ACCUCARE®

LDL CHOLESTEROL

Direct Enzymatic Colorimetric Method

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

ORDER INFORMATION

REF	Cont.
DLDL 20	1 X 20 mL
DLDL 40	1 X 40 mL
DLDL 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).
The measurement of LDL cholesterol (LDL-C) is a powerful predictor of CHD that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease

Method

Photometric Test method. The **Accucare LDL Cholesterol** Reagent is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of R2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

PRINCIPLE

The LDL Direct Cholesterol assay is a homogeneous method for directly measuring LDL-C levels in serum or plasma, without the need for any off-line pretreatment or centrifugation steps.
The method is in a two reagent format and depends on the properties of a unique detergent. This detergent (Reagent 1) solubilizes only the non LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction.
A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

REAGENT

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

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.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum or heparin plasma
It is very important to store the sample protected from light!
Stability: 1 day at 20 – 25°C
7 days at 4 – 8°C
3 months at –20°C in case of immediate freezing.
Freeze only once! Discard contaminated specimens!

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28°C, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	546 nm
Measurement	Against Reagent blank
Cuvette	1 cm light path
Reaction Temperature	37°C
Reaction Type	End Point
Reaction Direction	Increasing
Incubation	5 Min. + 5 Min.
Sample Volume	10 µl
Reagent I Volume	750 µl
Reagent II Volume	250 µl
Low Normal	0 mg/dl
High Normal	100 mg/dl
Linearity	400 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
After 5 minutes at 37°C. Read the absorbance (Ac) for calibrator and absorbance (As) for sample.		

SAMPLE DILUTIONS

- This method is linear upto a concentration of 400 mg/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CALCULATION

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

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

CALIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.
The assigned values of the calibrator have been made traceable to NIST SRM® 1951.
It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the

measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Level 1	121.42	4.74	3.91%
Level 2	400.46	4.42	1.10%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Level 1	117.58	3.53	3.05%
Level 2	400.21	4.36	1.90%

LINEARITY

The method is linear upto a concentration of 400mg/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for LDL Cholesterol is 2 mg/dL.

METHOD COMPARISON

A comparison of Accucare LDL Cholesterol with a commercially available assay (x) using 20 samples gave following results: R^2 = 0.9900

REFERENCE VALUES

Optimal	<100
Near or above optimal	100-129
Borderline high	130-159
High	160-189
Very high	>190

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

INTERFERENCE

- Hemoglobin: No interference found upto 500 mg/dL.
- Bilirubin: No interference found upto Bilirubin (free) 50 mg/dL, Bilirubin (Conjugated) 40m/dL.
- Ascorbic Acid: No interference found upto 50 mg/dL.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

1. Friedewald, W.T., Levy, R.I., and Fredrickson, D.S., Estimation of the low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge, Clin. Chem. 18, 1972, p. 449 - 502.

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
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