ACCUCARE®

HDL CHOLESTEROL

Precipitating Method

Quantitative determination of HDL Cholesterol in serum/plasma
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
HDL 50	2 x 25 mL

CLINICAL SIGNIFICANCE

HDL particles carry cholesterol from the cells back to the liver. HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease. A low HDL cholesterol levels, is considered a greater heart disease risk

Method

Precipitation – Photometric Test

PRINCIPLE

The very low density (VLDL) and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphortungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant containing high density lipoproteins (HDL) is used for the determination of HDL cholesterol.

REAGENT

Reagent : HDL Cholesterol Precipitating Reagent
HDL Cholesterol Standard : 50 mg/dl

REAGENT PREPARATION

Reagents are ready to use as supplied.

REAGENT STORAGE AND STABILITY

When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum or heparin plasma
It is very important to store the sample protected from light!
Stability: 1 day at 20 – 25°C
7 days at 4 – 8°C
3 months at –20°C in case of immediate freezing.
Freeze only once! Discard contaminated specimens!

MANUAL ASSAY PROCEDURE

Operating Instructions

1. Mix equal amount of serum and HDL cholesterol precipitating reagent in the glass tube and mix vigorously.
   E.g. 0.2 ml serum + 0.2 ml HDL precipitating reagent.
2. Centrifuge for ten (10) minutes at 1500 - 2000 rpm.
3. Separate supernatant from precipitate. The supernatant fraction contains HDL.

Determine the cholesterol content by the ACCUCARE CHOLESTEROL (CHOD-PAP) reagent.

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28°C, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	505nm (490-550nm)
Measurement	Against Reagent blank
Cuvette	1 cm light path
Reaction Temperature	37°C
Reaction Type	End point
Sample Volume	50 µl
Reagent Volume	1000 µl
Incubation	5 Minutes
Low Normal	40 mg/dL
High Normal	60 mg/dL
Linearity	400 mg/dlL

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

Test	Blank	Std	Sample (Supernatant)
Blank	-	-	-
Std	-	50 µl	-
Sample (Supernatant)	-	-	50 µl
Cholesterol Reagent	1000 µl	1000 µl	1000 µl

Mix and incubate for 5 min at 37 °C then Read the absorbance of Sample and standard at 505 nm against reagent blank

SAMPLE DILUTIONS

- This method is linear upto a concentration of 400 mg/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

$$\text{HDL Chol (mg/dL)} = \frac{\text{(Abs) of sample}}{\text{(Abs) of Standard}} \times \text{Standard value} \times 2.$$

Where, 2 is the serum dilution factor.

CALIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.
The assigned values of the calibrator have been made traceable to NIST SRM® 1951.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Level 1	75.62	2.87	3.95%
Level 2	149.52	3.16	2.78%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Level 1	72.69	2.89	3.97%
Level 2	150.50	2.88	1.91%

CE

Art No.: IFU/BC/DHDL
Effective Date: 20/09/2019
Revision No.: 01

LINEARITY

The method is linear upto a concentration of 400mg/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for HDL Cholesterol is 2 mg/dL.

METHOD COMPARISON

A comparison of Accucare HDL Cholesterol with a commercially available assay (x) using 20 samples gave following results: R^2 = 0.9900

REFERENCE VALUES

≥ 40mg/dL	Desirable(Normal)
≥ 60mg/dL	some protection against coronary heart disease
< 40mg/dL	significant independent risk factor for coronary heart disease

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

INTERFERENCE

- Hemoglobin: No interference found upto 500 mg/dL.
- Bilirubin: No interference found upto 40m/dL.
- Ascorbic Acid: No interference found upto 50 mg/dL.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

1. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
2. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed. AACC 2001.
3. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999. 7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 1995.

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

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