

# HDL - CHOLESTEROL PRECIPITATING REAGENT

## ORDER INFORMATION

CODE : DL6101 - 1 x 10ML  
DL6102 - 2 x 25ML

### INTENDED USE :

HDL - CHOLESTEROL Precipitating Reagent is for use in conjunction 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. Increased levels are found in cases of chronic hepatitis and intoxications, respectively. Decreased HDL-cholesterol levels are associated with increased risk of atherosclerotic diseases of blood vessels.

### PRINCIPLE :

Low density fractions (LDL, VLDL) of lipoproteids of the serum are precipitated with a mixture of phosphotungstic acid and magnesium 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  
CHOLESTEROL Standard : 200 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 & standard are Ready to use and are stable up to the expiry date stated on the label when stored tightly closed at 2° to 8°C.

### PROCEDURE :

#### HDL SEPARATION:

Bring all the reagents to room temperature.  
Pipette as follows:

Serum / Plasma	0.5 ml
HDL - Precipitating Reagent	0.5 ml

Mix thoroughly & centrifuge at 4000 R.P.M. For 10 minutes in a laboratory centrifuge. To obtain a clear supernatant.

#### HDL Cholesterol Determination:

### GENERAL SYSTEM PARAMETERS :

Reaction type	End Point (Increasing)
Wave length	505 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Cholesterol Reagent

Reagent Volume	1ml
Supernatant Volume	10 µl
Incubation Time	5 Minutes
Standard Concentration	200 mg/dl (See Calculation)
Linearity	400 mg/dl

### ASSAY PROCEDURE :

	Blank	Standard	Sample
Reagent	1 ml	1ml	1ml
Standard	-	10 µl	-
SUPERNATANT	-	-	10 µl

Mix and read the optical density (A) after a 5 - minute incubation at 37°C.

### CALCULATION :

$$\text{HDL Cholesterol Conc. (mg/dl)} = \frac{\text{OD of Sample}}{\text{OD of Standard}} \times 400 *$$

\* Use Standard concentration of 400 (not 200) for calculation due to serum dilution during precipitation step.

### LINEARITY :

Reagent is Linear up to 400 mg/dl  
Dilute the sample appropriately and re-assay if HDL-C Concentration exceeds 400 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.

### LIMITATION & PRECAUTIONS :

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

### BIBLIOGRAPHY :

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



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