



HDL - CHOLESTEROL KIT

PRINCIPLE:

The chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition of magnesium chloride. After centrifugation the supernatant fluid contains the HDL-fraction (high density lipoproteins), which is assayed for HDL Cholesterol using reagent supplied with Cholesterol kit.

REAGENT PREPARATION STABILITY:

1. HDL Reagent : Ready - To - Use
2. HDL Cholesterol Standard : Ready - To - Use

Avoid Contamination of Ready - To - Use Reagents. Always use fresh pipette tips. Keep always the caps tightly closed.

The above Reagent is Ready - To - Use and is stable until expiry dates mentioned on the label.

COLLECTION AND HANDLING OF SPECIMEN:

1. Serum, Heparinized or EDTA Plasma can be used as a sample. Avoid hemolysis.
2. Serum must be separated from the blood clot as rapidly as possible.

PROCEDURE:

ASSAY PARAMETERS :

Mode	: End point.
Wave Length	: 505
Temperature	: 37 ^o C.
Cuvette	: 1cm path length.
Reaction Slope	: Increasing.
Incubation	: 10 mins.
Blank	: Reagent Blank.
Reagent Volume	: 1000 ul.
Sample Volume	: 100 ul.
Standard Concentration:	25 mg/dl.
Linearity	: 200 mg/dl.
Units	: mg\dl.

Step 1.

Pipette into a centrifuge tube	Sample
Serum	200 ul
HDL reagent	200 ul
Mix well, leave to stand for 10 mins. at + 15 to 25 ^o C and centrifuge for 15 mins. at approx. 4000 rpm. Determine the cholesterol concentration of the supernatant within 1 hour after centrifugation.	

Step 2:

Pipette into cuvettes	Blank	Sample/Standard
Redistilled water	100 ul	-
Sample/Standard (supernatent)	-	100 ul
Cholesterol reagent (from Cholesterol kit)	1000 ul	1000 ul
Mix & Incubate Reagent Blank Std. and sample for 10 mins. at 20 ^o C (or) 5 min. at 37 ^o C, then measure absorbance of sample against Reagent Blank within 1 hour		

CALCULATION:

$$\text{HDL-Cholesterol Concentration} - \frac{\text{A. SAMPLE}}{\text{A. STANDARD}} \times 25 \times 2$$

(Standard Concentration)

LDL CHOLESTEROL: The following scheme is based on the friedewald formula which is reliable only if chylomicrons are absent in the sample and the Triglycerides concentration is below 400 mg/dl and the sample shows no sign of type III Hyperlipoproteinemia.

$$\text{LDL CHOLESTEROL (mg / dl)} = \text{TOTAL CHOLESTEROL} - (\text{HDL CHOLESTEROL} + \text{TGL}/5)$$

EXPECTED VALUES: HDL CHOLESTEROL

	MEN	WOMEN
Prognostically favourable	>55 mg/dl	>65 mg/dl
Standard risk level	35 - 55 mg/dl	45 - 65
Risk Indicator	<35 mg/dl	<45 mg/dl

REFERENCES:

1. burstein, M. Etal. (1970). J. Lipid Res. 11:583.
2. Lopes-Virella, M:F., Et Al. (1977), Clin. Chem. 23:882.
3. Fruchart J.C.: Rev. Fr. Des Laboratories 103 (1982) 7.
4. Warnick, G.R., et al: J. Lipid Res. 19, 65-76 9 (1978)