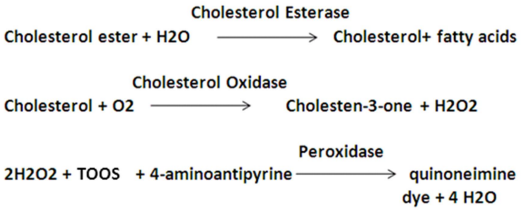
LDL

Direct Enzymatic Method

Intended Use:
LDL Cholesterol test reagent/kit is a medical device intended for the estimation of LDL Cholesterol in serum or plasma.

Clinical Significance
LDL Cholesterol circulates in the blood, it can slowly build up in the inner walls of the arteries that feed the heart and brain. Together with other substances, it can form plaque, a thick, hard deposit that can narrow the arteries and make them less flexible. This condition is known as atherosclerosis. In addition, a clot blocking a narrow artery results in a stroke or heart attack.
Low levels of LDL Cholesterol are not generally a concern and are not monitored. They may be seen in patients with an inherited lipoprotein deficiency and in patients with hyperthyroidism, infection, inflammation and cirrhosis.

Principle



In the first step HDL, VLDL and Chylomicrons are eliminated and transformed to non-reactive components under specific conditions for the reaction. The LDL cholesterol is subjected to color reaction by the second reagent.

Reagent Composition

R1 Enzyme Reagent	CHOD – 200U CE – 100U POD – 1000U Activators and stabilizers
R2 Buffer	Phosphate Buffer
LDL Calibrator	Refer vial label for conc.

Working Reagent Preparation
Reagent is ready to use.
LDL Calibrator Preparation & Stability
For reconstitution refer the Calibrator vial label.
After reconstitution Calibrator stable for 7 days at 2-8ºC

Stability and Storage
Store at 2-8ºC

All the kit contents are stable until the expiry date stated on the label. Do not use reagents beyond the expiration date.Store the vials tightly closed protected from light and prevent contaminations during the use.

Materials required

- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 630nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability

Serum or plasma
On empty stomach serum or heparinized plasma free of hemolysis. EDTA Plasma is acceptable but causes lower results do not freeze the samples. If any sample show precipitation, centrifuge before using.
LDL reported to be stable in serum or plasma for 7days at 2-8ºC.

Assay Procedure

Pipette into clean dry test tubes as follows:

Addition Sequence	Blank	Calibrator	Test
Calibrator	-	5µl	-
Sample	-	-	5µl
R1 Enzyme Reagent	300µl	300µl	300µl
Mix & Incubation for 5 mins at 37°C			
R2 Buffer	200µl	200µl	200µl

Mix well and incubate for 5min at 37°C and then read absorbance of standard (S) and Test (T) against Reagent blank (B) at 630 nm.

Calculation

$$\text{LDL (mg/dl)} = \frac{\text{Abs.of Test}}{\text{Abs.of Calibrator}} \times \text{conc. of Calibrator (Refer vial label)}$$

Quality Control
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity
The procedure is linear up to 500 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Reference Values

Serum, Plasma
Recommended : < 130mg/dl
Moderate Risk : 130 - 159mg/dl
Risk : > 160mg/dl

It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	630 nm
Blank with	Reagent
Sample Volume	5µl
Reagent Volume	R1:300 µl, R2: 200 µl
Calibrator Conc.	Refer vial label
Incub. Temp.	37°C
Incub. Time	5 min.
Normal Range	< 130 mg/dl
Linearity	Up to 500 mg/dl
Unit	mg/dl

Notes

1. No significant interference up to 79mg/dl Bilirubin, 1000mg/dl Haemoglobin and 1500mg/dl Triglycerides.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. Armstron, V. *et al.,* (1985), 31:325-330
2. Bablok, W. *et al.,*. (1988), Clin. Chem., 26:783-790.
3. Bachorik, P.S. *et al.,* (1995), Clin. Chem., 41:1414-1420.

For *in vitro* Diagnostic use only.