

HDL Precipitating Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2 - 30°C
Linear Range	:	200 mg/dL (5.2 mmol/L)
Specimen Type	:	Serum
Method	:	Precipitating
Reagent Preparation	:	Supplied ready to use.

INTENDED USE

For the quantitative separation of High-Density Lipoproteins (HDL) in Serum.

SUMMARY AND EXPLANATION

Castelli and co-workers have established an inverse relationship between serum high-density lipoprotein (HDL) cholesterol and the risk of coronary heart disease.¹ Total and HDL cholesterol assays, in conjunction with a triglyceride determination, provide valuable information for the prediction of coronary heart disease.¹

The Thermo HDL Precipitating Reagent offers a means for the rapid and complete separation of HDL from other serum lipoproteins. The isolated HDL fraction can then be analyzed for cholesterol content. The separation is based on isoelectric-polyanionic precipitation of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). The HDL Precipitating Reagent does not contain metal ions; thus, it differs from first generation precipitating reagents which use divalent metal ions (e.g. Mn⁺⁺, Mg⁺⁺, and Ca⁺⁺) to form complexes with LDL and VLDL. The molecular interactions involving metal ions are relatively weak and depend on time, temperature, ionic strength, and metal-binding agents. The polyanion component of HDL Precipitating Reagent acts directly on LDL and VLDL to form strong ionically bonded, insoluble complexes. This strong interaction assures rapid and complete separation of HDL from other serum lipoproteins. The precipitation is immediate.

PRINCIPLE

HDL Precipitating Reagent uses the well established precipitating properties of phosphotungstate.² Upon the addition of HDL Precipitating Reagent, serum pH is lowered to the isoelectric point of LDL and VLDL where the molecules have overall electrical neutrality.

Phosphotungstate then selectively forms insoluble ligand complexes with LDL and VLDL. Differences in ionic strength forcefully precipitate these complexes. Centrifugation removes them, leaving the HDL fraction in the supernatant.

REAGENTS PROVIDED (FOR IN VITRO DIAGNOSTIC USE)

REACTIVE INGREDIENTS

HDL PRECIPITATING REAGENT	
phosphotungstic acid	0.70 mmol/L
buffer	
preservative	

PRECAUTIONS

Do not ingest. If spilt thoroughly wash the affected area with plenty of water.

REAGENT PREPARATION

Reagent is ready to use as supplied.

STORAGE AND STABILITY

HDL Precipitating Reagent is stable until the expiration date stated on the label when stored at 2-30°C and tightly capped.










DETERIORATION

1. The reagent should be a clear, colorless to yellow or tan solution. If color change occurs, but remains as described, this is not an indication of deterioration.
2. If cloudiness develops, the reagent may have deteriorated and should not be used.

INSTRUMENTS

The procedure may be performed by using a suitable chemistry analyzer, spectrophotometer or colorimeter used for the determination of cholesterol.

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		

SPECIMEN COLLECTION

1. Non-hemolyzed serum is the recommended sample. The sample need not be fasting.³ However, if triglycerides are to be assayed at the same time, a fasting sample is required.
2. EDTA plasma may be used.³ No special additives or preservatives are required. Heparinized plasma should not be used.

INTERFERING SUBSTANCES

1. High abnormal levels of bilirubin, haemolysis and turbidity in the sample may cause negative interference.
2. Young has reviewed drug effects on serum HDL cholesterol levels.⁴

SAMPLE STORAGE

1. The sample may be stored at 2-8°C. Serum HDL cholesterol appears stable at least four days.⁵
2. Specimens frozen at -20°C may show decreases in HDL measured at 7 to 14 days after collection.⁵
3. Samples precipitated while cold may show values slightly lower than samples precipitated at room temperature.

PROCEDURE

Precipitation Temperature	15 - 30°C
Sample/Reagent Ratio	1:1
Precipitation Time*	Immediate
Centrifugation Time	10 Minutes

MATERIALS PROVIDED

HDL Precipitating Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes for accurately dispensing 0.50 mL volume.
2. Clinical centrifuge capable of 1,000 x g.
3. Thermo HDL Cholesterol Standard, 50 mg/dL, Cat. No. 2331-153 or equivalent.
4. Cholesterol Reagent, Thermo Cat. No. 2340 or equivalent.

PERFORMANCE OF PRECIPITATION

1. Dispense 0.50 mL of serum at room temperature into an appropriately labeled test tube.
2. Add 0.50 mL of HDL Precipitating Reagent. Mix well.*
3. Centrifuge for 10 minutes at full speed (at least 1000 x g). Do not use a warm or hot centrifuge.
4. Separate the supernatant, which contains the HDL, from the precipitate. Use supernatant as the sample in the Cholesterol Procedure.

