

# LiquiMAX HDL-Cholesterol

(Phosphotungstate Precipitation Method)

## ORDERING INFORMATION

Ref. No.	Pack Size
AVHDL1 - 50	2x25 ml
AVHDL1 -100	4x25 ml

## INTENDED USE:

Kit is use for the quantitative determination of HDL cholesterol in human serum and plasma by precipitation method

## PRODUCT FEATURES

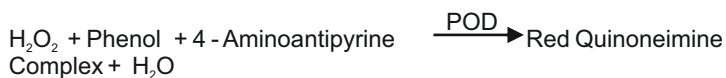
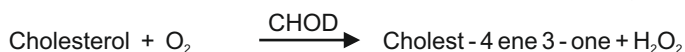
- To be used with LiquiMAX Cholesterol-SLR reagent kit. (To be purchased separately)
- Precipitation reagent is provided in the kit.
- Aqueous Standard provided (Standard Conc: 50 mg/dl)
- Linearity: 150 mg/dl
- Precipitation of Serum sample required (Read the insert carefully)
- Measuring Wavelength 505 nm (490 - 550 nm) at 37°C.
- Serum or Heparinized/EDTA Plasma are to be used as specimens.

## CLINICAL SIGNIFICANCE :

HDL (High Density Lipoproteins) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL- cholesterol in serum is of clin olesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL- cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk. A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, and precipitationbases methods. Of these precipitation-based methods are used routinely. HDL cholesterol is first separated by precipitating apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride. Such precipitation –bases method are, however, time consuming and not amenable to automated analysis. Thus, there is a great clinical need for a convenient and reliable method for measuring HDL-cholesterol in serum without any pretreatment. Several approaches for direct measurement of HDLcholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanionmetal combinations and the use of polyethylene glycol (PEG) with antiapoprotein B and antiapoprotein CIII antibodies.

## PRINCIPLE

The chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition phosphotungstec acid and magnesium chloride. HDL fraction remains in the supernatant. After centrifugation the supernatant fluid contains the HDL (high density lipoproteins)-fraction and their cholesterol content is determined enzymatically by the use of standard Total Cholesterol Reagents.



## STORAGE & STABILITY:

All the reagents should be stored at 2-8°C and are stable till the expiry date mentioned on the labels.

## KIT COMPONENTS

- Cholesterol Reagent (PPT)
- Cholesterol Standard : Concentration as stated on the label

## COMPOSITION

Phosphotungstic acid	0.60 mmol/l
Magnesium chloride	25 mmol/l

## REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

## MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

## REAGENT DETERIORATION

Discard any turbid reagent or blank reagent absorbance exceeds 0.8 at 505 nm against distilled water.

## WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

## SPECIMEN COLLECTION AND STORAGE

Serum / Heparinised or EDTA Plasma.

## SYSTEM PARAMETERS:

Reaction Type (Mode)	:	End Point
Units	:	mg/dl
Wave Length	:	505 nm (490-550)
Blanking with	:	Reagent
Flow Cell Temp.	:	37°C
Low Normal	:	30 (Male)
High Normal	:	70 (Male)
Supernatant Volume	:	100 µl
Reagent Volume	:	1000 µl
Linearity	:	150
Standard Conc.	:	50 (Set 125)

## TEST PROCEDURE

### Step-1:

Pipette into a centrifuge tube:

Serum / Plasma	0.2 ml
Precipitating Reagent	0.3 ml

Mix well and allow to stand at RT for 5 minutes. Centrifuge at 3000 rpm for 10 minutes to get a clear supernatant. If the supernatant is not clear (High TGL Level) dilute the sample 1:1 with normal saline and multiply the result with 2.

### Step-2:

Pipette into 3 test tubes labeled Blank (B), Standard(S) Test (T) as shown below:

Reagent	B	S	T
LiquiMAX Cholesterol Reagent	1.0 ml	1.0 ml	1.0 ml
HDL Cholesterol Standard (Conc. 50 mg/dl)	–	50µl	–
Supernatant (from Step 1)	–	–	50µl
Distilled Water	50µl	–	–

Mix well and incubate for 5 minutes at 37°C or 10 minutes at R.T. Read the absorbance of Standard (S), Test (T) against Reagent Blank (B) at 505 nm (490-550 nm).

#### CALCULATIONS

$$\text{HDL Cholesterol (in mg/dl)} = \frac{\text{Abs of (T)}}{\text{Abs. Of S}} \times 50 \times 2.5$$

#### EXPECTED RANGE:

HDL Cholesterol: Male: 30 - 70 mg/dl  
Female: 35 - 90 mg/dl

It is recommended that laboratories establish their own normal range.

#### QUALITY CONTROL & CALIBRATION

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality Control material checks both, the instrument and the reagent functions.

#### PERFORMANCE CHARACTERISTICS

##### 1. Linearity

Linearity : 150 mg/dl

##### 2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 3 mg/dl

##### 3. Interferences

No significant interference was observed from Bilirubin up to 20 mg/dl, Hemoglobin up to 450 mg/dl and Triglycerides up to 1250 mg/dl.

##### 4. Precision

Reproducibility was determined using controls. The following results were obtained:

##### Intra-Assay

Sample	Mean (mg/dl)	SD (mg/dl)	% CV
Control 1	45.1	1.35	1.32
Control 2	60.2	0.98	0.62
Control 3	120.5	0.99	0.55

##### Inter-Assay

Sample	Mean (mg/dl)	SD (mg/dl)	% CV
Control 1	45.2	1.71	1.38
Control 2	60.3	3.13	1.93
Control 3	120.8	1.97	1.06

##### 5. Method Comparison:

A comparison of the LiquiMAX HDL CHOLESTEROL (y) with a commercial obtainable assay (x) gave following result:  
y = 1.006 x + 0.258 ; r = 0.999

#### LIMITATIONS

3-150 mg/dl

Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl-solution or distilled/deionized water (e.g. 1 + 2). Multiply the result by the appropriate dilution factor (e.g. factor 3).

#### WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.














#### NOTES:

- 1) As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.
- 2) The supernatant obtained on centrifugation must be clear. If the sample has a high triglyceride content (above 1000mg/dl), lipoprotein precipitation may be incomplete (cloudy supernatant), or part of the precipitate may float on the surface. In these cases, dilute the specimen 1 : 1 with 0.9 % NaCl solution and repeat the precipitation step. The result of the cholesterol assay must then be multiplied by 2.

#### REFERENCES:

- 1) Allain, C.C. Clin. Chem 20, 470 (1974)
- 2) Assmann G. At. what levels of total low-or high-density lipoprotein cholesterol should diet/drug therapy be initiated European guidelines. Amer J Cardiol 1990;65:11F
- 3) Assmann G Schriewer H Schmitz G et al Qualification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid/Mg/Cl<sub>2</sub> Clin Chem 1983;29:2026-2030

#### Symbols Used on Pack

 REF	Catalogue Number		Warning/Caution
 LOT	Batch No.		In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		



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