Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Low Density Lipoprotein Cholesterol (LDL-C) Assay Kit

Cat #: KTB2260 Size: 48 T/48 S 96 T/96 S

	Micro Low Density Lipoprotein Cholesterol (LDL-C) Assay Kit		
REF	**Cat #:** KTB2260	LOT	**Lot #:** Refer to product label
	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		
	Storage: Stored at -20℃ for 6 months, protected from light		

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. CheKine™ Micro Low Density Lipoprotein Cholesterol (LDL-C) 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 Δ4-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	12 mL	24 mL	4℃
Chromogen	11 mL	22 mL	4℃, protected from light
Enzyme Mix	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Assay Buffer	5 mL	10 mL	4℃
Standard	0.25 mL	0.5 mL	-20℃, protected from light
Standard Diluent	5 mL	10 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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℃.

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

Note: Chromogen is toxic, so it is recommended to experiment in a fume hood.

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

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

Enzyme Mix： Add 7 mL Assay Buffer for 96 T or 3.5 mL Assay Buffer for 48 T to fully dissolve before use. This solution can be stored at 4℃ for one week or be stored at -20℃ for one month, protected from light after aliquoting to avoid repeated freezing and thawing.

Standard： Containing 5 mmol/L Cholesterol Standard. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

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 (μL)	The concentration of Standard（mmol/L)
Std.1	100 μL 5 mmol/L	0	5
Std.2	50 μL of Std.1 (5 mmol/L)	50	2.5
Std.3	50 μL of Std.2 (2.5 mmol/L)	50	1.25
Std.4	50 μL of Std.3 (1.25 mmol/L)	50	0.625
Std.5	50 μL of Std.4 (0.625 mmol/L)	50	0.313
Std.6	50 μL of Std.5 (0.313 mmol/L)	50	0.156
Std.7	50 μL of Std.6 (0.156 mmol/L)	50	0.078
Std.8	0	100	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ 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℃. 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℃ 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℃.
2. Add the following reagents respectively to the 96-well plate or microglass cuvette:

Reagent	Standard Well (μL)	Test Well (μL)
Stds.	20	0
Sample	0	20
Working Reagent	200	200

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

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

1. Drawing of standard curve

Each standard and sample absorption values subtract the zero standard (Std.8) absorption value to obtain ΔA.

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (mmol/L).

2. Calculate the content of LDL-C in sample

LDL-C (mmol/L)**=1.5×y**

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

Typical Data

Typical standard curve

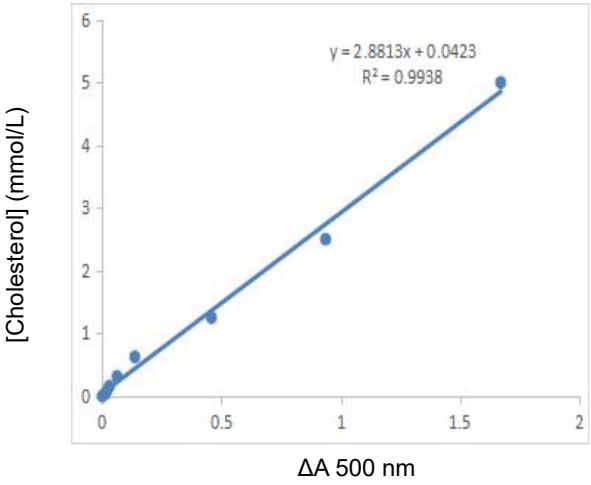

Figure 1. Standard curve of cholesterol in 96-well plate assay–data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB2210	CheKine™ Micro Free Cholesterol (FC) Assay Kit
KTB2220	CheKine™ Micro Total Cholesterol (TC) Assay Kit
KTB2230	CheKine™ Micro Free Fat Acid (FFA) Assay Kit

KTB2240	CheKine™ Micro Fatty Acid Synthetase (FAS) Activity Assay Kit
KTB2250	CheKine™ Micro High Density Lipoprotein Cholesterol (HDL-C) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.