AssayGenie

TECHNICAL MANUAL

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit

- **SKU CODE:** MAES0447
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Incubate and measure OD values
- Calculate GAPDH activity

2. Intended use

This kit can be used to detect the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in tissues and cell samples.

3. Detection principle

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), a key enzyme in the glycolytic pathway, catalyzes the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. This reaction is closely related to the gluconeogenic pathway, the maintenance of blood glucose concentrations in the body, and the development of diabetes. GAPDH gene is a housekeeping gene, which is expressed at a high level in almost all tissues. Therefore, GAPDH is widely present in tissues and cells and plays an important role in the disorders of glucose, lipid and protein metabolism in the body.

This kit can calculate the enzyme activity of GAPDH by measuring the OD value change at 450 nm per unit time of the product produced by GAPDH catalyzing the oxidation of glyceraldehyde-3-phosphate.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	10 mL x 1 vial	20 mL x 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	Liquid x 2 vials	Liquid x 4 vials	-20°C, 12 months, shading light
Reagent 3	Chromogenic Solution	1.5 mL x 1 vial	3 mL x 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 4	Standard	Powder x 2 vials	Powder x 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of working solution:** Dissolve one vial of substrate with 2 mL of buffer solution, mix well to dissolve. Transfer the solution to a 5 mL EP tube, then add 1 mL buffer solution, mix well. The working solution should be prepared fresh before use.
3. **Preparation of 1 mmol/L standard solution:** Dissolve one vial of standard with 2 mL of double distilled water, mix well to dissolve. Transfer the solution to a 10 mL EP tube, then add 3 mL double distilled water, mix well. Store at -20°C for 2 weeks protected from light.
4. **Preparation of 500 μmol/L standard solution:** Before testing, prepare sufficient 500 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 500 μmol/L standard solution (mix well 500 μL of 1 mmol/L standard solution and 500 μL of double distilled water). Store at -20°C for 3 days protected from light.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L.

6. Sample preparation

Tissue samples:

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×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 (animal: MAES0177; plant: MAES0126).

Cell (adherent or suspension) samples:

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

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

3. Homogenize 1×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

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

5. Determine the protein concentration of supernatant (MAES0177).

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 lung tissue homogenization: 1
- 10% Spinach tissue homogenization: 1
- 10% Squash tissue homogenization: 1
- 10% Cabbage tissue homogenization: 1
- 1×10^6 HL-60 cells: 1
- 1×10^6 CHO cells: 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.

7. Operating steps

1. Standard wells: Add 40 µL of standard to the corresponding wells. Sample wells: Add 40 µL of sample to the corresponding wells.
2. Add 100 µL of working solution to each well. Add 20 µL of chromogenic solution to each well.
3. Mix thoroughly with microplate reader for 5 s and measure the OD value (A1) of each well at 450 nm.
4. Incubate at 37°C for 10 min protected from light and measure the OD value (A2) of each well at 450 nm. (The standard curve is fitted to the standard well A2 values.)

8. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD (A_2) 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 corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Tissue and cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein that hydrolyzes the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

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

[Note]

ΔA: $\Delta A = A_2 - A_1$.

f: Dilution factor of sample before test.

T: Reaction time, 10 min.

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

9. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	15.00	20.00
%CV	3.2	4.3	5.0

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	15.00	20.00

Parameters	Sample 1	Sample 2	Sample 3
%CV	3.2	6.7	8.6

Recovery

	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	150	280	370
Observed Conc. (µmol/L)	142.5	277.2	373.7
Recovery rate (%)	95	99	101

Sensitivity

The analytical sensitivity of the assay is 0.49 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 data

Concentration (µmol/L)	OD value		Average OD	Absolute OD
0	0.05	0.05	0.05	0
100	0.378	0.382	0.38	0.33
200	0.737	0.731	0.734	0.684
250	0.922	0.924	0.923	0.873
300	1.1	1.117	1.109	1.059
350	1.298	1.298	1.298	1.248
400	1.498	1.486	1.492	1.442
500	1.811	1.784	1.798	1.748

