Low-Density Lipoprotein Cholesterol (LDL-C) Assay Kit

Catalog No.: abx298881

Size: 96 tests

Detection Range: 0.04 mmol/L – 12 mmol/L

Sensitivity: 0.04 mmol/L

Storage: Store all components at 4°C in the dark.

Application: For detection of low-density lipoprotein cholesterol (LDL-C) concentration in serum, plasma, cell culture supernatants, and tissue homogenates.

Introduction

Abbexa's Low-Density Lipoprotein Cholesterol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating low-density lipoprotein cholesterol concentration. Unlike other lipoproteins such as HDL, CM, and VLDL, LDL retains its structure and does not dissociate when under the action of surfactants. The intact LDL particles are then able to be dissociated by a coupling reagent to release cholesterol. The cholesterol is catalyzed by cholesterol esterase (CE) and cholesterol oxidase (CO) to produce hydrogen peroxide which can then be catalyzed by peroxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound with an absorbance maximum of 546 nm. The intensity of the color is proportional to the low-density lipoprotein cholesterol (LDL-C) concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme Working Solution 1: 18 ml
3. Enzyme Working Solution 2: 6 ml
4. Standard: 1 vial
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (546 nm) or Biochemical analyzer (546 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Normal saline (0.9 % NaCl)
5. Isopropanol
6. Pipette and pipette tips
7. 1.5 ml microcentrifuge tubes
8. Centrifuge
9. Vortex mixer
10. Incubator
11. Water bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly. Unused sample can be stored at -80°C for up to 1 month.
- **Cell Culture Supernatants:** Collect at least 1 x 10^6 cells and wash with PBS (0.01 M, pH 7.4). Add 300 µl – 500 µl of isopropanol and homogenize manually by ultrasonication at 4°C. Centrifuge at 10,000 × g for 10 minutes, then carefully remove the supernatant and keep on ice.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash with PBS (0.01 M, pH 7.4). Add 180 µl of isopropanol and homogenize manually using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes, then carefully remove the supernatant and keep on ice.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4) for serum (plasma) samples and isopropanol for tissue samples, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human plasma	1
Mouse serum	1
Rat Plasma	1
Porcine serum	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Working Solution 1 and 2:** Incubate the amount needed for the assay of Enzyme Working Solution 1 and Enzyme Working Solution 2 at 25°C for 15 minutes. The remaining Enzyme Working Solution 1 and Enzyme Working Solution 2 can be stored at 4°C.
- **Standard Solution:** Dissolve 1 vial of standard with 200 µl of double-distilled water. Unused Standard Solution can be stored at 4°C for up to 2 weeks in the dark.

Note:

- Allow all reagents (except Enzyme Working Solution 1 and 2) to equilibrate to room temperature before use.
- Take care to avoid bubbles when adding the liquid to the microplate.
- Avoid contamination of reagents with glucose, cholesterol, etc.
- The amount of reagent and sample can be adjusted proportionally according to the volume of the cuvette.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

96 Well Microplate Reader:

1. Assign microplate wells for each standard, sample, and blank. Each sample requires a corresponding blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 5 µl of double-distilled water to the blank wells.
3. Add 5 µl of Standard Solution to the standard wells.
4. Add 5 µl of Sample to the sample wells.
5. Add 180 µl of Enzyme Working Solution 1 to all wells.
6. Mix fully, then incubate at 37°C for 5 minutes.
7. Measure the OD (A_1) of each well with a microplate reader at 546 nm.
8. Add 60 µl of Enzyme Working Solution 2 to all wells.
9. Mix fully, then incubate at 37°C for 5 minutes.
10. Measure the OD (A_2) of each well with a microplate reader at 546 nm

Biochemical Analyzer:

1. Set up the biochemical analyzer using the following parameters:
    - Main Wavelength: 546 nm
    - Reaction Type: Terminal Method

- Reaction Direction: Up (+)

2. Add 5 µl of sample or double distilled water to the tube.
3. Add 180 µl of Enzyme Working Solution 1 to the tube. Mix fully.
4. Incubate at 37°C for 5 minutes. Measure the OD value at 546 nm using the biochemical analyzer. Record this as A_1.
5. Add 60 µl of Enzyme Working Solution 2 to the tube. Mix fully.
6. Incubate at 37°C for 5 minutes. Measure the OD value at 546 nm using the biochemical analyzer. Record this as A_2.

$\Delta A = A_2 - A_1$

C. **Calculation of Results**

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of low-density lipoprotein cholesterol in each sample well can be derived with the following formulae:

1. Serum (Plasma) and other liquid samples:

Calculations from microplate reader:

$$\mathbf{LDL\text{-}C\ (mmol/L) = F \times C \times \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}}}$$

Calculations from biochemical analyzer:

$$\mathbf{LDL\text{-}C\ (mmol/L) = F \times C \times \frac{\Delta A_{Sample}}{\Delta A_{Standard}}}$$

2. Cell Culture Supernatants:

Calculations from microplate reader:

$$\mathbf{LDL\text{-}C\ (mmol/10^6) = F \times C \times \frac{V \times (\Delta A_{Sample} - \Delta A_{Blank})}{N \times (\Delta A_{Standard} - \Delta A_{Blank})}}$$

Calculations from biochemical analyzer:

$$\text{LDL-C } (\text{mmol}/10^6) = \mathbf{F} \times \mathbf{C} \times \frac{\mathbf{V} \times (\mathbf{\Delta A_{Sample}})}{\mathbf{N} \times (\mathbf{\Delta A_{Standard}})}$$

1. Tissue samples:

Calculations from microplate reader:

$$\text{LDL-C } (\text{mmol/g fresh weight}) = \mathbf{F} \times \mathbf{C} \times \frac{\mathbf{V} \times (\mathbf{\Delta A_{Sample}} - \mathbf{\Delta A_{Blank}})}{\mathbf{W} \times (\mathbf{\Delta A_{Standard}} - \mathbf{\Delta A_{Blank}})}$$

Calculations from biochemical analyzer:

$$\text{LDL-C } (\text{mmol/g fresh weight}) = \mathbf{F} \times \mathbf{C} \times \frac{\mathbf{V} \times (\mathbf{\Delta A_{Sample}})}{\mathbf{W} \times (\mathbf{\Delta A_{Standard}})}$$

where:

ΔA_{Sample}	ΔA value of sample
$\Delta A_{Standard}$	ΔA value of standard
ΔA_{Blank}	ΔA value of blank
V	Volume of isopropanol (L)
C	Concentration of standard
W	The weight of the tissue sample (g)
N	Number of cells (x10^6). E.g. for 1 x 10^6 cells, N = 1
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.