150 µl	150 µl

Mix well & incubate for 5 min. at 37°C. Measure the absorbance of calibrator & sample against reagent blank.

CALCULATION

$$\text{Concentration in mg/dl} = \frac{\text{Abs.Test}}{\text{Abs.Calibrator}} \times \text{Calibrator Concentration}$$

REFERENCE VALUE

Normal	<130 mg/dl
Border High Value	130 to 159 mg/dl
High Risk Value	>160 mg/dl

It is recommended for each laboratory to establish it own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

Linearity: The procedure is linear upto 400 mg/dl Patient samples with LDL cholesterol levels exceeding 1000 mg/dl should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

Sensitivity / Limit of Quantitation: 2.2 mg/dl

Specificity / Interferences

Bilirubin up to 20 mg/dl, ascorbate up to 62 mg/l, haemoglobin up to 0.5 g/dl, and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	End Point
Wavelength	578 nm
Zero Setting	Reagent Blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	5 min. + 5 min.
Delay Time	-----
Read Time	-----
No. of Reading	-----
Interval Time	-----
Sample Volume	0.005 ml (5 ul)
Reagent Volume	0.6 ml (600 ul)
Standard Concentration	Refer Calibrator vial
Units	mg/dl
Factor	-----
Reaction Slope	Increasing
Linearity	400 mg/dl

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