

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

ORDER INFORMATION

REF	Cont.
DHDL 40	1 X 40 ML
DHDL 80	2 X 40 ML

CLINICAL SIGNIFICANCE

Lipoproteins serve to solubilise and transport cholesterol and other lipids in the bloodstream. Different lipoprotein classes have been shown to have various effects on the progression of coronary heart disease (CHD). High density lipoproteins are associated with decreased risk and seen as a protective factor.

The measurement of HDL cholesterol (HDL-C) is a powerful predictor of CHD that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease

PRINCIPLE

The method is in a two reagent format and depends on the properties of a unique detergent, as illustrated. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colorless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromagenic coupler to develop color for the quantitative determination of HDL-C.

REAGENT COMPOSITION

Reagent 1 : Enzyme Reagent
Reagent 2 : Developer Reagent
Calibrator : (Lyophilized) Human Serum.

SPECIMEN COLLECTION AND HANDLING.

Fresh serum is the recommended sample. EDTA or heparinized plasma are also acceptable.

Anticoagulants containing citrate should not be used. specimen.

Removed from the blood clot as soon as possible

Stability of the sample: 7 days at 2-8°C .

WORKING REAGENT PREPARATION.

Reagents are ready to use as supplied. **Reconstitute the calibrator with the exact volume of deionized water as mentioned on the label.** Mix well. Allow to stand at room temperature for 30 minutes.

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.

REFERENCE VALUES

	Male	Female
Low Risk	> 50 mg/dl	> 60 mg/dl
Normal Risk	35-50 mg/dl	45-60 mg/dl
High Risk	< 35 mg/dl	< 45 mg/dl

AUTOMATED PARAMETERS	
Wavelength	546 nm
Measurement	Against DI Water
Cuvette	1 cm light path
Reaction Temperature	37°C
Reaction Type	Fix time Kinetic
Reaction Direction	Increasing
Incubation	5 Min. + 5 Min.
Sample Volume	10 µL
Reagent I Volume	750 µL
Reagent II Volume	250 µL
Delay/Lag/Time	5 Sec
Interval Time	300 sec.
Low Normal	35 mg/dl
High Normal	60 mg/dl
Linearity	150 mg/dl

MANUAL ASSAY PROCEDURE

PIPETTE INTO TEST TUBES

	CALIBRATOR	SAMPLE
REAGENT I	750 µl	750 µl
CALIBRATOR	10 µl	-
SAMPLE	-	10 µl
Mix well and incubate for 5 mins at 37°C & Immediately Add		
REAGENT II	250 µl	250 µl
Read the absorbance (A1) at 546 nm immediately after 5 seconds after addition of R2 reagent .After 5 minutes read the absorbance A2		

CALCULATIONS:

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

$$\text{HDL Chol (mg/dL)} = \frac{(A2-A1) \text{ of Unknown} \times \text{Calibrator value}}{(A2-A1) \text{ of Calibrator}}$$

NOTES:

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.

LINEARITY

The method is linear upto a concentration of 150 mg/dl. Dilute samples above this concentration 1:1 and multiply the result by 2.

BIBLIOGRAPHY

Matsuzaki Y., Kawaguchi E., Norita Y. et al Evaluation of Two Kinds of Reagents for Direct Determination of HDL-Cholesterol. J.Anal Bio Sc 1996; 19:419-427.

