CheKine™ Micro Triglyceride (TG) Assay Kit

Cat #: KTB2200 Size: 48 T/48 S 96 T/96 S

	Micro Triglyceride (TG) Assay Kit		
REF	**Cat #:** KTB2200	LOT	**Lot #:** Refer to product label
	Detection range: 0.043-4.3 mg/mL		**Sensitivity:** 0.043 mg/mL
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Triglycerides (TG) are fat molecules formed by long-chain Fatty acids and Glycerol. They are not only the main components of cell membranes, but also important respiratory substrates. Serum triglycerides (TG) is an important index for clinical blood lipid measurement. CheKine™ Micro Triglyceride (TG) Assay Kit provides a simple method for detecting TG concentration in a variety of biological samples such as Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria. In the assay, TG can be extracted by Isopropanol and triglycerides (TG) can be hydrolyzed by lipoprotein lipase into glycerol and free fatty acids. Further, glycerol is phosphorylated to glycerol 3-phosphate by glycerol kinase in the presence of ATP, and then oxidized by glycerol phosphate oxidase to produce hydrogen peroxide. Under the action of catalase, the color substrate is converted to benzoquinimide, and there is an absorption peak at 505 nm. The content of glycerol can be calculated by measuring the absorbance of this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	15 mL	30 mL	4℃
Standard	1 mL	1 mL	4℃, 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
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, EP tubes
- Dounce homogenizer (for tissue samples)

Reagent Preparation

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

Working Reagent Ⅰ: Prepared before use. Add 6 mL Reagent Ⅲ for 48 T and 12 mL Reagent Ⅲ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Reagent Ⅱ: Prepared before use. Add 6 mL Reagent Ⅲ for 48 T and 12 mL Reagent Ⅲ for 96 T to fully dissolve. 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 Ⅲ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Solution: Prepared before use, each well requires 190 μL of Working Reagent. Mix Working Reagent Ⅰ and Working Reagent Ⅱ in a ratio of 1:1. Working Solution is freshly prepared.

Standard: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, 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 0.1 g tissue, add 1 mL Extraction Buffer and 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. Plant Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break 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. Cells or Bacteria: Collect 5 x 10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 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.
4. Serum, Plasma, Cell Supernatant or other Liquid samples: Tested directly by adding samples to the microplate.

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. Triglyceride content determination (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	10
Extraction Buffer	10	0	0
Standard	0	10	0

Working Solution	190	190	190

Mix well, Incubate for 20 min at room temperature. Then reading the values at 505 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 A_{Test} is greater than 1.0, 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

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. Calculation of TG concentration in liquid samples:

TG (mg/dL)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times100$**=$125\times\Delta A_{Test}\div\Delta A_{Standard}$**

2. Calculation of TG concentration in animal and plant tissues:

(1) Calculated by protein concentration

TG (mg/mg prot)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(Cpr\times V_{Sample})$**=$1.25\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

(2) Calculated by fresh weight of samples

TG (mg/g fresh weight)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(W\times V_{Sample}\div V_{Sample\ Total})$**=$1.25\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

3. Calculation of TG concentration in cells or bacteria:

TG (mg/10^4)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(500\times V_{Sample}\div V_{Sample\ Total})$**=$0.0025\times\Delta A_{Test}\div\Delta A_{Standard}$**

Where: $C_{Standard}$: 1.25 mg/mL; 100: 1 dL=100 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}=0.365, A_{Blank}=0.046, $A_{Standard}$=0.316; $\triangle A_{Test}$=0.365-0.046=0.319, $\triangle A_{Standard}$=0.316-0.046=0.27

Calculation of TG concentration (mg/dL)=$125\times\triangle A_{Test}\div\triangle A_{Standard}$=125×0.319÷0.27=147.69 mg/dL.

Recommended Products

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