CHOLESTEROL TEST KIT

CHOD-POD Enzymatic Method (Colorimetric)

Product Code: 10014 / 11014 / 12014	**Reaction Type:** End Point with Standard
Pack Size: 5x10 ml / 2x50 ml / 5x100 ml	**Matrix Target:** Human Serum & Plasma
Storage Temp: 2-8°C (Enzyme & Standard)	**Wavelength:** 500 nm (Green Filter)

INTENDED USE & CLINICAL SIGNIFICANCE

Intended Use: This liquid diagnostic reagent system is configured for the quantitative in vitro determination of Total Cholesterol and High-Density Lipoprotein (HDL) Cholesterol fractions in human fasting serum or plasma specimens.

Clinical Significance: Cholesterol is a vital precursor to bile acids, steroid hormones, and Vitamin D. Its measurement serves as an essential tool in diagnosing and classifying lipid disorders and hyperlipemia. High serum total cholesterol is recognized as a major independent risk factor for coronary heart disease. Clinical decisions should integrate holistic laboratory arrays rather than single tests.

METHOD PRINCIPLE

Cholesterol esters are enzymatically hydrolyzed by Cholesterol Esterase (CE) to free cholesterol and fatty acids. Free cholesterol is then oxidized by Cholesterol Oxidase (CHOD) to cholest-4-en-3-one and hydrogen peroxide (H_2O_2). In the presence of Peroxidase (POD), H_2O_2 couples with phenol and 4-Aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye:

$$\text{Cholesterol Esters} + H_2O \rightarrow \textit{[CE]} \rightarrow \text{Cholesterol} + \text{Free Fatty Acids}$$

$$\text{Cholesterol} + O_2 \rightarrow \textit{[CHOD]} \rightarrow \text{Cholest-4-ene-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{4-AAP} \rightarrow \textit{[POD]} \rightarrow \text{Quinoneimine Dye (Red)} + 4\ H_2O$$

TOTAL CHOLESTEROL ASSAY PROCEDURE

Reagent/Component Line	Blank	Std.	Test
R1 - Enzyme Reagent	1000 µl	1000 µl	1000 µl
Cholesterol Standard (200 mg%)	—	10 µl	—
Distilled Water	10 µl	—	—
Patient Serum / Plasma (Fasting)	—	—	10 µl

Operational Directive: Mix well and incubate at 37°C for exactly 5 minutes. Evaluate the absolute optical density of the Standard (S) and Test (T) against the Reagent Blank (B) at 500 nm.

> Serum Total Cholesterol (mg%) = ***(Abs. of Test / Abs. of Std.) × 200***

HDL CHOLESTEROL ASSAY PROCEDURE

STEP 1 (Precipitation): Pipette 200 µl of fasting serum into a clean test tube, add 300 µl of **R2-HDL PPT Reagent**, mix thoroughly, allow to stand at RT for 10 minutes, and centrifuge at 3000 RPM for exactly 10 minutes to separate non-HDL structures.

STEP 2 (Quantification): Pipette the supernatant into clean dry test tubes according to the matrix:

R1 - Enzyme Reagent	1000 µl (Blank)	1000 µl (Std.)	1000 µl (Test)
Cholesterol Standard (200 mg%)	—	10 µl	—
Distilled Water	100 µl	—	—
Separated Supernatant (From Step 1)	—	—	100 µl

Mix well, incubate at 37°C for 5 minutes, and evaluate the absorbance against the blank at 500 nm.

> HDL Cholesterol (mg%) = ***(Abs. of Test / Abs. of Std.) × 50***

TECHNICAL PARAMETERS & CLINICAL SUPPORT MATRIX

Universal Safeguards	Professional use only. Avoid detergents for cleaning glassware; trace surfactant impurities heavily degrade final color development. Discard reagents upon structural turbidity. Slight pink coloration (up to 0.15 Abs) does not alter baseline analytical performance. Contains sodium azide.
Expected Range	Total Cholesterol: up to 250 mg% | HDL Cholesterol: 30–60 mg% (Males) / 35–75 mg% (Females).
Analytical Linearity	Linear up to 700 mg%. If values exceed this parameter, dilute with saline, re-assay, and multiply by the dilution factor.

Manufactured by: M/s. SAWIN BIOMEDICALS PVT. LTD.
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