

## EZMeta™ Low Density Lipoprotein Cholesterol (LDL-C)

### Colorimetric Assay Kit

Cat #: D-AKC2260

Size: 48T / 96T

Storage: Stored at -20°C for 6 months, protected from light

### Product Information

**Detection range:** 0.078-5 mmol/L (The detection range corresponds to the standard, and the actual content of sample is 0.117-7.5 mmol/L)

**Sensitivity:** 0.039 mmol/L (The sensitivity corresponds to the standard, and the actual content of sample is 0.06 mmol/L)

**Applicable samples:** Serum

### Assay Principle

Low Density Lipoprotein Cholesterol (LDL-C) as one of the serum proteins, mainly synthesized by the liver. It's a lipoprotein particle that carries cholesterol into peripheral tissue cells. LDL can enter the cells of the artery wall and carry cholesterol into it. It can also be oxidized to oxidized LDL. When LDL, especially oxidized LDL (ox-LDL), is in excess, the cholesterol it carries can build up in the walls of arteries, causing atherosclerosis over time. Therefore, LDL is called the "bad cholesterol". Low density lipoprotein is positively correlated with the occurrence of coronary heart disease (CHD) and atherosclerotic damage, which is an important index for lipid disease classification and risk prediction. EZMeta™ Low Density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit provides a convenient tool for detection of LDL-C. The principle is that LDL-C in serum was separated with precipitant, and cholesterol esterase was used to catalyze the hydrolysis of cholesterol ester into free cholesterol (FC) and free fatty acid (FFA), so as to convert cholesterol ester into FC. Furthermore, cholesterol oxidase catalyzed FC oxidation to produce  $\Delta^4$ -cholestenone and  $H_2O_2$ . Then peroxidase catalyzes the oxidation of 4-amino-antipyrine and phenol with  $H_2O_2$  to produce red quinone compounds has a characteristic absorption peak at 500 nm.

## Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	10 mL	20 mL	4°C
Chromogen	7.5 mL	15 mL	4°C, protected from light
Enzyme Mix	1	1	-20°C, protected from light
Assay Buffer	5 mL	10 mL	4°C
Standard	0.25 mL	0.5 mL	-20°C, protected from light
Standard Diluent	5 mL	10 mL	4°C

## Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 500 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

## Reagent Preparation

**Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Chromogen:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

**Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Standard Diluent:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Enzyme Mix:** Add 5 mL Assay Buffer for 96 T or 2.5 mL Assay Buffer for 48 T to fully dissolve before use. This solution can be stored at 4°C for one week or be stored at -20°C, protected from light after aliquoting to avoid repeated freezing and thawing.

**Standard:** Containing 5 mmol/L Cholesterol Standard. Store at -20°C, protected from light.

**Working Reagent:** Prepare 200 µL Working Reagent for one well, add 50 µL dissolved Enzyme Mix and 150 µL Chromogen. Prepare Work Reagent before use and depend on your need. Working Reagent is freshly prepared.

**Standard curve setting:** dilute 5 mmol/L Cholesterol Standard with Standard Diluent to 5, 2.5, 1.25, 0.625, 0.313, 0.156,

0.078, 0 mmol/L standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Standard Diluent ( $\mu\text{L}$ )	The concentration of Standard (mmol/L)
Std.1	100 $\mu\text{L}$ 5 mmol/L	0	5
Std.2	50 $\mu\text{L}$ of Std.1 (5 mmol/L)	50	2.5
Std.3	50 $\mu\text{L}$ of Std.2 (2.5 mmol/L)	50	1.25
Std.4	50 $\mu\text{L}$ of Std.3 (1.25 mmol/L)	50	0.625
Std.5	50 $\mu\text{L}$ of Std.4 (0.625 mmol/L)	50	0.313
Std.6	50 $\mu\text{L}$ of Std.5 (0.313 mmol/L)	50	0.156
Std.7	50 $\mu\text{L}$ of Std.6 (0.156 mmol/L)	50	0.078
Std.8	0	100	0

**Note:** Always prepare fresh standards per use.

## Sample Preparation

**Note:** Fresh samples are recommended. If not assayed immediately, samples can be stored at  $-80^{\circ}\text{C}$  for one month.

Serum was separated within 3 h after blood collection and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at  $-80^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Thoroughly mix the serum sample with the Extraction Buffer in a ratio of 2:1 and let stand at  $25^{\circ}\text{C}$  for 15 min, then centrifuge at 2,000 g for 15 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 500 nm. Visible spectrophotometer was returned to zero with deionized water. Preheat the incubator to 37°C.

2. Add the following reagents respectively to the 96-well plate or microglass cuvette:

Reagent	Standard Well ( $\mu\text{L}$ )	Test Well ( $\mu\text{L}$ )
Stds.	20	0
Sample	0	20
Working Reagent	200	200

3. Mix well and incubate at 37°C for 30 min. Then reading the absorption values at 500 nm and record it as A.

## Data Analysis

1. Drawing of standard curve

Each standard and sample absorption values subtract the zero standard (Std.8) absorption value to obtain  $\Delta A$ .

With the concentration of the standard solution as the y-axis and the  $\Delta A_{\text{Standard}}$  as the x-axis, draw the standard curve.

Substitute the  $\Delta A_{\text{Test}}$  into the equation to obtain the y value (mmol/L).

2. Calculate the content of LDL-C in sample

$$\text{LDL-C (mmol/L)} = 1.5 \times y$$

Where: 1.5, Diluted multiples during sample preparation =  $(2+1)/2 = 1.5$ .

## Typical Data

Typical standard curve

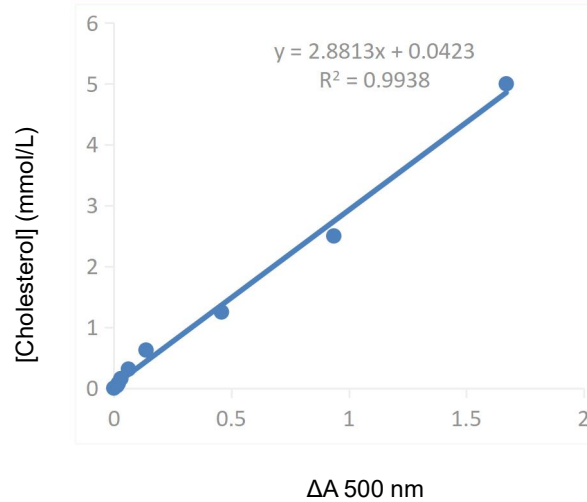


Figure 1. Standard curve of cholesterol in 96-well plate assay—data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.