NS Bio-Tec

HDL - Cholesterol

Precept. Reagent

HDL-MC-0150 (1x50ml) **HDL-MC-0520 (5x20ml)**
HDL-MC-0620 (6x20ml)

INTENDED FOR USE:

For the quantitative determination of HDL cholesterol in serum and plasma

PRINCIPLE :

HDL-Cholesterol is obtained trough selective precipitation of LDL and VLDL lipoproteins, thus HDL lipoproteins remain in solution. HDL-Cholesterol in supernatant is treated as a sample for cholesterol assay according to the following reaction:

$$\text{Cholesterol ester} \xrightarrow{\text{CEH}} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-ona + } H_2O_2$$

$$2H_2O_2 + \text{4-AAP + p-HBA} \xrightarrow{\text{POD}} \text{Colored Comp. +4H2O}$$

Formed color is measured at 546 nm and is proportional to HDL-Cholesterol concentration in sample when used as directed.

SPECIMEN COLLECTION:

Fresh (or just defrosted) not hemolized serum or plasma (EDTA Na_2 , Na Heparin) .
Centrifuge and collect serum as soon as possible .
HDL_C in serum or plasma is stable up to 7 days at +2-8ºC ,1 month at20ºC and 2 years at –70ºC Shake and bring the samples at room temperature (+15-25ºC.) before using .

REAGENTS COMPOSITON :

The reagents set are stored at ambient temperature. Storage must not exceed expiration date on box label.

Precipitating Reagent	
Phosphotungstic acid Magnesium Chloride	0.55 mM 25 mM

PACKAGE : Collection & Storage .

Store at +2-8ºC.
Stable until the expiration date reported upon the package.
After the unsealing and the taking of the reagent , it is advised to close up the bottle immediately in order to avoid evaporation , direct light exposure and bacterial contamination .

PRECAUTION & WARNING:

Avoid pipetting by mouth .
The preparation , according to current regulation . is classified as not dangerous.
The total concentration of non active components (preservatives , detergents ,stabilizers) is below the minimum required for citation .
Anyway handle with care , avoid ingestion , avoid contact with eyes , skin and mucous membranes . The samples must be handle as potentially infected from HIV or Hepatitis .

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations .

PROCEDURE :

This methodology describes the manual procedure to use the kit .
For automated procedure, ask for specific application .

Precipitation

Specimen	200 µl
Precipitant	500 µl

Mix and allow standing for 10 minutes at room temperature. Centrifuge for 10 minutes at 4000 rpm, or 2 minutes 12000rpm. Separate of the clear supernatant within two hours and determine the cholesterol content by the CHOD-PAP method. The supernatant may be stored up to five days at 2-8°C

Wavelength	546 nm
Optical path	1 cm
Incubation temperature	20, 25 or 37°C
Zero adjustment	Reagent blank

	Blank	Standard	Specimen
cholesterol reagent	1.0 ml	1.0 ml	1.0 ml
Specimen	—		100 µl
Distilled water	100 µl		—
Standard (R1)		100 µl	
	Mix, and incubate for 5 min. at 37°C or 10 min. at 20-25°C.Measure absorbance of specimen ($A_{specimen}$) against reagent blank within 30 minutes.		

CALCULATION :

1. HDL Cholesterol

$$\text{mg/dl} = 50 \times \frac{(A)\ \text{Sample}}{(A)\ \text{Standard}}$$

2. LDL Cholesterol

$$\text{LDL Cholesterol (mg/dl)} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}$$

EXPECTED VALUES :

HDL:
Women 30-85 mg/dL
Men 30-70 mg/dL
LDL: Adults 66-178 mg/dL
The above mentioned values are to be considered as a reference.
It is strongly recommended that each laboratory establish its own normal range according to its geographic area , according to IFCC protocol .
Since CHOL HDL has an elevated protective action against the risk of arising cardiovascular diseases , the following reference values can be used :

Protective Action	Men	Women
High	> 55mg/dL	> 65 mg/dL
None	35-55 mg/dL	45-65 mg/dL
Poor	< 35 mg/dL	< 45 mg/dL

WASTE DISPOSAL :

The disposal of the product must be in accordance with local regulation concerning waste disposal

QUALITY CONTROL :

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.

PERFORMANCE :

MEASURE INTERVAL L INEARITY :	2-200 mg/dl
LOWEST MEASURABLE LIMIT :	2 mg/dl
SENSITIVITY :	1mg/dl= ΔA

PRECISSION WITH SERIES : n=20

LOW LEVEL	M = 30.2 mg/dl	C.V = 1.50%
MEDIUM LEVEL	M = 42.7 mg/dl	C. V = 1.18%
HIGH LEVEL	M = 75.6 mg/dl	C.V = 1.04%

PRECISSION AMONG SERIES : n=20

LOW LEVEL	M = 29.4 mg/dl	C.V = 2.68%
MEDIUM LEVEL	M = 41.9 mg/dl	C.V = 1.89%
HIGH LEVEL	M = 73.2 mg/dl	C.V = 3.22%
INTER, ANALIZED	30-72 mg/dl	
CORRELATION	r = 0.999	n=50
LIN. REGRESSION	y= 1.01 ×- 3.39	n=50

INTERFERENCE:

Interferences are negligible up to :			
Bilirubin	20 mg/dl		
Hemoglobin	0.4 g/dl	Glucose	500 mg/dl
Ascorbic Acid	40 mg/dl	Triglycerids	2000 mg/dl

METHOD LIMITATIONS:

If Triglycrides levels are higher than 2000 mg/dl , repeat the measure on a sample diluted 1:2 with physiological solution e multiply the results × 2 .
Do not use Anticoagulants containing citrate .
For through evaluation of the interfering substances ,consult : Young , D. S ,et al , AACC Press , Washington DC , , 3-104 (1990)

REFERENCES:

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