TECHNICAL MANUAL

Glycerol Colorimetric Assay Kit

- **SKU CODE:** MAES0225
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure OD value

2. Intended use

This kit can be used to measure glycerol in serum (plasma), animal tissue, and cell samples.

3. Detection principle

Glycerol is an intermediate product of triglyceride metabolism in animal and plant tissues and blood. Glycerol is hydrolyzed to produce glycerol, which is further oxidized to provide energy for cell metabolism. Therefore, glycerol content is a reliable indicator of triglyceride hydrolysis reaction and detection is more convenient.

Glycerol is transformed by enzyme to produce hydrogen peroxide, which is catalyzed by oxidase in the presence of 4-amino-antipyrine and phenol to produce red quinones, the color of which is proportional to the content of glycerol.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	12.5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 2	1.0 mmol/L Standard	1 mL × 1 vial	1 mL × 2 vials	2-8°C, 12 months, protect from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Water bath, Microplate reader (500-520 nm, optimum wavelength: 510 nm)

Reagents:

Double distilled water, Isopropanol (AR), PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of the standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 mmol/L.

7. 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 PBS (0.01 M, pH 7.4).

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

4. Then inactivate by water bath at 70°C for 10 min, centrifuge at 10000×g for 10 min, take the supernatant for detection.

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 2×10^6 cells in 100 µL isopropanol with sonicate or mechanical homogenate at 4°C.

4. Then inactivate by water bath at 70°C for 10 min, centrifuge at 10000×g for 10 min, take the supernatant for detection.

Dilution of sample:

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

- 10% Mouse liver tissue homogenate: 2

- 10% Mouse kidney tissue homogenate: 1

- 10% Mouse spleen tissue homogenate: 1

- Cell: 1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. The key points of the assay

1. Touch the bottom of the plate when adding standards and samples.
2. There should be no bubbles in the wells of the microplate when measuring the OD value.

9. Operating steps

1. Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells. Sample well: Add 10 μL of sample to the corresponding wells.
2. Add 250 μL of working solution to each well.
3. Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min. Measure the OD value of sample well at 510 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

glycerol content (mmol/L) = (ΔA_510 - b) ÷ a × f

2. Tissue sample:

glycerol content (mmol/kg wet weight) = (ΔA_510 - b) ÷ a × V ÷ m × f

3. Cell sample:

glycerol content (μmol/10^6) = (ΔA_510 - b) ÷ a × V ÷ N × f

[Note]

ΔA_510: OD Sample – OD Blank (OD Blank is the OD value when the standard concentration is 0).

V: The volume of isopropanol, mL.

m: The weight of sample, g.

N: The number of cells, 10^6.

f: Dilution factor of sample before test.

11. Appendix I 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)

Inter-assay Precision

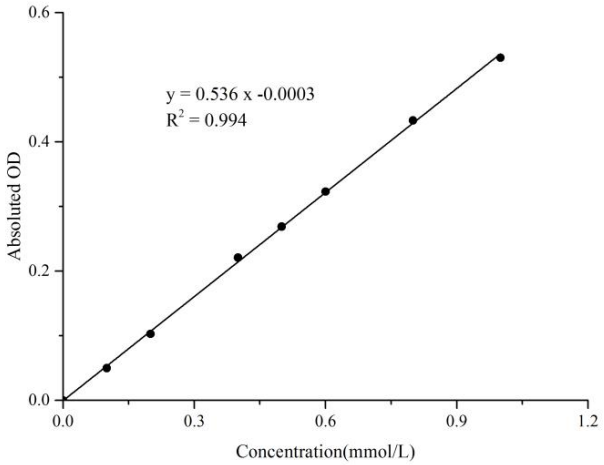
Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Sensitivity

The analytical sensitivity of the assay is 0.01 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:



12. Appendix II Example Analysis

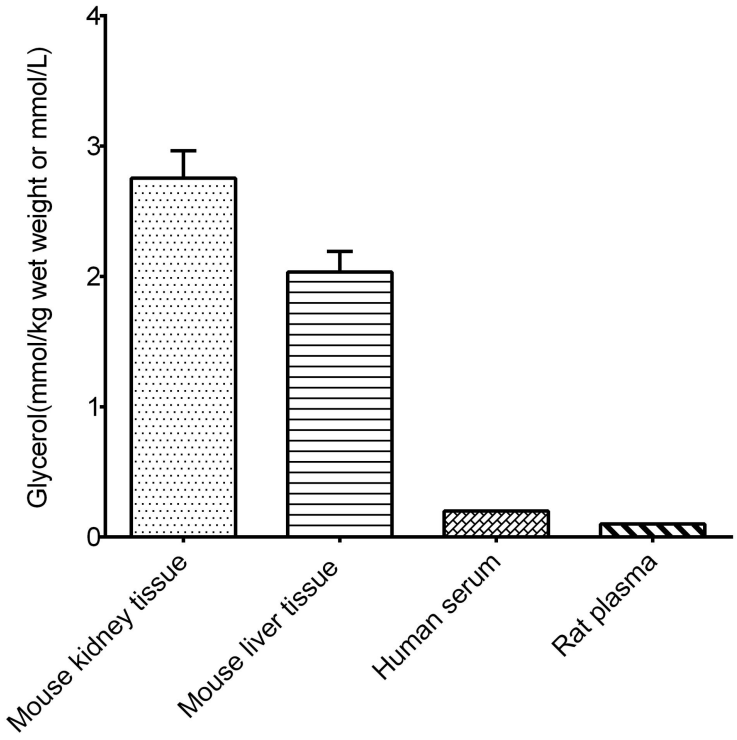
Example analysis:

For mouse kidney tissue, take 10 μL of 10% mouse liver tissue homogenate, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.536x – 0.003, the OD value of the blank is 0.072, the OD value of the sample is 0.244, and the calculation result is:

glycerol content (mmol/kg wet weight) = (0.244 - 0.072 + 0.003) ÷ 0.536 × 0.9 ÷ 0.1 = 2.93 mmol/kg wet weight

Detect 10% mouse kidney tissue homogenate, 10% mouse liver tissue homogenate, human serum and rat plasma according to the protocol, the result is 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. 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 pre-test if your sample type is not listed in the instruction manual.

6. 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 estimate 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.