TECHNICAL MANUAL

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

- **SKU CODE:** MAES0165
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

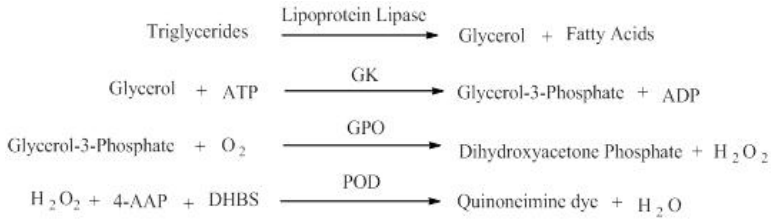
- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 10 min
- Measure OD at 510 nm

2. Intended use

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

3. 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 Trinder's reagent, hydrogen peroxide is catalyzed by peroxidase to produce quinones, which are proportional to the TG content.



4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	50 mL × 3 vials	2-8°C, 12 months, shading light
Reagent 2	2.26 mmol/L Glycerinum Standard	0.1 mL × 1 vial	0.1 mL × 5 vials	2-8°C, 12 months, shading light

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (510 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), Anhydrous ethanol

6. Reagent preparation

Equilibrate all reagents to room temperature (25°C) before use.

Note: The reagents must be stored strictly according to the preservation conditions in the table above. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to obtain sufficient amount of reagents.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold normal saline (0.9% NaCl).

3. Homogenize 20 mg tissue in 180 µL anhydrous ethanol with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

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

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) for serum and plasma; The diluent is anhydrous ethanol for tissue samples. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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, the volume of sample should be increased to 5-10 μL, and the volume of blank well and standard well should be increased at the same time.

9. Operating steps

1. Blank well: Add 2.5 μL of double distilled water to the wells. Standard well: Add 2.5 μL of 2.26 mmol/L glycerinum standard to the wells. Sample well: Add 2.5 μL of sample to the wells.

2. Add 250 μL of enzyme working solution to each well.

3. Incubate at 37°C for 10 min, then measure the OD values of each well with microplate reader at 510 nm.

10. Calculation

The sample:

1. Serum (plasma) and other liquid sample:

TG (mmol/L) = ΔA_1/ΔA_2 × c × f

2. Tissue sample:

TG (μmol/g wet weight) = ΔA_1/ΔA_2 × c × f ÷ (m/V)

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 2.26 mmol/L

f: Dilution factor of sample before test

m: The weight of tissue sample, g

V: The volume of the homogenate of tissue samples, mL

11. Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.60	7.40
%CV	4.6	4.1	3.6

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.60	7.40
%CV	9.1	8.5	10.0

Recovery

Three samples of high, middle, and low concentrations were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 105%.

Recovery

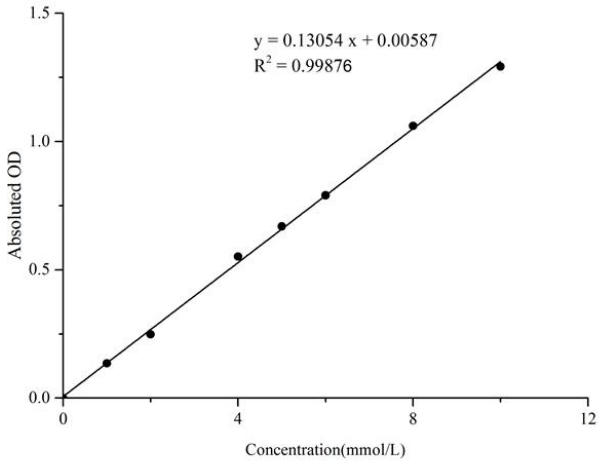
	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	1.6	3.7	8.5
Observed Conc. (mmol/L)	1.7	3.8	9.3
Recovery rate (%)	104	102	109

Sensitivity

The analytical sensitivity of the assay is 0.14 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	Average OD	Absolute OD
0	0.084	0
1	0.219	0.135
2	0.332	0.248
4	0.635	0.551
5	0.752	0.669
6	0.874	0.790
8	1.144	1.060
10	1.375	1.292

12. Example Analysis

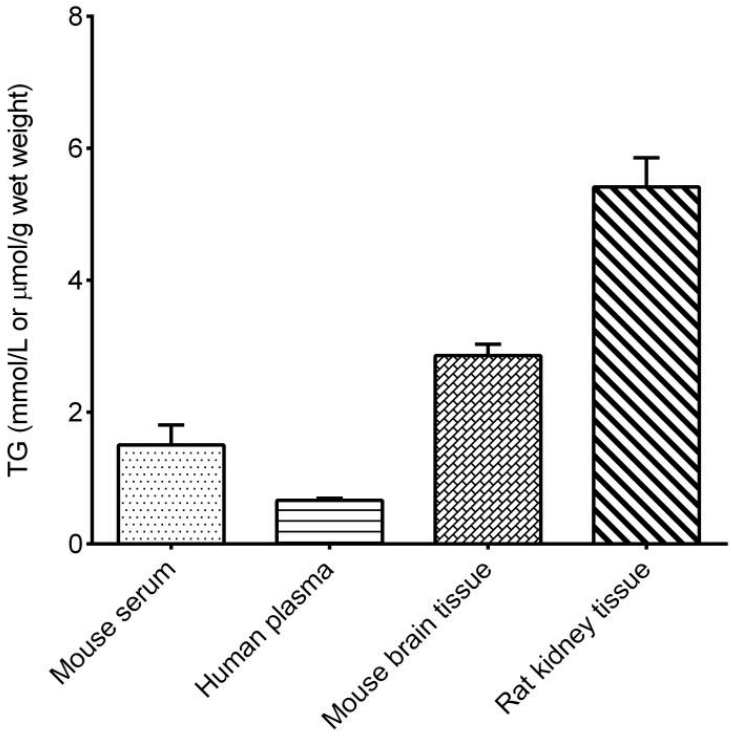
Example analysis:

Take 2.5 µL of mouse serum sample and carry out the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.314, the average OD value of the standard is 0.407, the average OD value of the blank is 0.080, and the calculation result is:

TG (mmol/L) = (0.314-0.080)/(0.407-0.080) × 2.26 = 1.62 mmol/L

Detect mouse serum, human plasma, 10% mouse brain tissue homogenate, and 10% rat kidney tissue homogenate according to the protocol; the results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Please follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.

6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to calculate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.