TECHNICAL MANUAL

Glucose-6-Phosphate Dehydrogenase (G-6-PD) Activity Assay Kit

- **SKU CODE:** MAES0064
- **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 and add reagents
- Measure OD values

2. Intended use

This kit can be used to measure Glucose-6-Phosphate Dehydrogenase (G-6-PD) activity in serum, plasma and animal tissue samples.

3. Detection principle

In the presence of G-6-PD, glucose-6-phosphate is oxidized to 6-PG, and NADP+ is reduced to NADPH. Under the action of electron coupling reagent 1-MPMS, NADPH reduces WST-8 to form orange formazan, which has a maximum absorption peak at approximately 450 nm. The formazan generated in the reaction system is proportional to the G-6-PD activity in the sample.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	50 mL × 2 vials	-20 °C, 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	-20 °C, 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20 °C, 12 months, shading light
Reagent 4	Buffer Solution	4 mL × 1 vial	-20 °C, 12 months
Reagent 5	Standard	Powder × 1 vial	-20 °C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (450 nm), Pipettor, Water bath, Centrifuge

6. Reagent preparation

1. Keep substrate on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of 5 mmol/L standard solution:** Dissolve one vial of standard with 720 µL of double distilled water. Mix well to dissolve. The standard solution should be prepared fresh before use.
3. **Preparation of 500 µmol/L standard solution:** For each well, prepare 10 µL of 500 µmol/L standard solution (mix well 1 µL of 5 mmol/L standard solution and 9 µL of buffer solution). The standard solution should be prepared fresh before use.
4. **Preparation of sample working solution:** For each well, prepare 50 µL of sample working solution (mix well 25 µL of substrate and 25 µL of chromogenic agent). The sample working solution should be prepared fresh before use.
5. **Preparation of control working solution:** For each well, prepare 50 µL of control working solution (mix well 25 µL of chromogenic agent and 25 µL of buffer solution). The control working solution should be prepared fresh before use.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 µmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 150, 250, 350, 400, 500 µmol/L. Reference is as follows: Concentration (µmol/L): 0, 50, 100, 150, 250, 350, 400, 500 500 µmol/L standard (µL): 0, 20, 40, 60, 100, 140, 160, 200 Reagent 1 (µL): 200, 180, 160, 140, 100, 60, 40, 0

7. Sample preparation

1. 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. Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10,000 xg for 10 min at 4 °C. Take the supernatant and preserve it on ice for detection. 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 reagent 1 with a dounce homogenizer at 4 °C.
4. Centrifuge at 10,000 xg for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample: The recommended dilution factor for different samples is as follows (for reference only): Human serum: 1 Rat serum: 1 Rabbit serum: 1 Cynomolgus monkey: 1 10% Mouse liver tissue homogenate: 3-5 10% Rat kidney tissue homogenate: 1 10% Rat spleen tissue homogenate: 5-10 10% Mouse brain tissue homogenate: 1 Note: The diluent is extracting solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. When the extracting solution is taken, it should be poured out partially to avoid contamination.
2. Prevent the formation of bubbles when the supernatant is transferred into the microplate.

9. Operating steps

1. Standard well: add 50 µL of standard solution with different concentrations into the corresponding wells.
   Sample well: add 50 µL of sample into the corresponding wells. Control well: add 50 µL of sample into the corresponding wells.
2. Add 50 µL of sample working solution into the sample wells and standard wells.
3. Add 50 µL of control working solution into the control wells.
4. Mix fully for 5 s with microplate reader and incubate at 37 °C for 10 min.
5. Measure the OD values of each well at 450 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 corresponding 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:

Unit definition: The amount of enzyme in 1 L of serum (plasma) that catalyzes the substrate to produce 1 μmol NADPH at 37 °C for 1 min is defined as 1 unit.

G-6-PD activity (U/L) = (deltaA - b) ÷ a ÷ T × f

2. Tissue sample:

Unit definition: The amount of enzyme in 1 g of tissue protein that catalyzes the substrate to produce 1 μmol NADPH at 37 °C for 1 min is defined as 1 unit.

G-6-PD activity (U/gprot) = (deltaA - b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

deltaA: OD_{Sample} – $OD_{Control}$.

f: Dilution factor of sample before test.

