COD 11523 2 x 50 + 2 x 50 mL
STORE AT 2-8ºC
Reagents for measurement of HDL cholesterol concentration Only for *in vitro* use in the clinical laboratory

CHOLESTEROL HDL

BioSystems
REAGENTS & INSTRUMENTS

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CHOLESTEROL HDL
PHOSPHOTUNGSTATE/Mg-CHOL.
OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1,2].

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + 4 - \text{Aminoantipyrine} + \text{DCFS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS AND COMPOSITION

A. Reagent: 2 x 50 mL. Phosphotungstate 0.4 mmol/L, magnesium chloride 20 mmol/L.
B. Reagent: 2 x 50 mL. PIPES 70 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, sodium cholate 0.5 mmol/L, dichlorophenolsulfonate 4 mmol/L, pH 7.0.
S. HDL Cholesterol Standard: 1 x 5 mL. Cholesterol 15 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:
- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagents and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Desktop centrifuge
- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm

SAMPLES

Serum or plasma collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

Precipitation

1. Pipette into labelled centrifuge tubes (Note 1):

Sample	0.2 mL
Reagent (A)	0.5 mL

2. Mix thoroughly and let stand for 10 minutes at room temperature.
3. Centrifuge at a minimum of 4000 r.p.m. for 10 minutes.
4. Carefully collect the supernatant (Note 2).

Colorimetry

5. Bring the Reagent B to room temperature.
6. Pipette into labelled test tubes: (Note 3)

	Blank	Standard	Sample
Distilled water	50 µL	—	—
HDL Cholesterol Standard (S)	—	50 µL	—
Sample supernatant	—	—	50 µL
Reagent (B)	1.0 mL	1.0 mL	1.0 mL

7. Mix thoroughly and incubate the tubes for 30 minutes at room temperature (16-25ºC) or for 10 minutes at 37ºC.
8. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

The HDL cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the HDL Cholesterol Standard provided has been used to calibrate (Note 4):

	Serum or plasma
$\frac{A_{Sample}}{A_{Standard}}$	x 52.5 = mg/dL HDL cholesterol x 1.36 = mmol/L HDL cholesterol

REFERENCE VALUES

HDL cholesterol concentrations vary considerably with age and sex. The following cut-off point has been recommended for identifiying individuals at high risk of coronary artery disease[3].

Up to 35 mg/dL = 0.91 mmol/L	High risk
> 60 mg/dL = > 1.56 mmol/L	Low risk

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005 and 18009) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 3.0 mg/dL = 0.078 mmol/L
- Linearity limit: 150 mg/dL = 3.9 mmol/L.
- Repeatibility (within run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	3.3 %	20
55 mg/dL = 1.42 mmol/L	2.0 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	4.2 %	25
55 mg/dL = 1.42 mmol/L	3.2 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 4). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) does not interfere. Bilirubin (10 mg/dL) and hemoglobin (5 g/L) may intefere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids.

Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[5,6].

There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, analphalipopro-teinemia, acute stress, some drugs and smoking[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Sample and Reagent A volumes may be varied as long as the same ratio is maintained.
2. Supernatant must be clear. When supernatant is turbid or the pellet floats, add again 0.5 mL of Reagent A, mix thoroughly and centrifuge. Multiply the obtained concentration by 1.7 (dilution).
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
4. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011).

BIBLIOGRAPHY

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3. National Cholesterol Education Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
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