ANA BIO ISP LDL CHOLESTEROL

(Homogenous direct Method)

For Miura Instruments

Intended Use
LDL Cholesterol is a reagent kit used for the determination of LDL-cholesterol based on enzymatic homogenous method.

Principle
The LDL cholesterol reagent is produced using a combination of detergents and phosphorus compounds which specifically bind HDL, VLDL and CM (chilomicrons) but not LDL. This combination impedes HDL, VLDL and CM from reacting with CO (cholesterol oxidase) and CE (cholesterol esterase), while LDL-cholesterol is able to react with both enzymes.

LDL (Cholesterol esters) + H_2O $\xrightarrow{CE}$ Cholesterol + Fatty Acid

Free cholesterol + O_2 $\xrightarrow{CO}$ $Delta_4$ –cholestenon + H_2O_2

$2H_2O_2$ + 4-AA + HDAOS $\xrightarrow{POD}$ $4H_2O$ + Quinone dye

The compound (Quinone dye) which forms is read at λ 578 nm, develops a color, the intensity of which is proportional to the LDL concentration in the test sample.

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Goods Buffer PH 7.0	20 mmol/L
	HDAOS	1 mmol/L
	Stabilizers, excipients & surface active agents	
Reagnet 2	Goods Buffer	20 mmol/L
	4-AAP	3 mmol/L
	CE	≥ 300 U/L
	COD	≥ 3000 U/L
	POD	≥ 1000 U/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 2° - 8℃ and is stable till the expiry date indicated on the label. **DO NOT FREEZE THE REAGENT.**

Reagent Preparation
Liquid reagents ready for use. After opening the reagents of R1 and R2 are stable for 60 days if closed, stored at 2° - 8℃, and protect from direct light. Do not mix different batches.

Specimen collection and preservation
Serum or heparinized plasma samples should be used. Samples can be stored for 7 days at 4-8 ℃ and 30 days at -20 ℃.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "HDL/LDL Calibrator"

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	LDL Cholesterol
Method Code	LDL
Type	Bichromatic – Substrate start
Unit	mg/dl
Filter F1	578 nm
Filter F2	700 nm
Blank in	Not Used

Step	Reaction Volume	U.M.
Volume reagent R1	150	µl
Sample Volume	2	µl
Incubation R1,S ⟶ R2	300	Sec.
Volume reagent R2	50	µl
Final Incubation	300	Sec.

Normal range
Serum/Plasma.
Men and Women:
- Normal values (no risk): <130 mg/dl (<3.37 mmol/L)
- Borderline (moderate risk): 130-159 mg/dl (3.37 - 4.12 mmol/L)
- High value (high risk): >160 mg/dl (>4.13 mmol/L)

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
Reaction is linear up to 245 mg/dl. If the LDL cholesterol value exceeds 245 mg/dl, then dilute the specimen suitability with normal saline and repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct LDL cholesterol value.

Quality Control
To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of qualitycontrol material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
The recovery of LDL Cholesterol from samples at known concentrations showed an accuracy of 100%.

Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 40 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 500 mg/dl and Ascorbic Acid in concentrations over 100 mg/dl does not cause interference.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	76.7	1.07	0.77	20
High	mg/dl	107.6	0.46	0.71	20
Between run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	75.9	0.91	0.66	60
High	mg/dl	108.2	0.61	0.93	60

Sensitivity
At 578 nm a concentration of 3.45mg/dl of LDL Cholesterol can estimate.

References

1. Butris, CA and Ashwood, E.R (ed), Tietz Fundamentals of Clinical Chemistry , 4th edition, W B Saunders Company, Philadelphia, 1996, pp. 382.
2. Thomas, L. (ed.), Clinical Laboratory Diagnostics; Use and Assessment of Clinical Laboratory Results, 1st edition, TH-Books Verlagsgesellachaft mbH, Franckufurt Main, Germany 1998, pp. 172.
3. Bachorik, Paul S. and Ross, John W., National Cholesterol Education Program Recommendations for Measurement of Low- Density Lipoprotein Cholesterol: Executive Summary Clin Chem. 1995;41;1714-1420.
4. Rifai, N. et al., Measurement. of Low-Density Lipoprotein Cholesterol in Serum: a Status report, Clin Chem. 1992;38.

5. Aufenanger, J. and Zawta, B., pre-analytical Aspects of Lipoprotein Measurement., Clin Lab 1999:45.

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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