ACCUCARE
HDL CHOLESTEROL
Precipitating Reagent

QUANTITATIVE DETERMINATION OF HDL CHOLESTEROL
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.

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 COMPONENT

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

WORKING REAGENT PREPARATION

Reagents are ready to use as supplied.

STABILITY AND STORAGE

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

SPECIMEN COLLECTION AND HANDLING

Serum or plasma : Free of hemolysis. Removed from the blood clot as soon as possible.
Stability : HDL Cholesterol is stable for 7 days at 2-8ºC.

MANUAL ASSAY PROCEDURE

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 g.
3. Separate supernatant from precipitate. The supernatant fraction contains HDL.

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

AUTOMATED PARAMETERS	
Wavelength	505nm (490-550nm)
Measurment	Against DI Water
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

ASSAY PROCEDURE FOR SEMIAUTOMATED ANALYZERS

1. Bring the Cholesterol Reagent and the photometer to 37ºC.
2. Pipette into a cuvette:

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

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

CALCULATIONS

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.

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with HDL values above 400 mg/dL should be diluted with isotonic saline and reassay. Multiply results by the dilution factor.

CALIBRATION

Calibration is recommended at anytime if one of the following event occurs-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical components replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

REFERENCE VALUES

According to NCEP, HDL Values description as follows

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

Each laboratory must establish its own range of expected values.

LINEARITY

The method is linear upto a concentration of 400 mg/dl (22.2 mmol/L). Dilute samples above this concentration 1:1 and multiply the result by 2.

BIBLIOGRAPHY

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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.

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