CheKine™ Micro High Density Lipoprotein Cholesterol (HDL-C) Assay Kit

Cat #: KTB2250 Size: 48 T/96 T

	Micro High Density Lipoprotein Cholesterol (HDL-C) Assay Kit		
REF	**Cat #:** KTB2250	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 0.156-10 mmol/L)		**Sensitivity:** 0.039 mmol/L (The sensitivity corresponds to the standard, and the actual content of sample is 0.078 mmol/L)
	Applicable samples: Serum		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

High Density Lipoprotein Cholesterol (HDL-C) as one of the serum proteins, mainly synthesized by the liver. It's carrying cholesterol from surrounding tissues, which is then converted into bile acids or directly excreted from the intestine through bile. It is an anti-atherosclerotic plasma lipoprotein, a protective factor for coronary heart disease (CHD), commonly known as "vascular scavenger", and is an important reference index for the clinical diagnosis of coronary heart disease (CHD). CheKine™ Micro High Density Lipoprotein Cholesterol (HDL-C) Assay Kit provides a convenient tool for detection of HDL-C. The principle is that HDL-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	10 mL	20 mL	4℃
Chromogen	7.5 mL	15 mL	4℃, protected from light
Enzyme Mix	1	1	-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℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 500 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, incubator, ice maker
- Deionized water
- Homogenizer (for tissue samples)

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.

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 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℃ for one week or be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Standard: Containing 5 mmol/L cholesterol Standard. Store at -20℃, protected from light.

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

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 a fresh set of standards per use.

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 1:1 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 teader 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 microplate or microglass cuvette:

Reagent	Standard Well（μL）	Test Well (μL)
Different Concentration Std.	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 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 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 HDL-C in sample

HDL-C (mmol/L)**=2×y**

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

Typical Data

Typical standard curve:

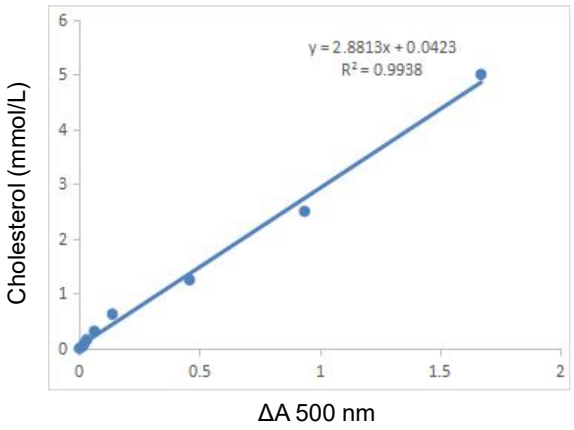

Figure 1. Standard curve for cholesterol.

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
KTB2260	CheKine™ Micro Low Density Lipoprotein Cholesterol (LDL-C) Assay Kit

Disclaimer

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