Technical Manual

Triglyceride (TG) Colorimetric Assay Kit (Single Reagent, GPO-PAP Method)

- Catalogue Code: MAES0166
- Size: 100Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100Assays

Range:

0.19-8.0 mmol/L

Sensitivity:

0.19 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

TG is the main component of vegetable oil, animal fat, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), and serves as a carrier and source of energy for fatty acids. Triglyceride turnover rate determines the utilization of fatty acids in mammalian tissues. Any dysfunction in this process may lead to changes in glucose metabolism, insulin resistance and type 2 diabetes.

3. Intended Use

This kit applies the GPO-PAP method and it can be used for in vitro determination of triglyceride (TG) content in serum, plasma, cells, culture supernatant and other samples.

4. Detection Principle

Triglycerides (TG) can be hydrolyzed by lipoprotein lipase into glycerol and free fatty acids. Glycerol produces glycerol-3-phosphate and ADP under the catalysis of glycerol kinase (GK). Glycerol-3-phosphate produces hydrogen peroxide under the action of glycerol phosphate oxidase (GPO). In the presence of 4-aminoantipyrine and phenol, hydrogen peroxide is catalyzed by peroxidase (POD) to produce quinones which is proportional to the content of TG.

5. Kit Components & Storage

Item	Specification	Storage
Enzyme Working Solution	100 mL×1 vial	2-8°C, 6 months, avoid direct sunlight
Glycerinum Standard (2.26 mmol/L)	0.1 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (510 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Isopropanol (AR)

6. Assay Notes:

Use the clean EP tubes to prevent contamination of glycerin, glucose and other reagents

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant and it is 10-12.5 IU of Heparin into 1 mL blood), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection.

3. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): Isopropanol (AR) (μL) =1: 200-300. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of Isopropanol (AR) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.19-8.0 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human plasma	1
Mouse plasma	1
Rat plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7. 4); The diluent of animal tissue and cells is isopropanol.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 510 nm

10. Operation Steps

The measurement of samples

1. **Blank tube:** Take 10 μL of double distilled water to the 2 mL EP tube.
   Standard tube: Take 10 μL of 2.26 mmol/L glycerinum standard to the 2 mL EP tube.
   Sample tube: Take 10 μL of sample to the 2 mL EP tube.

2. Add 1000 μL of enzyme working solution into each tube of step 1, mix thoroughly.

3. Incubate at 37°C for 10 min. Set the spectrometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Glycerinum standard (2.26 mmol/L) (μL)		10	
Sample (μL)			10
Enzyme working solution (μL)	1000	1000	1000
Incubate at 37°C for 10 min. Set the spectrometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.			

11. Calculations

1. Serum (plasma):

$$\text{TG (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard

f: Dilution factor of sample before tested

m: The weight of tissue sample, g

V: The volume of isopropanol, mL

N: The number of cells. For example, the number of cells is $5*10^6$, N is 5

2. Tissue sample:

$$\text{TG (μmol/g wet weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \left(\frac{m}{V}\right)$$

3. Cell sample:

$$\text{TG content (μmol/}10^6\text{ cells)} = \frac{\Delta A_1}{\Delta A_2} \times c \div \frac{N}{V} \times f$$

12. Performance Characteristics

Detection Range	0.19-8.0 mmol/L
Sensitivity	0.19 mmol/L
Average recovery rate (%)	94
Average inter-assay CV (%)	6.7
Average intra-assay CV (%)	2.4

Analysis

Take 10 μL of human serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.130, the average OD value of the blank is 0.050, the average OD value of the standard is 0.271, and the calculation result is:

$$\textbf{TG (mmol/L)} = \frac{\textbf{0.130-0.050.}}{\textbf{0.271-0.050}} \times \textbf{2.26 mmol/L}$$

$$= \textbf{0.82 mmol/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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