CheKine™ Micro Triglyceride (TG) Assay Kit

Cat #: KTB2200 Size: 48 T/96 T

	Micro Triglyceride (TG) Assay Kit		
REF	**Cat #:** KTB2200	LOT	**Lot #:** Refer to product label
	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 then saponified by KOH to produce Glycerol and Fatty Acid. Further, periodic Acid Oxidizes Glycerin to form Formaldehyde. In the presence of Chloride Ions, Formaldehyde can react with Acetylacetone to form a yellow substance which has a characteristic absorption peak at 420 nm. The Triglyceride (TG) present in the sample is proportional to the signal obtained.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	2 mL	4 mL	4℃
Reagent Ⅱ	3 mL	6 mL	4℃
Reagent Ⅲ	1 mL	2 mL	4℃, protected from light
Reagent Ⅳ	3 mL	6 mL	4℃, protected from light
Reagent Ⅴ	3 mL	6 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 420 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℃.
Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
ReagentⅢ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
ReagentⅣ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent Ⅴ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
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: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Catalog #KTD3002, if the content of TG 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 420 nm, Visible spectrophotometer was returned to zero with deionized water.
2. Preheat the incubator to 65℃.
3. Extraction of TG (The following operations are operated in the EP tubes)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Distilled Water	40	0	0
Standard	0	40	0
Supernatant	0	0	40
Extraction Buffer	125	125	125
Reagent Ⅰ	25	25	25

Place the supernatant, Standard and deionized water (Blank Control) into the marked EP Tube. Mix thoroughly after adding Extraction Buffer and Reagent Ⅰ. Shake vigorously for 30 s and let stand for 3-5 min, repeat 3 times. After stratification, transfer 15 μL of the upper layer solution to a new EP Tube.

Note: After adding Reagent Ⅰ, shake vigorously to fully extract Triglycerides in sample.

4. Triglyceride content determination

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Upper Layer Solution	15	15	15
Reagent Ⅱ	50	50	50
Reagent Ⅲ	15	15	15
Mix well and incubate at 65℃ for 3 min			
Reagent Ⅳ	50	50	50
Reagent Ⅴ	50	50	50
Mix well and incubate at 65℃ for 15 min			

Take out the tubes and after cooling down to room temperature, transfer 100 μL to 96-well plate or microglass cuvette, immediately read optical density at 420 nm. The blank tube is marked as A_{Blank}, the standard tube is marked as $A_{Standard}$, and the test tube is marked as A_{Test}.

Note: Blank Tube and Standard Tube 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 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)=$100\times C_{Standard}\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})$**=$100\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})$**

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

(1) Calculated by protein concentration

TG (mg/mg prot)=$C_{Standard}\times V\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div(Cpr\times V)$**=$(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div Cpr$**

(2) Calculated by fresh weight of samples

TG (mg/g fresh weight)=$C_{Standard}\times V\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div W$**=$(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div W$**

3. Calculation of TG concentration in cells or bacteria:

TG (mg/10^4)=$C_{Standard}\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div 500$**=$(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})\div 500$**

Where: $C_{Standard}$: 1 mg/mL; 100: 1 dL=100 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; V: Sample Preparation of added Extraction Buffer volume, 1 mL; 500: Total number of cells or bacteria, 5×10^6.

Typical Data

Add 40 μL mouse serum, according to the procedure, calculated:

A_{Test}=0.362, A_{Blank}=0.168, $A_{Standard}$=0.611;

Calculation of TG concentration (mg/dL)=$100\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})=100\times(0.362-0.168)\div(0.611-0.168)$=43.79 mg/dL.

Recommended Products

Catalog No.	Product Name
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.