HDL Direct Cholesterol

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Reagent for direct measurement of HDL Cholesterol concentration in human serum and plasma. For in Vitro Diagnostic Use. Do not freeze. Store at 2-8ºC .

REF: RHDL-10JA / RHDL-10JB

Summary:

This reagent is designed for quantitative determination of HDL Cholesterol (HDL-C) concentration in human serum and plasma.
High-density lipoproteins are one of the major classes of plasma lipoproteins. HDL Cholesterol is known as "good cholesterol" because high levels can help to lower the risk of heart disease and coronary artery disease (CHD).
A low HDL-C level, is considered as a greater heart disease risk.
Accurate measurement of HDL-C is a key parameter when assessing patient risk from CHD.

Principle:

After addition of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.
HDL-C in human serum is dissolved with a specific detergent, and makes color reactions with Cholesterol esterase, Cholesterol oxidase and Peroxidase.
Non-HDL-Lipoproteins such as chylomicron (CM), low density lipoprotein (LDL), very low density lipoprotein (VLDL) are inhibited by detergents so they don't react with the mentioned enzymes.
HDL Cholesterol concentration is determined by color intensity following Trinder's reaction.

Test Parameters

Method: Colorimetric, End Point Reaction
Wavelength: Main: 604 - 700 nm
Temperature: 37°C
Sample: Serum
Linearity: 3 mg/dL - 200 mg/dL

Composition:

R1: Dextran Sulfate - ≤ 10 gr/dL, Magnesium Chloride Hegzahydrate - ≤ 5 gr/dL, Preservative, Brij 35 - ≤ 10 gr/dL
R2: Detergent - ≤ 2 %, PEG - Cholesterol Esterase - ≤ 5 KU/L, PEG - Cholesterol Oxidase - ≤ 5 KU/L, 4 AAP - ≤ 1 gr/dL, Peroxidase - ≤ 8000 U/L

Reagent Stability And Storage:

Stability: up to expiration date on labels at 2-8°C.
Once opened vials are stable minimum 30 days at 2-8OC at optimum conditions. There is a strong relation between on board stability and auto analysers cooling specification and carry-over values.

Precautions:

Product to be used in professional laboratories by professional operators.
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

Preparation:

Reagents are ready to use, liquid.

Samples:

Fresh Serum, or EDTA and heparinized plasma. Samples are collected by standard procedures.
Note: Separate the serum or plasma as soon as possible after collection (within 3 hours). Store serum no more than 12 hours at room temperature, no more 7 days at 2-8 °C. HDL in sample is stable for 30 days at – 70 °C.

Procedure:

Mix well and incubate 10 minutes at 37°C.
Read Calibrator blank tube absorbance (A_{BCAL}) and Calibrator tube absorbance (A_{CAL}).
Then read sample blank tube absorbance (A_{SB}) and Sample tube absorbance (A_S).
ΔA Cal = (A_{CAL}-A_{BCAL}) ΔA Sample = (A_B-A_{SB})
Calibration stability: >30 days

Different incubation time gives differents absorbance values. Incubation time of sample and calibrator always have the same time duration.
Test time: 10 seconds

Calculations:

$$\frac{\Delta A \text{sample}}{\Delta A \text{CAL}} \times \text{Conc.Cal (mg/dL)} = \text{HDL Direct (mg/dL)}$$

Unit Conversion:
mmol/L*38.67= mg/dL
mg/dL*0.02586=mmol/L

Reference Intervals (Normal Values):

National Cholesterol Education Program (NCEP) guidelines:
<40 mg/dL: Low HDL (major risk factor for CHD)
≥60 mg/dL: High HDL ("negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercises, hormones, sex and age.
It is recommended that each laboratory establishes its own normal range.

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Performance characteristics:

Low Linearity (LOQ):
(Values are based on CV%≤ 20%): 3 mg/dL HDL Cholesterol.

High Linearity:
The test is linear up to 200 mg/dL.

Precision:
(Based on CLSI EP05A3 Doc.):

Intra-assay			Inter-assay		
Mean (mg/dL)	%CV	n	Mean (mg/dL)	%CV	n
106	1.70	20	80	1.90	20
22	2.80	20	22	2.95	20

Sensitivity (LOD):
2.7 mg/dL.

Accuracy:
No systematic differences seen in results obtained with this reagent when compared with reference reagents. It's available to get details of comparison experiments in case of requirement.

Interferences:
No interferences were observed to bilirubin T. and D. up to 60 mg/dL, hemoglobin up to 30 g/L or lipemia up to 2500 mg/dL.
A list of drugs and other interfering substances with HDL cholesterol determination has been reported by Young et. al.

Notes

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human & animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. Caps of the reagents bottles cannot be used between two different kind of reagent and between R1&R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The reagents contain sodium azide (< 0.1%) as a preservative.

References:

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10. Report on the Symposium "Drug effects in clinical chemistry methods", Breuer J, Eur J Clin Chem Clin Biochem 1996;34:385-386.
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