LDL Precipitant

Precipitation reagent for in vitro determination of LDL-cholesterol with the CHOD-PAP method on photometric systems

Order Information

Cat. No.	Kit size
1 4330 99 90 885	250 mL Precipitation reagent
1 1350 99 10 021	R 5 x 25 mL + 1 x 3 mL Standard
1 1350 99 10 026	R 6 x 100 mL
1 1350 99 10 023	R 1 x 1000 mL
1 1300 99 10 030	6 x 3 mL Standard

Principle

Low density lipoproteins (LDL) are precipitated by addition of heparin. High density lipoproteins (HDL) and very low density lipoproteins (VLDL) remain in the supernatant after centrifugation and are measured enzymatically by the CHOD-PAP method. The concentration of LDL cholesterol is calculated as the difference of total cholesterol and cholesterol in the supernatant.

Reagents

Components and concentrations

Heparin	100 000 U/L
Sodium citrate	64 mmol/L

Storage instructions and reagent stability

The precipitant is stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and contamination is avoided. The standard is stable up to the end of the indicated month of expiry, if stored at 2 – 25 °C.

Warnings and precautions

1. In very rare cases, samples of patients with gammopathy might give falsified results [7].
2. 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.
3. 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

Stability [5]:	1 day	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	100 µL
Precipitating reagent	1000 µL

Mix and incubate for 15 min. at room temperature, then centrifuge for 20 min. at 2500 g. Within one hour after centrifugation, transfer 100 µL of the clear supernatant to the reaction solution for the determination of cholesterol.

The cholesterol standard has to be diluted 1 + 10 with NaCl (9 g/L). After dilution the standard is treated like the supernatant.

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 10 min. at room temperature or 5 min at 37 °C, read absorbance of the sample for the standard within 45 min. against reagent blank.

Calculation

Cholesterol in supernatant

$$\text{Cholesterol in supernatant [mg/dL]} = \frac{\Delta E \text{ Sample}}{\Delta E \text{ Standard}} \times \text{Conc.Standard [mg/dL]}$$

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

LDL Cholesterol

LDL-Cholesterol [mg/dL] =

Total cholesterol [mg/dL] – Cholesterol in the supernatant [mg/dL]

Conversion factor

LDL-Cholesterol [mg/dL] x 0.02586 = LDL-Cholesterol [mmol/L]

Controls

For internal quality control DiaSys TruLab L controls should be assayed. 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 LDL-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

No interference was observed by bilirubin up to 30 mg/dL and hemoglobin up to 800 mg/dL. For further information on interfering substances refer to Young DS [6].

Limit of detection

The lower limit of detection is 2 mg/dL.

Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	20	0.81	4.1
Sample 2	57	2.47	4.3
Sample 3	141	1.39	1.0

Inter-assay n = 10	Mean [mg/dL]	SD [mg/dL]	VK [%]
Sample 1	62	1.90	3.0
Sample 2	131	2.80	2.1
Sample 3	283	2.09	0.7

Method comparison

A comparison of LDL-Cholesterol values obtained with the DiaSys LDL precipitant (y) with the Friedewald calculation (x) using 49 samples gave following results:
y = 1.121 x – 9.62 mg/dL; r = 0.947.

Reference range [4]

LDL cholesterol

Desirable	≤ 130 mg/dL (3.4 mmol/L)
Borderline high risk	130 –160 mg/dL (3.4 – 4.1 mmol/L)
High risk	> 160 mg/dL (> 4.1 mmol/L)

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

Clinical Interpretation [2]

Epidemiological studies have observed that low HDL-cholesterol concentrations < 39 mg/dL (0.9 mmol/L) in men and < 43 mg/dL (1.0 mmol/L) in women) especially if associated with fasting triglycerides > 180 mg/dL (2 mmol/L), predict a high risk of coronary heart disease.

The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L).

Literature

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd 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. Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press;1997.p.25–48.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st 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