

Triglyceride (TG) Colorimetric Assay Kit

Cat#: orb1173225 (User Manual)

Triglyceride (TG) Colorimetric Assay Kit

Lot #: Refer to product label

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria

Storage: Stored at 4°C for 6 months

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™ Triglyceride (TG) Colorimetric 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
	48T	96T	
Extraction Buffer	60 mL	60 mL×2	4°C
Reagent I	2 mL	4 mL	4°C
Reagent II	3 mL	6 mL	4°C
Reagent III	1 mL	2 mL	4°C, protected from light
Reagent IV	3 mL	6 mL	4°C, protected from light
Reagent V	3 mL	6 mL	4°C, protected from light
Standard	1 mL	1 mL	4°C, protected from light

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 420 nm
- Incubator, Ice Maker, Refrigerated Centrifuge

·96 well plate with clear flat bottom, Precision Pipettes, Disposable Pipette Tips

·Deionized Water

·Dounce homogenizer (for Tissue Samples)

Reagent Preparation

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

Reagent I : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

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

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

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

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

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

Sample Preparation

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 minutes at 4°C. 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 minutes at 4°C. Use supernatant for assay, and place it on ice to be tested.

3. Cells or Bacteria: Collect 5 x 10⁶ 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 10,000 g for 10 minutes at 4°C. 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.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 420 nm.

2. Preheat the incubator to 65°C.

3. Extraction of TG (The following operations are operated in the EP Tubes)

	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 μ L of the upper layer solution to a new EP Tube.

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

Triglyceride content determination

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

Take out the Tubes and after cooling down to room temperature, transfer 100 μ L to 96-well plate, immediately read optical density at 420 nm. The Blank Tube is marked as ABlank, the standard Tube is marked as AStandard, and the test Tube is marked as ATest.

Note: Blank Tube and Standard Tube only need to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

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:

$$TG(\text{mg/dL})=100 \times C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) = 100 \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}})$$

2. Calculation of TG concentration in Animal and Plant Tissues:

(1) Calculated by protein concentration

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

(2) Calculated by fresh weight of samples

$TG \text{ (mg/g fresh weight)} = C_{\text{Standard}} \times V \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div W$
 3. Calculation of TG concentration in Cells or Bacteria:

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

Where: C_{Standard} : 1 mg/mL; 100: unit conversion factor, 1 dL=100 mL; C_{pr} : 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 .

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), if the content of TG is calculated by protein concentration.

Typical Data

Add 40 μ L Mouse Serum, according to the procedure, calculated:

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

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