HDL CHOLESTEROL

Phosphotungstic - Precipitation Method

IVD CE

PRODUCT CODE
CS009

INTENDED USE
This reagent is intended for in vitro quantitative determination of HDL Cholesterol in serum & plasma

CLINICAL SIGNIFICANCE
Cholesterol is a fatty substance found in blood, bile and brain tissue, it serves as precursor to bile acids, steroids and vitamin D. The concentration of total cholesterol in serum has been associated with metabolic, infectious and coronary diseases. In the plasma, cholesterol is transported by three lipoprotein, high density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL-Cholesterol) and very low-density lipoprotein (VLDL-Cholesterol).
Castelli and co-workers have indicated that an inverse relationship exists between serum HDL-cholesterol and the risk of coronary heart disease. The measurement of total and HDL cholesterol and triglycerides provide valuable information for the prediction of coronary heart disease and for the lipoprotein phenotyping.

PRINCIPLE
Phosphotungstic acid and magnesium ions specifically precipitate low and very low-density lipoproteins (LDL and VLDL). After centrifugation the cholesterol content of the high-density lipoproteins (HDL) in the supernatant can be determined using Bio Research Cholesterol test kit (Product Code:CS005).

REAGENT COMPOSITION
HDL Cholesterol (Liquid) Reagent

Phosphotungstic acid	0.55 mmol/L
Magnesium Ion	25 mmol/L

REAGENT PREPARATION
1- Macro – assay: Reagent is ready for use
2- Semi Macro assay: pre-dilute the reagent with distilled water before use (80 ml of reagent and 20 ml water).

REAGENT STORAGE AND STABILITY
When stored at 2-8°C, the reagent is stable up to the expiry date. If cloudiness develops the reagent may have deteriorated and should not be used.

SPECIMEN
Serum heparinized or EDTA plasma, non-hemolyzed blood

PRECAUTION
To avoid contamination, use clean laboratory wares.
Avoid direct exposure of reagent to light.

PROCEDURE (Step 1):

	MACRO	SEMI MACRO
Sample	**500 µL**	**200 µL**
HDL reagent undiluted	**1000 µL**	**--**
HDL reagent diluted	**--**	**500 µL**
Mix and allow to stand for 10 minutes. Centrifuge for 10 minutes at 4000rpm. Determine the cholesterol content of the HDL supernatant by **using Bio Research Cholesterol test kit (Product Code: CS005).**		

ASSAY

Wavelength	:	546nm
Cuvette	:	1 cm light path
Temperature	:	20-25°C or 37°C
Measurement		Against reagent blank

PROCEDURE (Step 2):

Pipette into cuvettes	Blank	Sample
Distilled H_2O	100 µL	---
HDL supernatant	---	100 µL
Cholesterol reagent	1000 µL	1000 µL
Mix and incubate for 20 minutes at 25°C or 10 minutes at 37°C Measure the absorbance of sample against the reagent blank within 30 minutes (ΔA).		

CALCULATION
HDL Cholesterol Conc. (mg/dL) = ΔA X Factor

FACTOR

MACRO	SEMI-MACRO
274 mg/dL	**320 mg/dL**
7.05 mmol/L	**8.23 mmol/L**

To convert mg/dL to mmol/L divide by 38.9

LINEARITY
Up to 200 mg/dl, If the results obtained were greater than the linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2

NORMAL RANGE

Male	55 mg/dL	1.42 mmol/L
Female	65 mg/dL	1.67 mmol/L

QUALITY CONTROL
All control sera with HDL Cholesterol value determined by this method may be used.

For LDL Cholesterol
LDL Cholesterol: Friedwald Equation

$$\text{LDL Cholesterol (mg/dL)} = \text{Total Cholesterol} - \text{HDL} - \frac{\text{Triglyceride}}{5}$$

- For mmol/L, divide triglycerides by 2.2 instead of 5

NOTES
1- If supernatant is not clear, dilute sample 1+1 with 0.9% saline**.** Repeat the procedure and multiply by 2

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBILOGRAPHY

1- Trinder, P. Ann. Clin. Biochem, 6,24,1969.
2- Friedwald, W.T. et al. 18 499 1972
3- Castelli, W.P.et al.,Circulations 55.767-772 (1977)

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