10. Appendix II Example Analysis

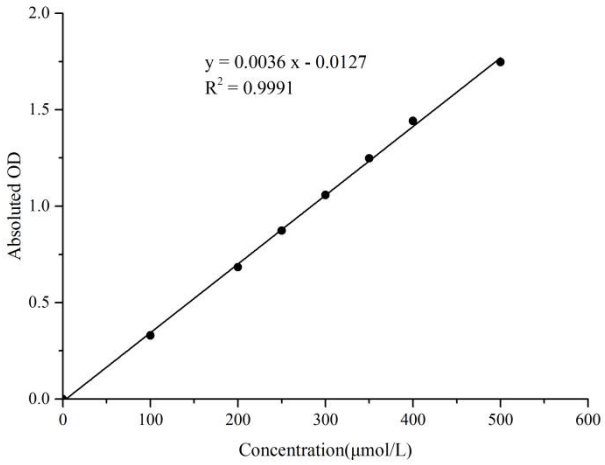
Example analysis:

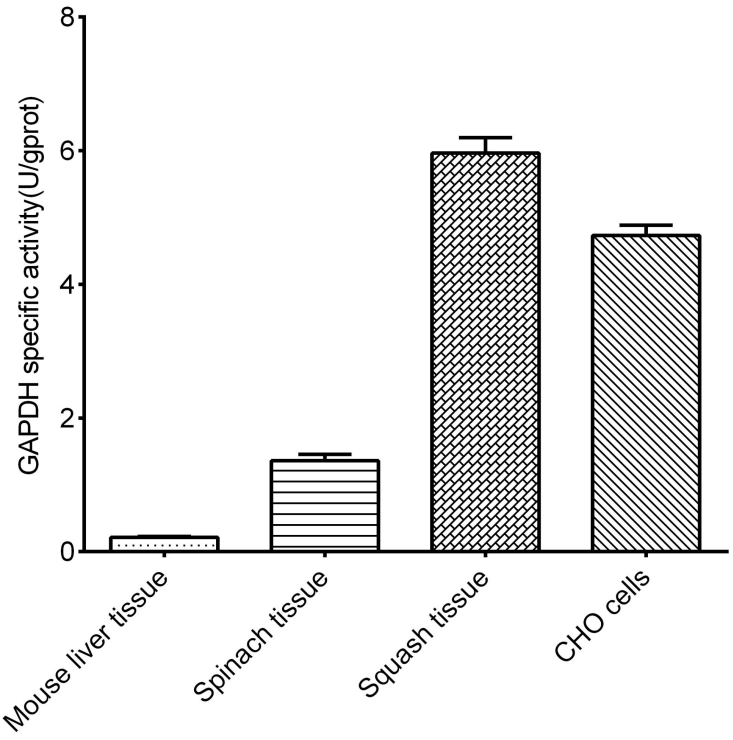
Take 40 μL of 10% mouse liver tissue homogenization and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0036x - 0.0127, the average A_1 of the sample is 0.138, the average A_2 of the sample is 0.236, the concentration of protein in sample is 14.06 gprot/L and the calculation result is:

GAPDH activity (U/gprot) = (0.236 – 0.138 + 0.0127) ÷ 0.0036 ÷ 14.06 ÷ 10 = 0.22 U/gprot

Detect 10% mouse liver tissue homogenization (the concentration of protein in sample is 14.06 gprot/L), 10% spinach tissue homogenization (the concentration of protein in sample is 2.00 gprot/L), 10% squash tissue homogenization (the concentration of protein in sample is 0.08 gprot/L) and 1×10^6 CHO cells (the concentration of protein in sample is 0.59 gprot/L) according to the protocol. The results are as follows:





11. 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.