AssayGenie

TECHNICAL MANUAL

6-Phosphogluconate Dehydrogenase (6-PGDH) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0513
- **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
- Add working solution
- Incubate and measure OD values
- Calculate 6-PGDH activity

2. Intended use

This kit can be used to measure the 6-phosphogluconate dehydrogenase (6-PGDH) activity in serum (plasma), plant, animal tissue and cell samples.

3. Detection principle

6-phosphogluconate dehydrogenase (6-PGDH) is the rate-limiting enzyme of the pentose phosphate pathway, which is responsible for the production of NADPH, energy balance, growth rate, and cell viability. 6-PGDH is also associated with a variety of tumor signaling pathways, including epidermal growth factor signaling pathway and nuclear factor E2 related factor signaling pathway.

The principle of this kit is that the product generated by the 6-PGDH catalytic substrate reacts with the chromogenic agent to generate the chromogenic substance, which has the maximum absorption at 450 nm, and is calculated by measuring the OD value at 450 nm 6-PGDH enzyme activity.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL ×1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	0.55 mL × 1 vial	1.1 mL × 1 vial	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Coenzyme	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Standard	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator

Reagents:

PBS(0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all the reagents to 25°C before use.
2. **The preparation of coenzyme working solution::** Dissolve coenzyme with 850 µL of double distilled water, mix fully. Aliquoted storage at -20°C for a week protect from light.
3. **The preparation of working solution::** Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 400 µL of working solution (mix well 250 µL of buffer solution, 20 µL of substrate, 30 µL of coenzyme working solution and 100 µL of chromogenic agent). The working solution should be prepared on spot and store protect from light, and used up on the same day.
4. **The preparation of 1000 µmol/L standard solution::** Dissolve standard with 750 µL of double distilled water, mix fully. Aliquoted storage at -20°C for 1 week protect from light.
5. **The preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1000 µmol/L standard

solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 400, 600, 800, 1000 μmol/L.

7. Sample preparation

Plasma or serum samples: Detect directly.

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell 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 PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection, detect on the same day.

8. Operating steps

1. Standard well: Add 20 μL of standard with different concentrations into the standard wells. Sample well: Add 20 μL of sample into the sample wells.
2. Add 200 μL of working solution into each well.
3. Mix well with microplate reader for 5 s. Measure the OD values of each well at 450 nm with microplate reader, as A1. Incubate 10 min at 37°C, measure the OD values of each well at 450 nm with microplate reader, as A2. (The standard curve is calculated as A2 value).

9. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean A_2 value of the blank (Standard #1) from all standard readings. This is the absoluted A_2 value.

3. Plot the standard curve by using absoluted A_2 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. Plasma (serum) samples:

Definition: The amount of 6-PGDH in 1 L of plasma (serum) sample that catalyze decomposition of 1 μmol product at 37°C for 1 min is defined as 1 unit.

6-PGDH activity (U/L) = (ΔA_450 - b) ÷ a ÷ T × f

2. Tissue sample:

Definition: The amount of 6-PGDH in 1 g of tissue sample that catalyze decomposition of 1 nmol product at 37°C for 1 min is defined as 1 unit.

6-PGDH activity (U/g wet weight) = (ΔA_450 - b) ÷ a × V_1÷ m ÷ T × f × 1000

3. Cell sample:

Definition: The amount of 6-PGDH in 1×10^6 cell sample that catalyze decomposition of 1 nmol product at 37°C for 1 min is defined as 1 unit.

6-PGDH activity (U/10^6) = (ΔA_450 - b) ÷ a × V_2 ÷ n ÷ T × f × 1000

[Note]

ΔA_450: The change value of sample well, $A_2 – A_1$.

T: Reaction time, 10 min

f: Dilution factor of sample before test.

V_1: The volume of tissue homogenate, 0.0009 L

m: The weight of the sample, 0.1 g.

V_2: The volume of cell homogenate, 0.0002 L

n: The number of cells, 1×10^6

1000: 1 μmol/L = 1000 nmol/L

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

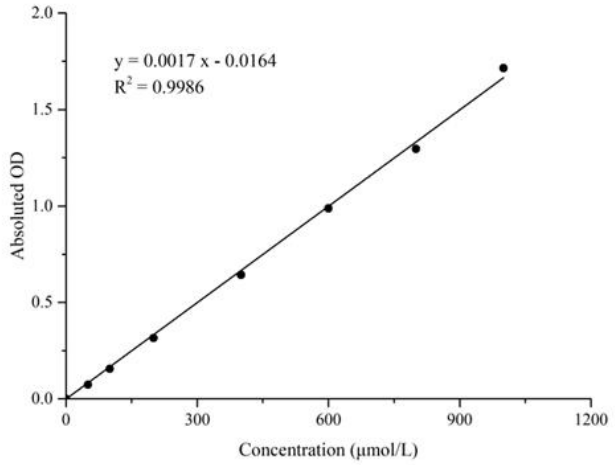
Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100.7%.

Sensitivity

The analytical sensitivity of the assay is 1.26 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.



10. Appendix II Example Analysis

Example analysis:

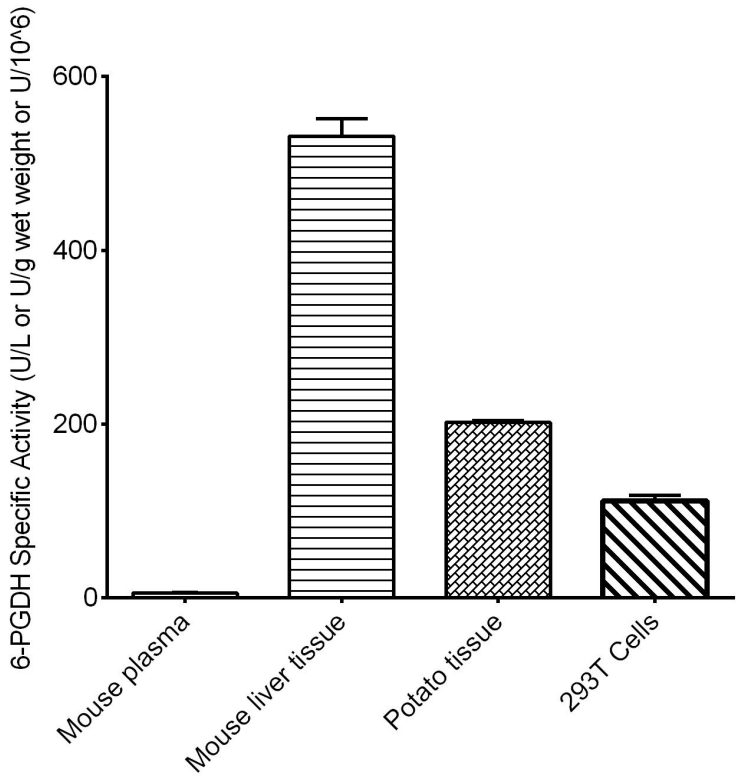
Take 20 μL of 10% mouse kidney tissue homogenate and dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.002x - 0.0117, the A_1 value of the sample well is 0.21, the A_2 value of the sample well is 0.5, $\Delta A_450 = A_2 - A_1 = 0.5 - 0.21 = 0.29$, and the calculation result is:

6-PGDH activity(U/g wet weight) = (0.29 + 0.0117) ÷ 0.002 × 0.0009 ÷ 0.1 ÷ 10 × 5 × 1000

= 678.83 U/g wet weight

Detect mouse plasma, 10% mouse liver tissue homogenate, potato tissue homogenate and 1×10^6 293T cells (dilute for 5 times) according to the protocol, the result is 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.