

Liquid Reagents – ready to use

## CHOLESTEROL HDL

**Precipitation**  
Single Reagent

**Reagent for precipitation of non-HDL lipoproteins in tests for determination of high density lipoprotein cholesterol (HDL-C) in human serum or plasma on photometric systems**

REF

Cont.

**D00127 5 x 50 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
D95116	5 x 100 ml	Cholesterol, CHOD-PAP	Reagent
D96112B	1 x 1000 ml	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, heparinized or EDTA plasma

Linearity: up to 750 mg/dl

### REAGENT COMPOSITION

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

### REAGENT PREPARATION

**Macro Assay:**  
Precipitation reagent is ready to use.

**Semimicro Assay:**  
Mix 4 parts of reagent 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

### SAMPLE STABILITY AND STORAGE

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.  
Discard contaminated specimens.

### STANDARD

(has to be ordered separately)

Concentration: 200 mg/dl

Storage: 2 – 8°C

Stability: up to the expiration date

**CLOSE IMMEDIATELY AFTER USE!**

### 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 Reagent, Cat. No. D95116 / D96112B.

#### 2. Cholesterol Determination

Pipette into test tubes	Blank	Supernatant (Std.)	Supernatant (Sample)
Reagent	1000 µl	1000 µl	1000 µl
Supernatant (Sample)	-	-	100 µl
Supernatant (Std.)	-	100 µl	-

Mix. Incubate for 20 min. at 20–25°C or for 10 min. at 37°C. Measure absorbance A of Sample and Std./Cal. within 60 minutes against Reagent Blank.

### CALCULATION (light path 1 cm)

#### HDL Cholesterol

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

#### LDL- Cholesterol

LDL Cholesterol values can be calculated using the Friedewald formula 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 0.0259 = mmol/L

## REFERENCE RANGE \*(mg/dl)

<b>HDL – Cholesterol:</b>	≥ 35
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### LDL – Cholesterol:

Desirable	≤ 130
Borderline high risk	130 –160
High risk	> 160

\* It is recommended that each laboratory establishes its own normal range.

## 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 measured with DIALAB Cholesterol reagent.

## QUALITY CONTROL

All control sera with HDL Cholesterol values determined by this method can be used.

We recommend:

<b>REF</b>	<b>Cont.</b>
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**D99486** 3 x 3 ml **DIACON LIPIDS** Assayed Control Serum Normal

## CALIBRATION

The assay requires the use of a Cholesterol Standard.

We recommend:

<b>REF</b>	<b>Cont.</b>
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**D95114** 1 x 3 ml **CHOLESTEROL STANDARD**

## AUTOMATION

not possible for this test.

## WARNINGS AND PRECAUTIONS

Take the necessary precautions for the use of laboratory reagents.

## WASTE MANAGEMENT

Please refer to local legal requirements

## REFERENCES

1. Friedewald, W.T. et al., **CLIN.Chem.** **18** (1971) 499

2°C → 25°C



DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.  
A – 2351 Wiener Neudorf, Austria  
IZ-NÖ Süd, Hondastrasse, Objekt M55  
Phone: ++43 (0) 2236 660910-0  
Fax: ++43 (0) 2236 660910-30 e-mail: [office@dialab.at](mailto:office@dialab.at)