

# HDL Precipitant

Precipitation reagent for in vitro determination of high density lipoprotein cholesterol (HDL-C) according to the CHOD-PAP-method on photometric systems

## Order Information

Cat. No.	Kit size
1 3540 99 90 885	250 mL Precipitation reagent
1 1350 99 10 021	5 x 25 mL + 1 x 3 mL Standard
1 1350 99 10 026	6 x 100 mL
1 1350 99 10 023	1 x 1000 mL
1 1300 99 10 030	6 x 3 mL Standard

## Principle

Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only HDL in the supernatant the concentration of which is determined enzymatically by using DiaSys Cholesterol FS.

## Reagents

### Components and concentrations

Phosphotungstic acid	1.4 mmol/L
Magnesium chloride	8.6 mmol/L

**Standard:** 200 mg/dL (5.2 mmol/L)

### Storage instructions and reagent stability

The reagent and standard are stable up to the end of the indicated month of expiry, if the reagent is stored at 15–25°C and the standard is stored 2–8°C and contamination is avoided. Do not freeze the reagents. Protect the standard from light.

## Warnings and precautions

- In very rare cases, samples of patients with gammopathy might give falsified results [7].
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only.

## Waste management

Please refer to local legal requirements.

## Reagent Preparation

The precipitant is ready to use.

## Material required but not provided

NaCl-Solution 9 g/L  
General laboratory equipment

## Specimen

Serum, heparin plasma or EDTA plasma

Stability [5]:	2 days	at	20 – 25°C
	7 days	at	4 – 8°C
	3 months	at	–20°C

Freeze only once!

Discard contaminated specimens!

## Assay procedure

### Precipitation

Sample/Standard	200 µL
Precipitation reagent	500 µL
Mix and incubate for 15 min. at room temperature, then centrifuge for 20 min at 2500 g. Within 2 hours after centrifugation transfer 0.1 mL of the clear supernatant to the reaction solution for the determination of cholesterol.	

After centrifugation, the supernatant should be clear. Serum or plasma with triglyceride contents > 1000 mg/dL tends to produce turbid supernatants or floating precipitates. In this case dilute the sample 1 + 1 with NaCl solution (9 g/L) and then perform the precipitation. Multiply the result by 2.

### Cholesterol determination

Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 – 25°C, 37°C
Measurement	Against reagent blank

	Standard	Sample
Supernatant	-	100 µL
Standard	100 µL	-
Cholesterol reagent	1000 µL	1000 µL
Mix and incubate for 10 min at room temperature or 5 min at 37°C. Then measure the absorbance of the sample or the standard against the reagent blank value within 45 min.		

## Calculation

### With Standard

$$\text{HDL - Cholesterol [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Conc. Standard [mg/dL]}$$

The concentration of the standard is the concentration of the total cholesterol in the cholesterol standard solution.

### Conversion factor

$$\text{Cholesterol [mg/dL]} \times 0.02586 = \text{Cholesterol [mmol/L]}$$

## Controls

Use DiaSys TruLab L for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 3 mL

## Performance characteristics

### Measuring range

The test has been developed to determine HDL-Cholesterol concentrations up to 400 mg/dL. When values exceed this range, samples should be diluted 1+4 with NaCl solution (9 g/L) and the result multiplied by 5.

### Specificity/Interferences

Bilirubin and hemoglobin interfere in low concentrations. For further information on interfering substances refer to Young DS [6].

### Limit of detection

The lower limit of detection is 2 mg/dL.

### Precision

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	29	0.60	2.1
Sample 2	61	0.86	1.4
Sample 3	105	1.49	1.4

Inter-assay n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27	0.80	3.0
Sample 2	45	0.51	1.1
Sample 3	56	1.34	2.4

### Method comparison

A comparison of DiaSys Cholesterol FS + HDL Precipitant (y) with a commercially available cholesterol test + HDL precipitant (x) using 60 samples gave following results:  
 $y = 0.98 x + 1.65$  mg/dL;  $r = 0.996$ .

### Reference range [4]

National Cholesterol Education Program (NCEP) guidelines:

Low HDL-cholesterol (major risk factor for CHD):  
< 40 mg/dL (< 1.04 mmol/L)

High HDL-cholesterol ("negative" risk factor for CHD):  
≥ 60 mg/dL (≥ 1.55 mmol/L)

A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and progestational agents, genetic factors.

Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

## Literature

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998;19: 1434-503.
3. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.
4. Third Report of the National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No. 02-5215; September 2002.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: GIT Verlag; 2001. p. 22-3.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

### Manufacturer



DiaSys Diagnostic Systems GmbH  
Alte Strasse 9 65558 Holzheim Germany