

BHAT BIOSCAN™

Pack size. HDL -20ml

HDL-CHOLESTEROL

Phosphotungstic precipitation method
Cat. No. HDL -20

Principle:

LDL, VLDL and chylomicrons are precipitated by phosphotungstate and magnesium ions and are then separated by centrifugation. The HDL remaining in the supernatant is analysed for cholesterol content using cholesterol assay kit.

Reagent Composition:

1. Reagent-1 (HDL- Precipitating reagent)

Phosphotungstic acid 150mmol/L

Reagent Preparation:

Reagent is ready to use.

Storage & Stability:

Store at 2-8° C, and keep away from light. Unopened reagent is stable until expiry date stated on the label.

Sample:

Unhemolysed serum or heparinised plasma can be used.

Procedure:

1. Let stand reagents and specimens at room temperature.
2. Take 0.2ml serum in a clean tube.
3. Add 0.2ml of reagent -1 to above and mix well.
4. Allow the mixture to stand at room tempt. for 15min.
5. Centrifuge the mixture at 4000rpm for 30min.
6. Use the supernatant (50 µl) sample for the HDL assay by using cholesterol assay kit.

Calculations:

Calculate the result as follows:

$$\text{HDL- Cholesterol (mg/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Std.}$$

Multiply the final value by 2 as serum is diluted with precipitating reagent in (1: 1).

Expected Value:

Adult male : 30- 64 mg/dl

Adult female: 36- 77 mg/dl

Each lab should optimize its own normal range.

Quality Control:

The assay linear up to triglyceride concentration of 500mg/dl. The supernatant obtained after centrifugation must be clear. If, it is turbid, then dilute it with saline and use for the assay and use the appropriate dilution factor to get the result.

Reference:

1. Friedewald, WT, et al., Clin chem.18: 499(1972).