COMMENT ON CHOLESTEROL ASSAY

Thermo has designed this HDL Precipitating Reagent for use with enzymatic cholesterol reagents. The supernatant volume used in the HDL procedure should be approximately 4 times the sample volume used in the total cholesterol assay.

QUALITY CONTROL

Normal and abnormal control sera with known concentrations of HDL cholesterol should be analyzed routinely with each group of unknown samples.

*Quality control of HDL cholesterol by use of lyophilized lipid control sera is often questionable, probably owing to the influence of lyophilization and the addition of stabilizers.⁶ Due to these influences, it is recommended that control materials be incubated for 10 minutes prior to centrifugation.

LINEARITY

The procedure is linear to 200 mg/dL (5.2 mmol/L)

CALCULATION OF RESULTS

Use the equation below to calculate the HDL cholesterol concentration of a sample.

$$\frac{\text{Abs. of Unk.}}{\text{Abs. of Std.}} \times \text{Conc. Of Std.} \times 2 = \text{HDL Chol. mg/dL}$$

The factor 2 corrects for sample dilution during the precipitation step.

Example:

Absorbance of Unknown Supernatant	=	0.450
Absorbance of Standard	=	0.486
Concentration of Standard	=	50 mg/dL

$$\frac{0.450}{0.486} \times 50 \times 2 = 93 \text{ mg/dL}$$

LIMITATIONS

HDL cholesterol analysis is intended for the assessment of coronary heart disease risk in apparently normal individuals. Results obtained from patients with hyperlipoproteinemia, liver disease or recent myocardial infarction have no correlation with the coronary heart disease risk factors.

EXPECTED VALUES

HDL Cholesterol⁷

Males: 27 - 75 mg/dL (0.7 - 2.0 mmol/L)
Females: 33 - 96 mg/dL (0.89 - 2.5 mmol/L)

LDL Cholesterol⁸

Desirable: <130 mg/dL (< 3.4 mmol/L)
Moderate Risk: 130 - 159 mg/dL (3.4 - 4.1 mmol/L)
High Risk: ≥160 mg/dL (≥ 4.2 mmol/L)

These ranges should serve only as guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE DATA

The following data was obtained using Thermo HDL PTA and the Thermo Cholesterol reagent set following the NCCLS EP-5 procedure.¹⁰

IMPRECISION

Within Run	Level I	Level II
Number of Samples	20	20
Mean (mmol/L)	0.97	3.1
Mean (mg/dL)	37.3	120.8
SD (mmol/L)	0.02	0.03
SD (mg/dL)	0.8	1.3
CV(%)	2.1	1.0
Total	Level I	Level II
Number of Samples	20	20
Mean (mmol/L)	0.97	3.1
Mean (mg/dL)	37.3	120.8
SD (mmol/L)	0.07	0.2
SD (mg/dL)	2.5	6.0
CV(%)	6.6	4.9

ACCURACY

Comparison studies were carried out using a similar commercially available HDL Precipitating reagent. Normal and abnormal patient serum was assayed in parallel. The following results were obtained.

Number of sample pairs	67
Range of sample results	31-59 mg/dL (0.81-1.5 mmol/L)
Slope	1.062
Intercept	3.7 mg/dL (0.1 mmol/L)
Correlation coefficient	0.977

SENSITIVITY

Sensitivity is a function of the cholesterol reagent used. Employing the Thermo cholesterol reagent set as recommended for HDL Precipitating reagent, the sensitivity of the assay is such that a change in absorbance of 0.001 equals 0.333mg/dL (0.009mmol/L).

INTERPRETATION OF RESULTS

Serum HDL cholesterol is an important indicator of coronary heart disease risk in an apparently normal individual.

A general assessment can be established by considering the HDL cholesterol expressed as percent of total cholesterol. To calculate this value, use the equation:

$$\frac{\text{HDL Chol. Conc.}}{\text{Total Chol. Conc.}} \times 100 = \text{HDL Chol (\% of Tot Chol.)}$$

The following chart indicates risk of coronary heart disease (CHD).⁷

CHD Risk	HDL Chol. % of Total Chol.	
	Male	Female
Dangerous	<7	<12
High	7 - 15	12 - 18
Average	15 - 25	18 - 27
Below Average	25 - 37	27 - 40
Protection Probable	>37	>40


NOTE: If the triglyceride is less than 400 mg/dL,⁵ the LDL cholesterol can be calculated by using the Friedewald equation.⁹ Assay triglyceride, total cholesterol, and HDL cholesterol on a fasting sample and calculate the LDL cholesterol concentration by the following formula:

$$\text{LDL} = \text{TC} - [\text{HDL} + (\text{Triglycerides}/5)]$$

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REF	Reorder Information	
	Catalogue No.	Configuration
	1335-250	2 x 125 mL
	1335-500	2 x 250 mL