LDL CHOLESTEROL
Friedwald Method

IVD CE

PRODUCT CODE
CS011

INTENDED USE
This reagent is intended for in vitro quantitative determination of LDL Cholesterol in serum & plasma

CLINICAL SIGNIFICANCE
Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, low density lipoprotein cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported. LDL Cholesterol acts as a key factor in the pathogenesis of atherosclerosis and coronary artery disease

PRINCIPLE
The Cholesterol content of low-density lipoproteins (LDL) can be extrapolated using the Friedwald equation.

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

$$\text{LDL Cholesterol (mmol/L)} = \text{Total Cholesterol} - \frac{(\text{Triglyceride})}{2.2} - \text{HDL}$$

5: 1 ratio exists between plasma triglycerides & VLDL Cholesterol over a broad range. If the total triglyceride is greater than 400 mg / dl, this approximation for VLDL Cholesterol is no longer valid and the sample will need to be diluted.

REAGENT VOLUMES AND PREPARATION

1	Cholesterol Reagent	Bio Research (CS005)	Ready for use
2	Cholesterol Standard	Bio Research (CS005)	Ready for use
3	HDL-Precipitant	Bio Research (CS009)	Macro assay Ready for use Micro assay, dilute 1:4 with dist. Water
4	Triglyceride Enzyme Conc. (Reagent 1)	Bio Research (CS016)	Diluent 1:100 with Triglyceride buffer, stable for 3 weeks at 2-8°C
5	Triglyceride Buffer (Reagent 2)	Bio Research (CS016)	Diluent for working reagent.
6	Triglyceride Standard	Bio Research (CS016)	Ready for use

REAGENT STORAGE AND STABILITY
When stored at 2-8°C, the reagent is stable up to the expiry date. If cloudiness develops the reagent may have deteriorated and should not be used.

SPECIMEN
Serum, heparinized or EDTA plasma.

PRECAUTION
To avoid contamination, use clean laboratory wares.
Avoid direct exposure of reagent to light.

1- CHOLESTEROL (PRODUCT CODE CS005)
ASSAY

Wavelength	:	546 nm
Cuvette	:	1 cm light path
Temperature	:	20-25°C or 37°C
Measurement	:	Against reagent blank

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Cholesterol reagent	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Sample	--	--	10 µL

Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C
Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank

CALCULATION

$$\text{Cholesterol Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 200 \text{ (Std.conc.)}$$

To convert mg/dL to mmol/L divide by 38.9

LINEARITY
This reagent is linear up to 750 mg/dL or 19.30 mmol/L
If the concentration is greater than linearity (750 mg/dL), dilute the sample 1+2 with physiological saline (0.9%) and repeat the assay. Multiply the result by 3.

NORMAL RANGE

Desirable	<200mg/dL	<5.1mmol/L
Suspect	200 – 240mg/dL	5.1 – 6.2 mmol/L
High	> 240mg/dL	> 6.2 mmol/L

2- HDL CHOLESTEROL (PRODUCT CODE CS009)

-REAGENT PREPARATION

1- Macro – assay   -Reagent is ready for use

2- Semi Macro assay   - pre-dilute the reagent with distilled water before use (80 ml of reagent and 20 ml water).

PROCEDURE (Step 1):

	MACRO	SEMI MICRO
Sample	500 µL	200 µL
HDL reagent undiluted	1000 µL	--
HDL reagent diluted	--	500 µL

Mix and allow to stand for 10 minutes. Centrifuge for 10 minutes at 4000 rpm . Determine the cholesterol content of the HDL supernatant by using **Bio Research Cholesterol test kit (Product Code: CS005).**

ASSAY

Wavelength	:	546nm
Cuvette	:	1 cm light path
Temperature	:	20-25°C or 37°C
Measurement	:	Against reagent blank

PROCEDURE (Step 2):

Pipette into cuvettes	Blank	Sample
Distilled H$_2$O	100 µL	---
HDL supernatant	---	100 µL
Cholesterol reagent	1000 µL	1000 µL

Mix and incubate for 20 minutes at 25°C or 10 minutes at 37°C Measure the absorbance of sample against the reagent blank within 30 minutes (ΔA).

CALCULATION
HDL Cholesterol Conc. (mg/dL) =   ΔA X Factor

FACTOR

MACRO	SEMI-MICRO
274 mg/dL	320 mg/dL
7.05 mmol/L	8.23 mmol/L

To convert mg/dL to mmol/L divide by 38.9

NORMAL RANGE

Male	55 mg/dL	1.42 mmol/L
Female	65 mg/dL	1.67 mmol/L

LINEARITY
up to 200 mg/dl, If the results obtained were greater than the linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2

3- TRIGLYCERIDES (PRODUCT CODE CS016)

-REAGENT PREPARATION
-To prepare working reagent, dilute 1 part of Reagent 1 (Enzyme concentrate) with 100 parts of Reagent 2 (buffer), e.g.: 1 mL / 100mL, 100µL/10mL
-Mix gently and allow equilibrating to room temperature before use, stable for 3 weeks at a 2-8°C

ASSAY

Wavelength	:	546nm
Cuvette	:	1 cm light path
Temperature	:	25°C or 37°C
Measurement	:	Against reagent blank

PROCEDURE

	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Sample	--	--	10 µL

Mix and incubate for 10 minutes at 25°C or 5 minutes at 37°C.Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank within an hour.

CALCULATION

$$\text{Triglyceride Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 200 \text{ (Std.conc.)}$$

To convert mg/dL to mmol/L, divide by 88.50

NORMAL RANGE
36 – 165 mg/dl; 0.4 – 1.86 mmol/l: > 200 mg/dl elevated
It is strongly recommend each laboratory establish its own normal

LINEARITY

up to 1000 mg/dl (11.3mmol/l), Sample with higher values should be diluted 1+5 with physiological saline (0.9 % Nac1), multiply the values by 6.

4-   LDL CHOLESTEROL (PRODUCT CODE CS011)

- **CALCULATION**

(LDL) can be extrapolated using the Friedwald equation:

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

NORMAL RANGE

Optimal	< 100 mg/dL
Near or above optimal	100-129 mg/dL
Borderline high	130-160 mg/dL
High	> 160 mg/dL

To convert mg/dL to mmol/L divide by 38.9

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
(hourglass)	Expiry Date	VOL	Volume
(thermometer)	Storage Condition	LOT	Lot Number
(book)	Instruction for Use	IVD	In Vitro Diagnostics
(factory/date)	Manufacturing Date	(factory)	Manufacturer
Σ	Number of Tests	②	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBILOGRAPHY

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3-   Trinder, P. Ann. Clin. Biochem, 6,24,1969.
4-   Allain, C.C. et al.; Clin. Chem., 20, 470, 1974.