HDL CHOLESTEROL - PPT

METHOD – PRECIPITATING (PHOSPHOTUNGSTIC ACID)
PRODUCT CODE – LH01

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for estimation of HDL Cholesterol in serum / plasma using Phosphotungstic Acid method.

SUMMARY AND PRINCIPLE

The determination of serum HDL Cholesterol is a useful tool in identifying patients at risk of developing coronary heart disease. Low HDL Cholesterol levels are associated with an increased risk of coronary artery disease. HDL Cholesterol precipitating reagent is for use in conjunction with Cholesterol reagent for enzymatic determination of HDL Cholesterol in serum or plasma. Compared to the conventional Ultra centrifugation method, the precipitation method is simple and time saving particularly when combined with single step enzymatic Cholesterol reagent. Phosphotungstate/Mg^{2+} precipitates Chylomicrons, LDL and VLDL fractions. High density lipoprotein (HDL) fraction remains unaffected in supernatant. Cholesterol content of HDL fraction is assayed using Cholesterol reagent.

KIT COMPONENTS

Reagent 1: HDL Cholesterol Precipitating Reagent
Reagent 2: HDL Cholesterol Standard (50 mg/dL)

REAGENT PREPARATION, STORAGE & STABILITY

HDL-Cholesterol Precipitating Reagent is ready to use as supplied. The kit should be stored at 2-8 °C and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDELING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	HDL Chol PPT
Reaction Type	End Point
Primary Wavelength	546 nm
Flow Cell Temp.	37 °C
Blank setting	Reagent
Blank abs. limit	< 0.300

Reagent Volume	1000 µl
Sample Volume	50 µl
Incubation Time	5 mins
Incubation Temperature	37 °C
Standard Conc.	50 mg/dL
Linearity	400 mg/dL

MATERIALS REQUIRED BUT NOT PROVIDED

Cholesterol kit, Test tubes, Micropipette with tips, Analyzer, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Fasting blood is preferred for HDL Cholesterol assay. Serum or Heparinised / EDTA plasma can be used. HDL-Cholesterol in the serum is stable for 24 hrs when stored at 2-8 °C and 30 days when stored at -20 °C.

COMPONENTS OF REAGENT

Component	Concentration
Phosphotungstic Acid	2.4 mmol/l
Magnesium chloride	40 mmol/l
Stabilizers, inactive ingredients and surface-active agents.	-

ASSAY PROCEDURE

	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Standard		50 µl	
Sample (supernatant)			50 µl
Mix the reagent and sample/standard in the above-mentioned ratio and incubate for 5 mins at 37 °C or 10 mins at R.T.			
Aspirate reaction mixture into flow cell and measure the absorbance.			
The final colour is stable for 2 hours if not exposed to light.			

CALCULATION

$$\text{HDL Cholesterol (mg/dL)} = \frac{\text{Abs. of sample} \times 100^*}{\text{Abs. of standard}}$$

100* = 50x2 (For serum dilution HDL separation).

REFERENCE VALUES FOR NORMAL PEOPLE

HDL Cholesterol: 30 to 70 mg/dL. (<30 mg/dL indicates cardiac risk)

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 3.5 – 400 mg/dL. If the HDL Cholesterol value exceeds linearity limit (above 400 mg/dL), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct HDL Cholesterol value of the specimen.

Interference: There is no significant interference in samples containing Bilirubin upto 20 mg/dL, Ascorbic Acid upto 4 mg/dL and Haemoglobin upto 400 mg/dL.

Precision: Precision studies has been carried out using quality control sera as shown below:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (mg/dL)	SD (mg/dL)	CV %	Mean (mg/dL)	SD (mg/dL)	CV %
Low Value Serum	28.8	0.65	2.2	26.22	0.71	2.7
High Value Serum	70.9	0.73	1.0	65.02	0.72	1.1

Note: We recommend all the laboratories to establish its own accuracy and precision data.

QUALITY CONTROL

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for HDL Cholesterol. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

Discard the working reagent if its absorbance exceeds 0.300 at 546 nm against distilled water. The standard is a viscous solution. Use broad mouth pipette for accurate pipetting.

BIBLIOGRAPHY

1. Lang,P.D. ,and G. schettler, (1985), in Schettler, G and R.Gross Arteriosklerose, Grundlageon-Diagnostik-Therapie.Deutschar Arzte Verlag GmbH, Cologne/W. Germany.

2. Assmann, G. (1979). Lipiddiagnostik heute. Page 29ff, in Greten H, *et al.* Lipoprotein and Herzinfarkt. Witzstrock-Verlag, Baden/W.Germany.

3. NIH Consensus conference (1984),JAMA 251:1196

Symbol	Explanation	Symbol	Explanation
	Manufactured By	IVD	In Vitro Diagnostic Use
LOT	Lot Number		Read Instructions Before Use
REF	Catalogue Number		Storage Temperature
	Manufacturing Date	Σ	Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

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ISO 9001: 2015
ISO 13485 : 2016
GMP
CE