

Reagent kit for determination of HDL cholesterol concentration in serum. (Precipitating reagent)

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.

Reference values

Normal values:

Female: >1.7 mmol/l (>65 mg/dl)

Male: >1.4 mmol/l (>55 mg/dl)

It is recommended that each laboratory should assign its own normal range.

Estimation of atherogenic risk

Moderate risk:

Female: 1.2-1.7 mmol/l (45-65 mg/dl)

Male: 0.9-1.4 mmol/l (35-55 mg/dl)

Increased risk:

Female: <1.2 mmol/l(45 mg/dl)

Male: <0.9 mmol/l(35 mg/dl)

Reagents

1.Reagent (R1)

Precipitating reagent:

Phosphotungstic acid: 14 mmol/l

Magnesium chloride: 2 mmol/l

2. HDL cholesterol standard

Ready for use. For details please check the insert.

Available only in Cat. No. 41411S and 41461S.

Cholesterol reagents

Cholesterol PAP Cat. No.: 47061, 47062, 47063

Cholesterol ADPS liquid Cat. No.: 46061, 46062, 46063

Sample

Serum.

PROCEDURE

Preparation of reagents

1. Reagent 1 (R1) is ready for use. The reagent can be used until its expiry date as indicated on the package label.

2. Preparation as well as stability of cholesterol reagent is described in instructions for use of CHOLESTEROL Kit.

Performance of assay

1. Separation of lipoprotein fractions

Serum	500µl
(R1) Precipitating reagent	50µl

Mix and incubate the mixture for 10 minutes between 2-25°C, then centrifuge at 4000 g for 20 minutes.

2. Reaction

Determine the cholesterol concentration from the supernatant based upon the instructions of CHOLESTEROL Kit.

	Blank	Standard	Sample
Cholesterol working reagent	1 ml	1 ml	1 ml
HDL-standard		20 µl	
Sample (clear supernatant)			20µl

The concentration of LDL-cholesterol can be calculated according to Friedewalds formula:

LDL(mg/dl) = total cholesterol-(triglycerides/5)-HDL-cholesterol

LDL(mmol/l) = total cholesterol-(triglycerides/2.2)-HDL-cholesterol

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C). By the measurement we used Cholesterol PAP reagent.

Linearity

The test is linear up to 7.15 mmol/l (275 mg/dl) cholesterol concentration.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 0.015 mmol/l (0,58mg/dl) cholesterol concentration at 492nm.

Precision

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
sample I	1.33	0.053	4.01
sample II	0.95	0.027	2.85

	Repeatability		
	Average concentration (mmol/l)	SD	CV%
sample I	0.44	0.013	2.92
sample II	2.01	0.042	2.13

Correlation

Comparative studies were done to compare our reagent with another commercial HDL-cholesterol assay.

The results from these studies are detailed below.

Correlation coefficient: $r = 0.9862$

Linear regression: $y \text{ (mmol/l)} = 1.049x - 0.003$


(x= other commercial reagent, y= own reagent).

Note

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

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)