LDL CHOLESTEROL (2-8°C)

(PRECIPITANT)

DESCRIPTION	MPRLDL8
LDL Precipitant Reagent	2x60 ml
Cholesterol Standard	5 ml

Intended Use:
For *In Vitro* diagnostic use by trained professionals only.
This reagent is intended for the quantitative determination of LDL Cholesterol in human serum and plasma.

Clinical Significance:
Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and coronary sclerosis in particular. LDLs are derived from Very Low Density Lipoproteins rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The majority of cholesterol stored in atherosclerotic plaques originates from LDL and there is a correlation between serum LDL concentration and incidence of coronary heart disease. Therefore, serum LDL-cholesterol concentration is considered the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Accurate measurement of LDL-cholesterol is of vital importance in monitoring therapies focused on lipid reduction to prevent atherosclerosis or reduce its progression and to avoid plaque rupture.

Test Principle:
Low Density Lipoproteins are precipitated by heparin at their isoelectric pH (pH 5.12). After centrifugation HDL and VLDL remain in the supernatant and can then be determined enzymatically. The LDL cholesterol can be calculated as the difference between supernatant cholesterol and total serum.
Exercise normal precautions required for handling all laboratory reagents.

Reagent Composition

REAGENT	COMPONENT	CONCENTRATION
Precipitant R1	Heparin	0.68 g/l
	Sodium Citrate, stabilizers	0.064 mol/l 2%
Standard	Cholesterol	As given on the vial label

Reagent Preparation and Stability:
R1: Liquid, ready to use
Standard: Liquid, ready to use
R1 and Standard are stable to the stated expiry date when stored unopened at 2 - 8°C
Dispose of reagents carefully in line with local guidelines.

Sample Collection, Preparation and Stability:
Collect serum and Li-heparin or Na-heparin plasma using standard venepuncture techniques. Do not use citrate, oxalate, fluoride or EDTA anticoagulation tubes. Cholesterol is stable in samples up to 7 days at 2 - 8°C or for 3 months at -20°C.
Fasting and non fasting samples can be used. Centrifuge samples containing precipitate before performing the assay.

Assay Procedure:
Cholesterol Reagent (MPRCHO1) is also required for LDL cholesterol determination.
Precipitation Step:
Mix 100 µl of sample or standard with 1000 µl of the precipitant reagent R1. Allow to stand for 10 minutes at room temperature, mix again and centrifuge for 10 mins at 10000 rpm.

Treat the standard supplied in the same way as the samples.
Determine the cholesterol concentration within 1 hour

WAVELENGTH	546nm
TEMPERATURE	37°C
CUVETTE	1cm Path Length
BLANK	Reagent Blank

	Blank	Standard	Sample
Sample	-	-	100 µl
Standard	-	100 µl	-
Reagent	1000 µl	1000 µl	1000 µl
Mix and incubate for 5 minutes at assay temperature. Read the absorbance (Δ Abs) of Sample/Standard against the Reagent Blank.			

Calculation:
Concentration = $\frac{\Delta Abs\ Supernatant\ Sample}{\Delta Abs\ Supernatant\ Standard}$ x Concentration of Standard

Concentration of LDL Cholesterol = Concentration of Total Cholesterol – Concentration of Cholesterol in the supernatant.

Calculation by Factor:
LDL Cholesterol (mg/dl) = 1000 x ΔAbs Sample
Concentration of LDL Cholesterol = Concentration of Total Cholesterol – Concentration of Cholesterol in the supernatant.

Performance Characteristics:
Measuring range:
3 – 800 mg/dl (0.08 – 20.7 mmol/l)
Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with normal saline or distilled water (1+2). Multiply the result by 3.

Analytical Sensitivity: (Lowest detection limit):
3.00 mg/dl (0.08 mmol/l)

Imprecision
Intra-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Level 1	30.13	0.75	2.49
Level 2	113.51	0.75	0.66

Inter-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Level 1	59.4	2.15	3.61
Level 2	84.2	2.64	3.14
Level 3	142.7	4.29	3.00

Method Comparison:
A comparison of the AMS LDL (y) with a commercial assay (x) gave the following comparison:
y = 0.956 x + 8.049, r = 0.956

Reference Range:
Concentration of LDL as an indicator for clinical intervention:
No treatment required: < 150 mg/dl
Indeterminate range: 150 - 190 mg/dl
Treatment required: > 190 mg/dl
For diagnostic purposes the LDL results should always be used in conjunction with the patient's medical history and other clinical findings. Each laboratory should establish its own mean reference range according to the population.

Limitations:
The values obtained are reliable provided:
- There is no presence of chylomicrons in the sample.
- Triglyceride concentration does not exceed 400 mg/dl
- The sample does not show signs of type III hyperlipoproteinemia.
- The absorption of haemoglobin at 546nm simulates elevated LDL values with interference occurring greater than 200 mg Hb/100 ml.
- The supernatant obtained on centrifugation must be clear. If the sample has a high triglyceride content (above 1000 mg/dl) lipoprotein precipitation may be incomplete or part of the precipitate may float on the surface. In these cases, dilute the specimen 1+1 with normal saline and repeat the precipitation step (dilution factor = 2).
- High concentrations of ascorbic acid may result in artificially low values.

The result from this test should not be used as the sole criteria for the diagnosis of lipid disorders, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Interferences:
Criterion: Recovery within +/- 10% of initial value.
Icterus: No significant interference up to 20 mg/dl of Bilirubin.
Haemolysis: No significant interference up to 300 mg/dl of Haemoglobin.
LIpaemia: (Intralipid): No significant interference up to 400 mg/dl of Triglycerides. There is poor correlation between turbidity and triglyceride concentration.

References:
1. Assman G. At what levels of total low or high density lipoprotein cholesterol should diet/drug therapy be initiated? European guidelines. Amer J Cardiol 1990: 65:11F.
2. Hatch FT and Lees RS. Practical methods for plasma lipoprotein analysis. Adv Lipid Res, 1968; 6: 1-68.
3. Glick M. R. Ryder K W, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. ClinChem 1986; 32:470-474

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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