HDL CHOLESTEROL (PPT) TEST KIT

INTENDED USE : HDL - CHOLESTEROL Precipitating Reagent is for use in cojunction with DELTA CHOLESTEROL Reagent for **"in vitro"** quantitative determination of High Density Lipoprotein Cholesterol (HDL-C) in serum & plasma.

CLINICAL SIGNIFICANCE : Determination of the concentration of High Density Lipoprotein (HDL) cholesterol plays an important role in examination of lipid metabolism. Increases level are found in cases of chronic hepatitis and intoxication, respectively. Decreased HDL cholesterol levels are associated with increased risk of atherosclerotic diseases of blood vessels.

PRINCIPLE : Low density fractions (LDL, VLDL) of iporoteids of the serum are precipitated with a mixture of phosphotungstic acid and mangnesium chloride solutions and removed by centrifugation. Concentration of high density lipoproteids (HDL) in the clear supernatant can be measured. The reagent used for the determination is identical with that applied for assay of total cholesterol.

REAGENT COMPOSITION :
Reagent 1 : HDL - CHOLESTEROL Precipitating Reagent
Standard : 50 mg/dl

SAMPLES : Serum or heparinized plasma, free of hemolysis, removed from the blood clot as soon as possible.

WORKING REAGENT PREPARATION & STABILITY :
HDL - CHOLESTEROL Precipitating Reagent and standard are Ready to use & stable up to the expiry date stated on the lable when stored tightly closed at 2^{o} to $8^{o}C$.

PROCEDURE :

HDL SEPARATION :

Serum / Plasma	**500 µl**
HDL-Precipitating Reagent	**500 µl**

Mix thoroughly and leave for 10 min. at room temp. and then centrifuge at 4000 R.P.M. for 10 minutes in a laboratory centrifuge. After centrifugation separate supernatant solution & use for HDL - Cholesterol estimation by using Cholesterol Reagent.

ASSAY PROCEDURE :

	Blank	Standard	Sample
Cholesterol Reagent	**1000 µl**	**1000 µl**	**1000 µl**
Standard	**-**	**50 ml**	**-**
Supernatant	**-**	**-**	**50 ml**

Mix and read the optical density (A) after a 10 - Minute incubation at $37^{o}C$, against Blank.

GENERAL SYSTEM PARAMETERS :

Reaction Type	**End Point**
Wavelength	**510 nm**
Light Path	**1cm**
Reaction Temperature	**37°C**
Blank / Zero Setting	**With Cholesterol Reagent**
Reagent Volume	**1000 µl**
Supernatant Volume	**50 µl**
Incubation Time	**10 Minutes**
Standard Concentration	**50 mg/dl**
Linearity	**200 mg/dl**

CALCULATION :

$$\text{HDL Cholesterol Conc. (mg/dl)} = \frac{\text{OD of Sample}}{\text{OD of Calibrator}} \times 50 \times 2 \text{ mg/dl}$$

LINEARITY : Reagent is linear up to 200 mg/dl.
Dilute the sample appropriately and re-assay if HDL-C concentration exceeds 200 mg/dl.

REFERENCE NORMAL VALUE :
Male : > 55 mg/dl
Female : > 65 mg/dl
It is recommended that each laboratory should assign its own normal range.

QUALITY CONTROL : For accuracy it is necessary to run known controls with every assay.

SENSITIVITY / LIMIT DETECTION : The Lower Limit of detection is 10 mg/dl.

LIMITATION & PRECAUTIONS :
- Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- Before the assay bring all the reagents to room temperature.
- Avoid contamination of the reagent during assay process.
- Use clean glassware free from dust or debris.

BIBLIOGRAPHY :

1) Burstein M., Selvenick H. R. : Lipid Res. 11, 583 (1970).
2) Lopes Virella M. : Clin. Chem. 23, 882 (1977)
3) Friedewald W. T. : Clin. Chem. 14, 449 (1972).

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