

PRINCIPLE:

Chylomicrons(CM), LDL Cholesterol and VLDL Cholesterol only from serum are precipitated with phosphotungstate reagent in presence magnesium ions while HDL cholesterol remains unaffected in the supernatant, which is estimated using total cholesterol reagent. The colour formation of supernatant with cholesterol reagent is directly proportional to the HDL cholesterol present in the sample.

serum+Phosphotungstate reagent-----HDL in supernatant + ppt.
(CM+LDL+VLDL)

KIT CONTENTS:

Pack Size :	1 x 50ml	1 x 100ml
R₁-HDL Chol.ppt. Regt.	1 x 50ml	1 x 100ml
S₁-HDL Chol. Standard (50 mg/dl)	1 x 01 ml	1 x 01ml

STORAGE & STABILITY:

All the reagents are stable at 2-8°C. upto the expiry date mentioned on the label.

SAMPLES:

Serum or plasma (Free from haemolysis.)

REFERENCE VALUES:

Serum / plasma - 45 - 60 mg / dl.
[It is recommended that each laboratory should establish its own normal range]

REAGENT PREPARATION:

All reagents are ready to use.

ASSAY PROCEDURE:

STEP 01: PRECIPITATION

R₁-HDL Cholesterol reagent: 100ul.
Serum : 100ul.

Mix well & stand for precipitation for 02 minutes & centrifuge for 05 minutes @ 3000 RPM to get clear supernatant.

STEP 02:

Wavelength / Filter : 505 nm (492 - 550 nm)
Temperature : 37°C
Cuvette : 1 cm light path.

	B	S	T
R₁-Total Chol. reagent (From Cholesterol Kit)	1000ul	1000ul	1000ul
S₁-HDL Chol. Standard(50mg/dl)	-----	50ul	-----
Supernatant from step 1st	-----	-----	50ul

Mix well and incubate at 37°C for exactly 05min. and measure the absorbance of the Standard (Abs.S) and Test Sample (Abs.T) against the reagent blank (Abs.B).

CALCULATION:

$$\text{HDL Cholesterol in mg/dl} = \frac{(\text{Abs.T}) - (\text{Abs.B})}{(\text{Abs.S}) - (\text{Abs.B})} \times 50 \times 2$$

Where, 2 = dilution factor for serum.

LINEARITY:

The reagent is linear upto 125 mg / dl.
If values exceed this limit, dilute the sample with DI water & repeat the assay. Multiply the result with proper dilution factor.

ASSAY PARAMETERS:

- Mode of reaction : End point
- Slope of reaction : Increasing
- Wavelength : 505 nm (492 - 550 nm)
- Temperature : 37°C
- Blanking : Reagent blank
- Standard Conc. : 50 mg/dl
- Reaction time : 07 min + 5 min
- Reagent Volume : 1000 ul
- Sample Volume : 50 ul
- Linearity : 125 mg/dl
- Low Linearity : 0 mg/dl

REFERENCES:

Trinder, P., (1969) Ann.Clin. Biochem. 6 : 24.
Allain C.C and al., Clin. Chem., 20, (1974), 470.

Mfg. By: BIOGENIX INC. PVT. LTD.

Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India

Email: biogenix2007@yahoo.com, info@biogenixinc.com

Website: www.biogenixinc.com

Customer care no: +919140971443

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