ESTROM HDL Cholesterol
(PTA Method)

ANGSTROM BIOTECH PVT. LTD

This reagent kit is for in-vitro use to measure the concentration of high density lipoprotein cholesterol in serum or plasma.

PRINCIPLE:
The chylomicrons, VLDL and LDL are precipitated by addition of phosphotungstic acid and magnesium chloride. After centrifugation, high density lipoproteins fraction recovered as clear supernatant its cholesterol content is estimated by enzymatic method.

SERUM + PHOSPHOTUNGSTIC ACID

⟶ SUPERNATANT (HDL) + PRECIPITATES (OTHER FRACTIONS)

CLINICAL SIGNIFICANCE:
HDL, VLDL, LDL are responsible for transport of cholesterol from liver to various tissue cells. Lower values of HDL Cholesterol and increased ratio of total cholesterol to HDL Cholesterol can be related to risk factor for coronary heart disease and complications like hypertension. Excess of cholesterol is brought back by HDL to liver where it is converted to bile salts or excreted in bile.

PRECAUTION:
Reagent is for in-vitro diagnostic use only.

SPECIMEN COLLECTION AND STORAGE:
Fresh, fasting, unhemolysed serum is preferred. Plasma collected with heparin or EDTA as anti coagulant may be used. Samples are stable for 2 days when stored at 2-8°C.

REAGENTS:

1. Reagent 3	PPT Reagent	1
2. Standard	50 mg/dl	1

REAGENT PREPARATION, STABILITY AND STORAGE:
All the reagents are stable up to expiry date indicated on the bottle label.
Ready to use reagents.

GENERAL INSTRUMENT PARAMETERS:

Reaction Type	:	End Point
Slope of reaction	:	Increasing
Wavelength	:	505 nm (490-530 nm)
Flowcell Temperature	:	37°C
Reagent Volume	:	1000 µl (1.0 ml)
Sample Volume (Supernatant)	:	50 µl
Standard Concentration	:	50 mg/dl
Dilution Factor	:	2
Units	:	mg/dl
Incubation	:	5 minutes
Zero Setting	:	Reagent Blank
Path Length	:	1 cm

PROCEDURE:

Dispense into Centrifuge tube	Test
Sample	200 µl
Precipitating Reagent (Reagent 3)	200 µl

Mix well. Centrifuge for 10 minutes at 3000 rpm. Separate clear supernatant and determine HDL Cholesterol as given below.

Dispense into test tube	Blank	Std.	Test
Cholesterol Reagent	1000 µl	1000 µl	1000 µl
Standard	-	50 µl	-
Sample (Supernatant)	-	-	50 µl
Mix well. Incubate for 5 minutes at 37°C. Read absorbance at 505 nm (490-530 nm) or against green filter.			

The color of the reaction mixture is stable for 30 minutes when stored at room temperature, protected from light.

CALCULATION:
Concentration of HDL Cholesterol in sample (mg/dl)

$$= \frac{\text{Abs. of test}}{\text{Abs. of Std.}} \times \text{Conc. of Std.} \times 2$$

Factor 2 is applied to account for the dilution of the sample in the precipitation procedure

EXPECTED VALUES:

		Normal level	Risk Indicator
Men	:	30 – 55 mg/dl	Less than 30 mg/dl
Women	:	45 – 65 mg/dl	Less than 45 mg/dl

* It is recommended that each laboratory establishes its own normal range.

The values of LDL Cholesterol can be calculated if the value of triglyceride is known by using Friedewald's equation:

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \{(\text{HDL-Cholesterol}) + \frac{\text{Triglycerides}}{5}\}$$

The equation is valid only if triglyceride values are normal; or not very high.

BIBLIOGRAPHY:

- Friedewald W.T., et al. Clin. Chem 18, 499 (1972)
- Burstein M. Scholnick H.P. and Morfin, R (1970) Cholesterol in high density lipoprotein using Mg++/PTA; J. Lipid Res. 19.583.

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⚠	Attention,see instructions for use		Consult Instructions For Use
IVD	For in vitro diagnostic use only	REF	Catalog #
2°C 8°C	Store between 2-8°C	LOT	Lot Number
	Do not use if package is damaged		Date of Manufacturing
	Manufacturer		Use by