Technical Manual

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

- Catalogue Code: MAES0160
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.14-9.5 mmol/L

Sensitivity:

0.14 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	25 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
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Centrifuge
- Microplate Reader (510 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Isopropanol (AR)
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. Prevent the formation of bubbles when adding the liquid to the microplate.

2. Protect the reagent from contamination of glucose, cholesterol, etc.

3. When measuring low content samples such as cells, the volume of sample should be increased to 5-10 μL, and the volume of the blank well and standard well should be increased at the same time.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample 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. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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. If not detected on the same day, the plasma can be stored at -80°C for a month.

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 (4×10^6): isopropanol (AR) (μL) =1: 400. Sonicate the sample on an 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) (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 (0.14-9.5 mmol/L).

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

Sample Type:	Dilution Factor
Human serum	1
Mouse serum	1
Rat plasma	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
HepG2 cells	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

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank wells; B, standard wells; S1-S92, sample wells.

10. Operation Steps

Operation with microplate reader

	Blank well	Standard well	Sample well
Double distilled water (μL)	2.5		
Glycerinum standard (μL) (2.26 mmol/L)		2.5	
Sample (μL)			2.5
Enzyme working solution (μL)	250	250	250
Mix thoroughly, incubate at 37°C for 10 min, measure the OD value at 510 nm with microplate reader.			

Operation with biochemical analyzer

Sample value/ Double distilled water (μL)	2.5
Enzyme working solution (μL)	250
Incubate at 37°C for 10 min, set zero with distilled water + working solution, measure the absorbance value A at 510 nm.	
Main wavelength (nm)	510
Reaction type	Endpoint method
Reaction direction	(+)

11. Calculations

1. Serum (plasma) and other liquid sample:

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

2. Tissue sample:

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

3. Cell sample:

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

ΔA1: $OD_{Sample} - OD_{Blank}$

ΔA2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard.

f: Dilution factor of sample before test.

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.

12. Performance Characteristics

Detection Range	0.14-9.5 mmol/L
Sensitivity	0.14 mmol/L
Average recovery rate (%)	105
Average inter-assay CV (%)	9.2
Average intra-assay CV (%)	4.1

Analysis

Take 2.5 μL of mouse serum sample and carry the assay with microplate reader according to the operation table.

The results are as follows:

The average OD value of the sample is 0.195, the average OD value of the standard is 0.250, the average OD value of the blank is 0.088, and the calculation result is:

$$\text{TG content (mmol/L)} = \frac{(0.195 - 0.088)}{(0.250 - 0.088)} \times 2.26$$

$$= 1.49 \text{ mmol/L}$$

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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com