



## CheKine™ Micro Total Cholesterol (TC) Assay Kit

Cat #: KTB2220

Size: 48 T/96 T

	<b>Micro Total Cholesterol (TC) Assay Kit</b>		
<b>REF</b>	Cat #: KTB2220	<b>LOT</b>	Lot #: Refer to product label
	<b>Applicable samples:</b> Serum, Plasma, Animal Tissues, Cells, Bacteria		
	<b>Storage:</b> Stored at 4°C for 12 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  $\Delta^4$ -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	
TC Working Solution	10 mL	20 mL	4°C
Standard	1 mL	1 mL	4°C, protected from light

### 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
- Ice maker, refrigerated centrifuge, water bath
- Anhydrous ethanol
- Homogenizer (for tissue samples)

### Reagent Preparation

**TC Working Solution:** Ready to use as supplied. Equilibrate to 37°C before use. Store at 4°C.

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

**Standard Curve Setting:** Dilute the 2 µmol/mL standard with absolute ethanol to 1, 0.5, 0.25, 0.125, 0.063, 0.031 µmol/mL as indicated in the table below.

Num.	Volume of Standard	Volume of Anhydrous Ethanol (µL)	The Concentration of Standard (µmol/mL)
Std.1	200 µL of Standard	0	2
Std.2	100 µL of Std.1 (2 µmol/mL)	100	1
Std.3	100 µL of Std.2 (1 µmol/mL)	100	0.5
Std.4	100 µL of Std.3 (0.5 µmol/mL)	100	0.25
Std.5	100 µL of Std.4 (0.25 µmol/mL)	100	0.125
Std.6	100 µL of Std.5 (0.125 µmol/mL)	100	0.063
Std.7	100 µL of Std.6 (0.063 µmol/mL)	100	0.031

## Sample Preparation

**Note: Fresh samples are recommended, if not assayed immediately, samples can be stored at -80°C 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°C. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect  $5 \times 10^6$  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°C. Use supernatant for assay, and place it on ice to be tested.
3. Serum (plasma) samples: Directly test.

**Note: (1) The extract contains components that denature the protein. If the protein concentration is calculated, it is necessary to extract the protein from the lysate again for determination. (2) 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 500 nm, visible spectrophotometer was returned to zero with deionized water.
2. Warm TC Working Solution to 37°C for more than 30 min before use.
3. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Anhydrous Ethanol	50	0	0
Standard	0	50	0
Sample	0	0	50
TC Working Solution	150	150	150

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

**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  $\Delta A_{\text{Test}}$  is less than 0.001, increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is greater than 2.0, 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. Drawing of standard curve

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 ( $\mu\text{mol/mL}$ ).

2. Calculate the content of TC in sample

(1) By sample fresh weight

$$\text{TC } (\mu\text{mol/g}) = y \div (W \div V_{\text{Extraction}}) \times n = \mathbf{y \div W \times n}$$

(2) Calculated by protein concentration

$$\text{TC } (\mu\text{mol/mg prot}) = \mathbf{y \div Cpr \times n}$$

(3) Calculated by cells or bacteria number

$$\text{TC } (\mu\text{mol}/10^4) = y \div (\text{cells or bacteria number} \div V_{\text{Extraction}}) \times n = \mathbf{y \div 500 \times n}$$

(4) Calculated by liquid volume

$$\text{TC } (\mu\text{mol/mL}) = \mathbf{y \times n}$$

Where: W: sample weight, g;  $V_{\text{Extraction}}$ : Anhydrous ethanol volume added, 1 mL; n: dilution factor; Cpr: supernatant protein concentration, mg/mL; 500: Total number of cells or bacteria,  $5 \times 10^6$ .

## Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

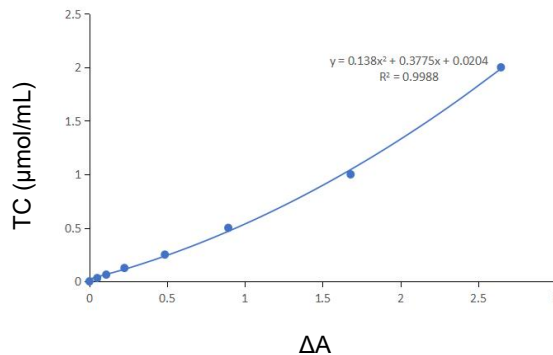


Figure 1. Standard curve for TC.

Examples

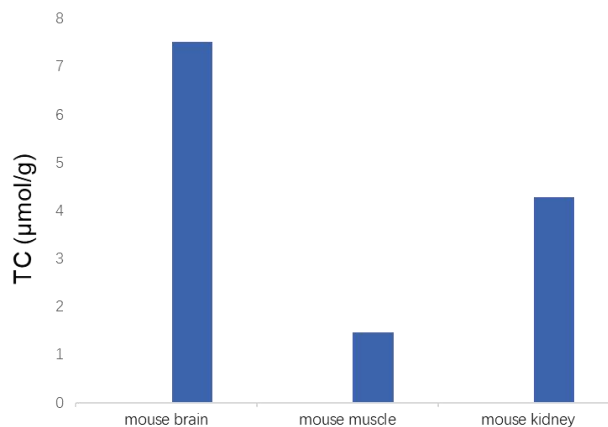


Figure 2. TC content in animal tissues. Assays were performed following kit protocol.

## 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.