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Liquid Reagents – ready to use

Cholesterol HDL Precipitant

Single Reagent

Precipitation reagent for the in vitro determination of HDL-cholesterol in human serum or plasma with the CHOD-PAP method on photometric systems

REF	Kit Size	Configuration
D96122B	**1 x 1 L**	Single Reagent
D14125	**5 x 100 mL**	Single Reagent
D00127	**5 x 50 mL**	Single Reagent
D00142	**5 x 25 mL**	Single Reagent
D00129	**5 x 10 mL**	Single Reagent

Additionally offered:

D95114	1 x 3 mL	Cholesterol Standard	
D99486	3 x 3 mL	Lipid Control normal	Diacon Lipids
D99486SV	1 x 3 mL	Lipid Control normal	Diacon Lipids
D11487	3 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D11487SV	1 x 3 mL	Lipid Control abnormal	Diacon Lipids High

DIALAB Cholesterol CHOD-PAP Reagent

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, precipitation
Wavelength:	500 nm, Hg 546 nm
Temperature	20 – 25 °C or 37 °C
Sample:	Serum, heparin or EDTA plasma

TEST PRINCIPLE

Low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons contained in serum are precipitated by the addition of phosphotungstic acid and magnesium chloride. High density lipoproteins (HDL) which remain in the supernatant (obtained after centrifugation) can be enzymatically determined with DIALAB Cholesterol CHOD-PAP reagent.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Phosphotungstic Acid	0.55	mmol/L
Magnesium Chloride	25	mmol/L

REAGENT PREPARATION

Macro Assay:
The precipitatant is ready to use.
Semimicro Assay:
Mix 4 parts of precipitant and 1 part of dist. water.

REAGENT STABILITY AND STORAGE

Conditions:	close immediately after use avoid contamination of opened reagent
Storage:	at 2 – 25 °C
Stability:	up to the expiration date indicated on labels

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment
Total cholesterol reagent (CHOD-PAP)

STANDARD

(not included in the kit – has to be ordered separately)

Concentration:	200 mg/dL
Storage:	2 – 8 °C
Stability:	up to the expiration date

Close immediately after use. Avoid contamination.
Protect from light.

SAMPLE STABILITY AND STORAGE [6]

Stability:	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at -20 °C	3 months

Serum must be separated from the blood clot as rapidly as possible.
Freeze only once! Discard contaminated specimens.

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

1. Precipitation

	MACRO	SEMIMICRO
Sample or standard	500 µL	200 µL
Precipitant undiluted	1000 µL	---
Precipitant diluted (4:1 with dist. water)	---	500 µL
Mix. Let stand for 10 min. at 20 – 25°C. Centrifuge for 2 minutes at 10000 g or for 10 minutes at 4000 g.		

After centrifugation separate the clear supernatant from the precipitate within 1 hour and determine the cholesterol concentration using Dialab Cholesterol CHOD-PAP Reagent.

2. Cholesterol Determination

Pipette into test tubes	Blank	Supernatant (Std.)	Supernatant (Sample)
Reagent	1000 µL	1000 µL	1000 µL
Supernatant (Sample)	-	-	100 µL
Supernatant (Standard)	-	100 µL	-
Mix. Incubate for 20 min. at 20–25°C or for 10 min. at 37°C. Measure absorbance A of Sample and Standard within 60 minutes against reagent blank.			

CALCULATION

HDL Cholesterol

$$\text{HDL (mg/dL)} = \frac{\Delta A \text{ Supernatant Sample}}{\Delta A \text{ Supernatant Standard}} \times \text{Conc. Std. (mg/dL)}$$

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

LDL- Cholesterol

LDL Cholesterol values can be calculated using the Friedewald formula [4] which is reliable only if chylomicrons are absent in the sample, the triglycerides concentration is < 400 mg/dl and the samples are not derived from patients with type III hyperlipoproteinemia.

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

UNIT CONVERSION

mg/dL x 0.02586 = mmol/L

REFERENCE RANGE [5] *

	mg/dL	mmol/L
HDL – Cholesterol:	≥ 35	≥ 0.9
LDL – Cholesterol:		
Desirable	≤ 130	3.4
Borderline high risk	130 –160	3.4 – 4.1
High risk	> 160	> 4.1

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

CALIBRATION

The assay requires the use of a Cholesterol Standard.
We recommend the Dialab **Cholesterol Standard**

QUALITY CONTROL

All control sera with HDL Cholesterol values determined by this method can be used.
We recommend the Dialab lipid control sera **Diacon Lipids** and **Diacon Lipids High.**

AUTOMATION

Not possible for this test.

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.
3. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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CE 2°C 25°C IVD