T: Reaction time: 10 min.

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

11. Appendix I Performance Characteristics

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	11.50	38.50
%CV	2.4	2.0	1.9

Inter-assay Precision

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

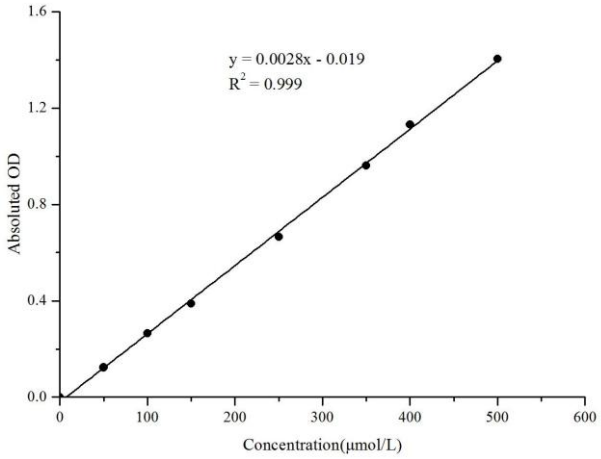
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	11.50	38.50
%CV	5.7	6.1	5.6

Sensitivity

The analytical sensitivity of the assay is 0.01 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:



Concentration (μmol/L)	Average OD	Absolute OD
0	0.059	0.000
50	0.182	0.124
100	0.323	0.265
150	0.447	0.388

Concentration (μmol/L)	Average OD	Absolute OD
250	0.724	0.666
350	1.019	0.961
400	1.190	1.132
500	1.463	1.404

12. Appendix II Example Analysis

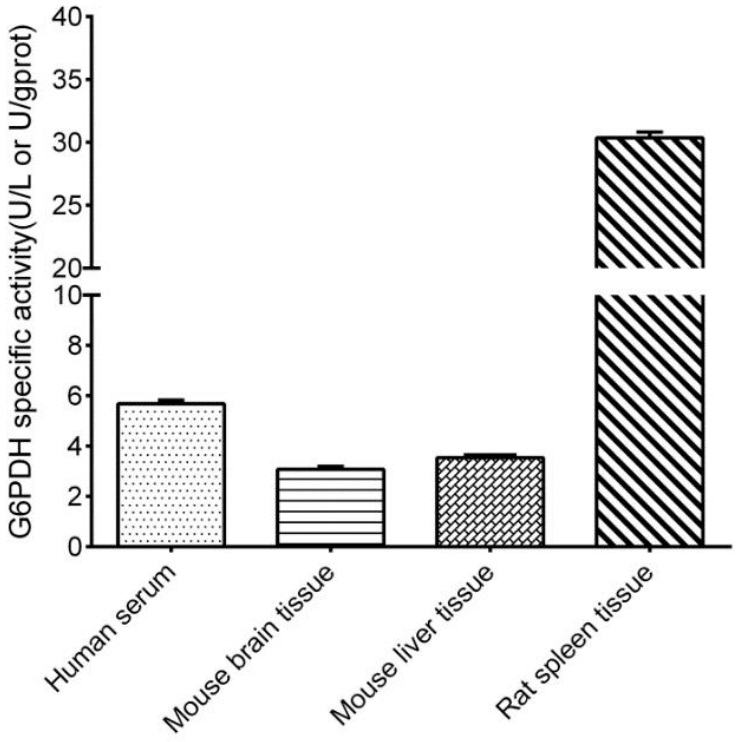
Example analysis:

For mouse brain tissue, take 50 μL of mouse brain tissue homogenate, and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0031x – 0.0149, the average OD value of the sample is 0.904, the average OD value of the control is 0.180, deltaA = (A_{Sample} – $A_{Control}$); the concentration of protein in sample is 7.77 gprot/L, and the calculation result is:

G-6-PD activity (U/gprot) = (0.904 - 0.180 + 0.0149) ÷ 0.0031 ÷ 10 ÷ 7.77 = 3.07 U/gprot

Detect human serum, 10% mouse brain tissue homogenate (the concentration of protein is 7.77 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 11.52 gprot/L), 10% rat spleen tissue homogenate (the concentration of protein is 6.16 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 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.

Notes:

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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.