



# LDL CHOLESTEROL (DIRECT Method-End Point)

## Intended Use

The reagents are used for the quantitative determination of LDL cholesterol in serum or plasma. For in-vitro diagnostic use only.

## Introduction

LDL transports cholesterol and triglycerides from the liver to peripheral tissues. LDL Cholesterol is called bad cholesterol because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis. The measurement of LDL cholesterol (LDL-C) is a powerful predictor of coronary heart disease that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease.

## Method

Direct Method, End Point.

## Principle

In the first step HDL, VLDL & chylomicrons are eliminated & transformed to non-reactive components under specific conditions for the reaction. By the second reagent only LDL-cholesterol is subject to colour reaction.



## Reagent Composition

### Reagent 1:

Peroxidase	2.5 KU/I
Cholesterol esterase(CHE)	1 KU/I
Cholesterol Oxidase(CHO)	1 KU/I

### Reagent 2:

4-aminoantipyrine	2.5 mmol/L
Pipes	50 mmol/L

\*Calibrator is not provided with this kit.

## Precautions

Following precaution should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

## Reagent Preparation

Reagent are ready for use.

## Reagent Storage and Stability

Unopened reagents are stable till expiry mentioned on the label when stored at 2-8°C temperature.

## Reagent Deterioration

Reagents should be clear. Turbidity and/or precipitation may be because of reagent deterioration. R2 is clear light yellow solution.

## Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 7 days at 2-8°C and two weeks when frozen.

## GENERAL ASSAY PARAMETERS

Mode	End Point
Wavelength 1 (nm)	546
Wavelength 2 (nm)	700
Sample Volume (µl)	6
Reagent R1 (µl)	600
Reagent R2 (µl)	200
Incubation Time (min.)	5 + 5
Incubation Temp. (°C)	37
Normal Level (mg/dl)	<130
Linearity (mg/dl)	Upto 400
Concentration of Calibration (when provided)	As on vial
Blank with	Reagent
Units	mg/dl

## PROCEDURE

Pipette into test tubes as given below:

Addition Seq.	Blank	Calibrator	Sample
Sample	-	-	6µl
Calibrator	-	6µl	-
Dist. Water	6µl	-	-
Reagent R1	600µl	600µl	600µl
Mix and incubate for 5 min. at 37°C			
Reagent R2	200 µl	200 µl	200 µl

Mix and incubate for 5 min. at 37°C. Measure the Abs. of calibrator & sample against reagent blank.

## Calculation

$$\text{Conc. of LDL-Cholesterol (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Calib.}}} \times \text{Conc. of Calibrator (mg/dl)}$$

Example: If the absorbance of sample is 0.218 and the absorbance of calibrator is 0.162. The calculation shall be:

$$\frac{0.218}{0.162} \times 103 = 138.6 \text{ mg/dl}$$

Here the concentration of calibrator is 103 mg/dl. However, for different calibrator, the concentration of calibrator varies. If the LDL cholesterol concentration exceeds 400mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

## Reference value

Desirable : 130mg/dl (3.4mmol/L)  
 Borderline Risk : 130-160mg/dl (3.4-4.1mmol/L)  
 High Risk : >160mg/dl (>4.1mmol/L)

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

### Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample:reagent, ratio remains same.
2. Gross Hemolysis and lipemia may result in false elevated results.

### Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for LDL cholesterol.

### Performance

Linearity Limit: 400mg/dl

### Precision:

### Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	138.36	65.57
S.D.	1.07	0.46
C.V. %	0.77	0.71

### Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	138.48	65.03
S.D.	0.91	0.61
C.V. %	0.66	0.93

### References

1. Tietz NW. Clinical guide to laboratory tests, 2nd ed. Saunders Co., 1991.

2. Superko HR, Nejedly M, Garrett B (2002). "Small LDL and its clinical importance as a new CAD risk factor: a female case study". Prog Cardiovasc Nurs 17 (4): 167-73. doi:10.1111/j.08897204.2002.01453.x.PMID12417832.http://www.lejacq.com/articleDetail.cfm?pid=ProgCardiovascNu rs\_17;4:167.Retrie ved on 2007-12-04.
3. Effect of antioxidants on oxidative modification of LDL. Esterbauer H, Puhl H, Dieber-Rotheneder M, Waeg G, Rabl H. Ann Med. 1991;23(5):573- 81. http://www.inc-opc.com/content/view/67/66/
4. Okada M. et al. Low-density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Med., 1998; 132, 195-201.
5. Gotto, A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital Practice, 23. Suppl. 1, 4-13, 1988.

### Pack Presentation

Product Code/ Catalogue No.	Pack Size*	Reagent 1	Reagent 2
KGLCH103.3.1	1x15, 1x5ml	1x15ml	1x5ml
KGLCH103.3.2	1x30, 1x10ml	1x30ml	1x10ml
KGLCH103.3.3	2x30, 2x10ml	2x30ml	2x10ml

\* Pack size may vary on market demand.

Revision No: (ver: KGLCH103.3/1)

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### Symbols

Following symbols are used in the labeling of KEE GAD kits:



Catalogue No.



Batch No.



CE Mark



Read instructions



In Vitro Diagnostics



Storage temperature



Expiry Date



Content



Product Name



Manufactured By



**Manufactured by:**  
**KEE GAD Biogen Pvt. Ltd.**  
 A-8, Third Floor, Naraina Industrial Area,  
 Phase-II, New Delhi-110028 (India)