TECHNICAL MANUAL

Glycerol Kinase (GK) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0448
- **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
- Measure OD value (A1)
- Incubate at 37°C for 25 min
- Add stop solution
- Measure OD value (A2)

2. Intended use

This kit can be used to detect glycerol kinase (GK) activity in animal tissue samples.

3. Detection principle

Glycerol kinase (GK) is a rate-limiting enzyme in glycerol metabolism, and the lack of this enzyme directly results in the inability of cells to utilize glycerol.

This kit produces hydrogen peroxide under the action of glycerophosphate oxidase. The reaction between hydrogen peroxide and the chromogenic agent has a maximum absorption peak at 555 nm. The enzyme activity of GK can be determined by measuring the increase rate at 555 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	6 mL x 1 vial	12 mL x 1 vial	-20°C, 12 months, shading light
Reagent 2	Stop Solution	6 mL x 1 vial	12 mL x 1 vial	-20°C, 12 months, shading light
Reagent 3	Standard	Powder x 1 vial	Powder x 1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (545-565 nm, optimum wavelength: 555 nm)

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use. The working solution can be aliquoted and stored at -20°C for 6 months protected from light.
2. **Preparation of 4.25 mmol/L standard solution:** Dissolve one vial of standard with 5 mL of double distilled water, mix well to dissolve. Store at -20°C for 1 month.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 4.25 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.85, 1.28, 1.70, 2.13, 2.98, 3.40, 4.25 mmol/L.

7. Sample preparation

1. **Tissue sample preparation:** 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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant.
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 10% Mouse liver tissue homogenization: 1; 10% Mouse kidney tissue homogenization: 1; 10% Mouse small intestine tissue homogenization: 1. Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

When using the pipette to take 4.25 mmol/L standard solution, carefully aspirate to avoid bubbles.

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 100 μL of working solution to each well.
3. Mix fully with microplate reader for 5 s and measure the OD value of sample well at 555 nm, as A1.
4. Incubate at 37°C for 25 min protected from light. Add 100 μL of stop solution to each well. Measure the OD value of each well at 555 nm, as A2. (The standard curve is fitted to the standard well A2 value)

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

The sample:

Tissue sample:

Definition: The amount of enzyme in 1 g tissue protein per 1 min that produces 1 μmol glycerol 3-phosphate at 37°C is defined as 1 unit.

GK activity (U/gprot) = (ΔA - b) ÷ a ÷ C_{pr} × f ÷ T × 1000*

[Note]

ΔA: $\Delta A = A_2 - A_1$.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: Reaction time, 25 min.

1000*: Unit conversion, 1 mmol/L = 1000 μmol/L.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three mouse liver tissue 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 (U/L)	25	45	120
%CV	3.0	3.2	3.6

Inter-assay Precision

Three mouse liver tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	25	45	120
%CV	6.5	7.8	8.0

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration assayed 6 times in parallel to obtain an average recovery rate of 98%.

Recovery

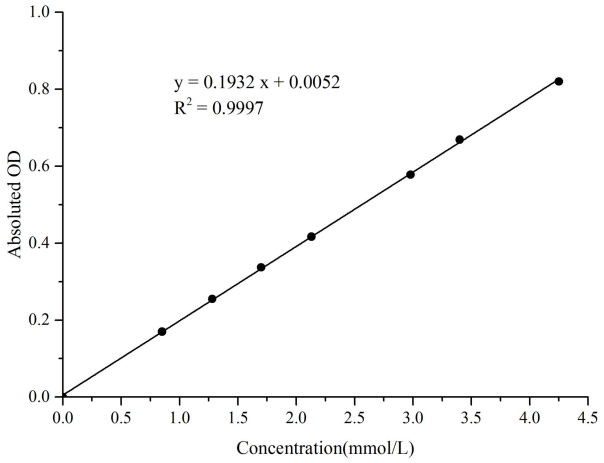
	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	25	45	120
Observed Conc. (U/L)	24	45	117.6
Recovery rate (%)	96	100	98

Sensitivity

The analytical sensitivity of the assay is 9.28 U/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)	OD value		Average OD	Absolute OD
0	0.046	0.046	0.046	0
0.85	0.215	0.216	0.216	0.170
1.28	0.300	0.302	0.301	0.255

Concentration (mmol/L)	OD value		Average OD	Absolute OD
1.70	0.383	0.383	0.383	0.337
2.13	0.463	0.462	0.463	0.417
2.98	0.621	0.626	0.624	0.578
3.40	0.712	0.718	0.715	0.669
4.25	0.865	0.866	0.866	0.820

12. Appendix II Example Analysis

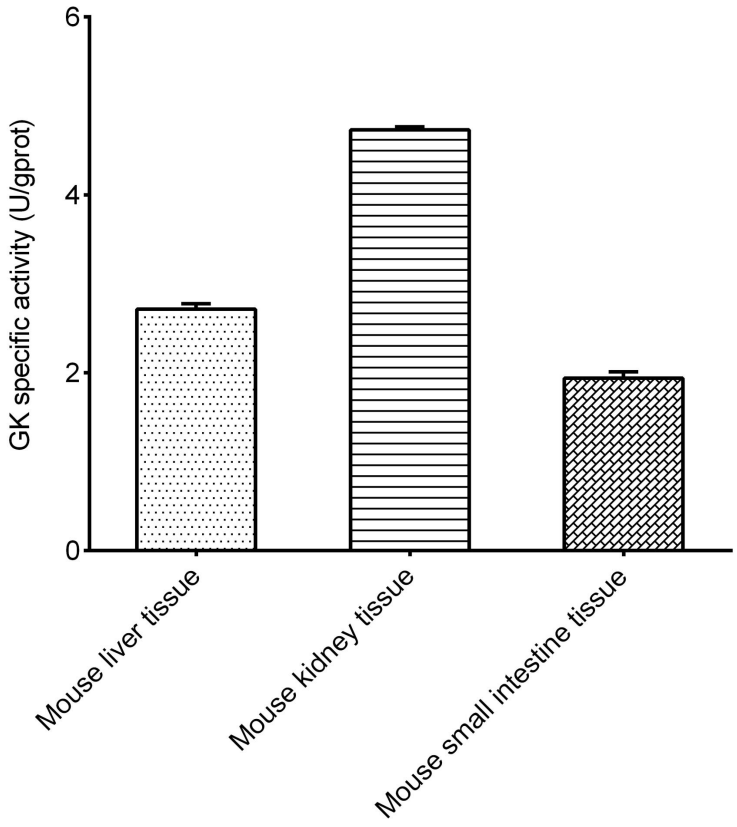
Example analysis:

Take 10 μL of 10% mouse liver tissue homogenization and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.1932x + 0.0052. The A_1 of sample is 0.060, the A_2 of sample is 0.240, the concentration of protein in sample is 13.20 gprot/L, and the calculation result is:

GK activity (U/gprot) = (0.240 – 0.060 – 0.0052) ÷ 0.1932 ÷ 13.20 ÷ 25 × 1000 = 2.74 U/gprot

Detect 10% mouse liver tissue homogenization (the concentration of protein is 13.20 gprot/L), 10% mouse kidney tissue homogenization (the concentration of protein is 10.55 gprot/L) and 10% mouse small intestine tissue homogenization (the concentration of protein is 7.75 gprot/L), 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 is not within 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. 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.