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CheKine™ Micro Total Cholesterol (TC) Assay Kit

Cat #: KTB2220 Size: 48 T/48 S 96 T/96 S

	Micro Total Cholesterol (TC) Assay Kit		
REF	**Cat #:** KTB2220	LOT	**Lot #:** Refer to product label
	Detection range: 0.07-7 μmoL/mL		**Sensitivity:** 0.07 μmoL/mL
	Applicable samples: Serum, Plasma, Animal Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Total Cholesterol (TC) includes free cholesterol and cholesterol esters. Tissue TC refers to the sum of cholesterol contained in all lipoproteins in the tissue. CheKine™ Micro Total Cholesterol (TC) Assay Kit provides a simple method for detecting TC concentration in a variety of biological samples such as serum, plasma, animal tissues and cells, bacteria. In the assay, esterase catalyzes the hydrolysis of cholesterol esters to produce Free Cholesterol (FC) and Free Fatty Acids (FFA). Then Cholesterol oxidase catalyzes FC to produce Δ4-cholestenone and H_2O_2. Further, H_2O_2, 4-aminoantipyrine and phenol can be catalyzed by peroxidase to form red quinone compounds which has a characteristic absorption peak at 500 nm, the color depth is proportional to the TC content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Working Reagent Ⅰ	Empty Bottle×1	Empty Bottle×1	RT
Reagent Ⅱ	5 mL	10 mL	4℃
Reagent Ⅲ	10 mL	20 mL	4℃, protected from light
Standard	1 mL	1 mL	-20℃, protected from light

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 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, water bath

- Anhydrous ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

Working Reagent Ⅰ: Prepared before use.For the 48 T kit, dissolve the Reagent Ⅰ thoroughly in 4 mL Reagent Ⅱ and transfer it to the Working Reagent I bottle. For the 96 T kit, dissolve the Reagent Ⅰ thoroughly in 8 mL Reagent Ⅱ and transfer it to the Working Reagent I bottle. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Solution: Prepared before use, each well requires 200 μL of Working Reagent. Mix Working Reagent Ⅰ and Reagent Ⅲ in a ratio of 1:3. Working Solution is freshly prepared.

Standard: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal tissues: Weigh about 0.1 g tissues and add 1 mL anhydrous ethanol. Homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, discard the supernatant after centrifugation; add 1 mL anhydrous ethanol to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (plasma) samples: Directly test.

Note: The extraction buffer contains components that denature the protein. If the protein concentration is calculated, the protein needs to be extracted with deionized water for determination. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Anhydrous Ethanol	10	0	0
Standard	0	10	0
Sample	0	0	10
Working Solution	200	200	200

Mix well and then incubatefor 15 min at room temperature. Then reading the values at 500 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. 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 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than the $\Delta A_{Standard}$ value of the 2 μmoL/mL Standard, the sample can be appropriately diluted with anhydrous ethanol, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. The test time should not exceed 1 h.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculate the content of TC in sample

(1) By sample fresh weight

TC (μmol/g)=$C_{Standard}$×△A_{Test}÷△$A_{Standard}$×V_{Sample}÷(W×V_{Test}÷$V_{Sample\ Total}$)**=1.25×△A_{Test}÷△$A_{Standard}$÷W**

(2) Calculated by protein concentration

TC (μmol/mg prot)**==**$C_{Standard}$×△A_{Test}÷△$A_{Standard}$×V_{Sample}÷(Cpr×V_{Sample})**=5×△A_{Test}÷△$A_{Standard}$÷Cpr**

(3) Calculated by cells or bacteria number

TC (μmol/10^4)=$C_{Standard}$×△A_{Test}÷△$A_{Standard}$×V_{Sample}÷(500×V_{Sample}÷$V_{Sample\ Total}$)**=0.01×△A_{Test}÷△$A_{Standard}$**

(4) Calculated by liquid volume

TC (μmol/mL)**=**$C_{Standard}$×△A_{Test}÷△$A_{Standard}$**=5×△A_{Test}÷△$A_{Standard}$**

Where: $C_{Standard}$: 5 μmol/mL; Cpr: Sample protein concentration, mg/mL; V_{Sample}: Sample volume added to the reaction system, 0.01 mL; W: Sample weight, g; $V_{Sample\ Total}$: Sample Preparation of added Extraction Buffer volume, 1 mL; 500: Total number of cells or bacteria, 5×10^6.

Typical Data

Add 10 μL mouse serum, according to the procedure, calculated:

A_{Test}=0.302, A_{Blank}=0.092, $A_{Standard}$=0.889; △A_{Test}=0.302-0.092=0.21, △$A_{Standard}$=0.889-0.092=0.797

Calculation of TC concentration (μmol/mL)=5×△A_{Test}÷△$A_{Standard}$=5×0.21÷0.797=1.32 μmol/mL

Recommended Products

Catalog No.	Product Name
KTB2200	CheKine™ Micro Triglyceride (TG) Assay Kit
KTB2210	CheKine™ Micro Free Cholesterol (FC) 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.