HDL-CHOLESTEROL

(PTA, Mg++/CHOD-PAP)

ENZOPAK

Last update 09-2020

Ref. CC2-HDC.013, 2x25 ml

INTENDED USE

Reagent kit for quantitative estimation of high density lipoprotein (HDL) cholesterol in serum or plasma.

PRODUCT HIGHLIGHTS

- Liquid Stable Reagents.
- Two reagent (Enzyme+Buffer) with long (6 months) stability.
- Pack sizes offered suitable to every laboratory.
- Colorimetric procedure with economy.
- Stabilized aqueous base standard.
- Low blank all throughout reagent life.

HDL-PPT Internationally accepted PTA reagent giving results parallel to ultracentrifuge method.

INTRODUCTION

The common classification of lipoproteins (a) high density (HDL) (b) low density (LDL) (c) very low density (VLDL) comes mainly from ultracentrifugation of serum or plasma. As the word indicates, it is based on the density of lipoproteins. Chylomicrons are formed from lipid and protein associations giving opalescent appearance to the plasma. These are even lighter than very low density lipoproteins.

High density lipoproteins (specific gravity more than 1.063) can be separated by using polyionic substances along with bivalent metal ion. Cholesterol distribution in the different fractions of lipoproteins i.e. HDL, LDL, VLDL and chylomicrons is of particular interest in understanding the metabolic status and risk of various diseases like artherosclerosis, coronary heart disease (CHD), etc.

DIAGNOSTIC SIGNIFICANCE

Cholesterol from liver is transported to various tissue cells by low density lipoproteins, very low density lipoproteins and chylomicrons through blood (plasma) circulation. Cholesterol is also synthesized by the cells as needed. The excess cholesterol is transported back to liver by HDL. HDL-Cholesterol level, therefore indicates excess cholesterol received by liver, where it is converted to bile salts and excreted in bile. Elevated levels of HDL-Cholesterol suggest a balanced status of cholesterol metabolism in tissues. Lower levels of HDL cholesterol are associated with higher risks of artherosclerosis (i.e. deposition of cholesterol in cells of blood vessels) and complications like hypertension, coronary heart disease (CHD) related to it.

PRINCIPLE

High density lipoproteins (HDL) are separated from other lipoprotein fractions by treating serum with phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated,

$$\text{Serum + PTA Reagent} \longrightarrow \begin{matrix}\text{Supernatant (HDL)} \\ + \\ \text{Precipitates (other fractions)}\end{matrix}$$

HDL-Cholesterol (Supernatant) content of which is estimated by enzymatic method as bellow,

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{CO} \text{Cholesten-4-en-3-one+} H_2O_2$$

$$\left.\begin{matrix}H_2O_2 + 4AAP \\ + \\ \text{Phenolic Compound}\end{matrix}\right\} \xrightarrow{\text{Peroxidase}} \left\{\begin{matrix}\text{Quinoneimine dye} \\ + \\ 4H_2O\end{matrix}\right.$$

The intensity of colour produced is directly proportional to the concentration of HDL cholesterol in the sample.

PRESENTATION

All reagents to be stored at 2-8^{0}C.	NO. of bottles 2 x 25 ml
• 1 HDL-Cholesterol (Enzymes-Chromogen Buffer)	2
• 2 HDL-Cholesterol (Diluent)	2
• 3 HDL - Cholesterol (Precipitating Reagent) Ready for use	1
• HDL - Cholesterol Standard. (50 mg/dl)	1

FINAL REAGENT COMPOSITION

ACTIVE INGREDIENTS	Concentration
• Cholesterol Oxidase	≥500 U/L
• Cholesterol Esterase	≥600 U/L
• Peroxidase	≥6000 U/L
• 4- Amino Antipyrine	0.5 mmol/L
• Buffer	100 mmol/L
• Phosphotungstic Acid	2 mmol/L
• pH 7.00 ± 0.5 at 25^{0}C	

HDL -Cholesterol Standard (50 mg/dl)
Also contains non-reactive fillers & stabilizers.

PRECAUTION

HDL – Cholesterol is for *IN -VITRO* diagnostic use only.
Reagent contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT

Transfer contents of one vial of 1 HDL-Cholesterol to the bottle containing 2 HDL - Cholesterol. Mix, dissolve the contents, wait for 10 minutes and use as given in the procedure.

REAGENT STORAGE AND STABILITY

All the reagents included in the kit are stable at 2-8°C until the expiry date stated on the label.
The working reagent is stable for 180 days at 2-8°C.

SPECIMEN COLLECTION

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice. However, plasma collected using heparin as an anticoagulant may also be used.

SEPARATION OF HDL - FRACTION

Pipette into Test Tubes	TEST
Sample (ml)	0.2
3-HDL-Cholesterol (ml)	0.2

Mix well and centrifuge at 3500-4000 rpm for ten minutes.
Separate the clear supernatant immediately and determine cholesterol content as follows,

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REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 505nm
- Flowcell temperature : 37 ^{0}C
- Sample Volume : 50 µl (0.05 ml.)
- Reagent Volume : 1.0 ml
- Incubation Time : 10 min. at 37^{0}C
- Factor (Std. Conc.x2) : (50x2) = 100 mg/dl
- Light Path : 1.0 cm
- Zero setting with : Reagent Blank

TEST PROCEDURE

Pipette into Test Tubes	BLANK	STANDARD	TEST
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.05	-
Supernatant (ml)	-	-	0.05

Mix well and incubate for ten minutes at 37^{o}C and read absorbance of test and standard against reagent blank at 505nm (500-540 Green filter).

TEST RESULTS

$$\text{HDL-Cholesterol} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{Conc. of std. x dilu. factor}$$

$$= \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times 50 \times 2$$

NORMAL VALUES

	Men	Women
Normal Level (mg/dl)	30-55	45-65
Risk Indicator (mg/dl)	Less than 30	Less than 45

The values of LDL-Cholesterol can be calculated, if the value of triglycerides is known by using Friedewald's equation.

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

The formula is valid only if Triglyceride values are normal or not very high.

LINEARITY

This procedure is linear upto 250 mg/dl. For sample values higher than 250 mg/dl, dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

NOTE

Do not subject HDL - Cholesterol Standard (50 mg/dl) provided in the kit to separation procedure of HDL.

REFERENCES

1. Burstein M. Scholnick, H.P. and Mortin, R Cholesterol in High Density lipoprotein : Using Mg^{++} /PTA; J. Lipid Res. 19. Pg. 583 (1970).

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