TECHNICAL MANUAL

Glucose-6-phosphate (G6P) Colorimetric Assay Kit

- **SKU CODE:** MAES0025
- **SIZE:** 96 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 sample
- Measure the OD values

2. Intended use

This kit can be used to measure glucose-6-phosphate (G6P) content in serum, plasma and animal tissue samples.

3. Detection principle

In the presence of glucose-6-phosphate dehydrogenase, glucose-6-phosphoric acid is oxidized to gluconolactone-6-phosphate (6-PG), while 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 total G6P content in the sample.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20 °C, 12 months
Reagent 2	Buffer Solution	5 mL × 1 vial	-20 °C, 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20 °C, 12 months, protect from light
Reagent 4	Enzyme Agent	Powder × 1 vial	-20 °C, 12 months
Reagent 5	10 mmol/L G6P Standard	0.5 mL × 1 vial	-20 °C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (450 nm), Micropipettor, Incubator, Centrifuge

Reagents:

Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of 1 mmol/L standard solution:** Dilute 38 µL of 10 mmol/L G6P standard with 342 µL of extracting solution and mix well. The 1 mmol/L standard solution should be prepared immediately before use.
3. **Preparation of enzyme working solution:** Dissolve one vial of enzyme agent with 1.8 mL of buffer solution and mix well. The enzyme working solution should be prepared immediately before use. Can be stored in aliquots at -20 °C for up to 7 days.
4. **Preparation of control working solution:** For each well, prepare 50 µL of control working solution by mixing 25 µL of buffer solution with 25 µL of chromogenic agent. The control working solution should be prepared immediately before use. Store protected from light.
5. **Preparation of sample working solution:** For each well, prepare 50 µL of sample working solution by mixing 25 µL of chromogenic agent with 25 µL of enzyme working solution. The sample working solution should be prepared immediately before use. Store protected from light.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with extracting solution to create a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 300, 350, 400, 500 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not analyzed on the same day, serum or plasma can be stored at -80 °C for one 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 extracting solution with a Dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 × g for 10 min at 4 °C to remove insoluble material.

5. Collect supernatant and keep on ice for detection.

6. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

- Human plasma: 1

- Mouse serum: 2-3

- Rat plasma: 1

- Porcine serum: 1

- 10% Rat spleen tissue homogenate: 2-3

- 10% Rat heart tissue homogenate: 2-3

- 10% Rat liver tissue homogenate: 2-3

- 10% Mouse lung tissue homogenate: 1

Note: The diluent is extracting solution. For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

Prevent bubble formation when adding liquid to the microplate.

Operating steps:

1. Standard well: Add 50 µL of standards with different concentrations into the standard wells.

Sample well: Add 50 µL of sample into the sample wells.

Control well: Add 50 µL of sample into the control wells.

2. Add 50 µL of sample working solution to the sample wells and standard wells.

Add 50 µL of control working solution to the control wells.

3. Mix thoroughly for 5 seconds with microplate reader and incubate at 37 °C for 10 min.

4. Measure the OD values of each well at 450 nm with microplate reader. Record the OD values of sample wells as A_2, the OD values of control wells as A_1, then deltaA = A_2 - A_1.

9. Calculation

The standard curve:

1. Average the duplicate readings 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 the absolute OD value of standards and corresponding concentrations 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:

G6P content (μmol/L) = (deltaA - b) ÷ a × f

2. Tissue sample:

G6P content (μmol/g_{prot}) = (deltaA - b) ÷ a ÷ C_{pr} × f

[Note]

deltaA$_{450}$: A_2 - A_1

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, g_{prot}/L

10. 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 20 times in duplicate by three operators to determine precision between assays.

Recovery

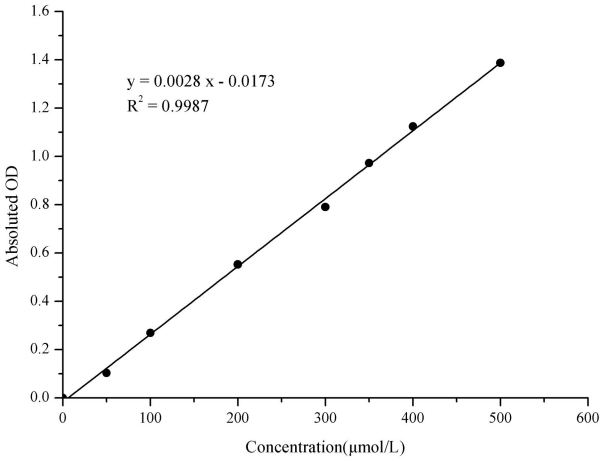
Three samples of high, medium, and low concentrations were tested with 6 replicates for each concentration to obtain an average recovery rate of 95%.

Sensitivity

The analytical sensitivity of the assay is 5.6 µmol/L G6P. 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

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:



11. Appendix II Example Analysis

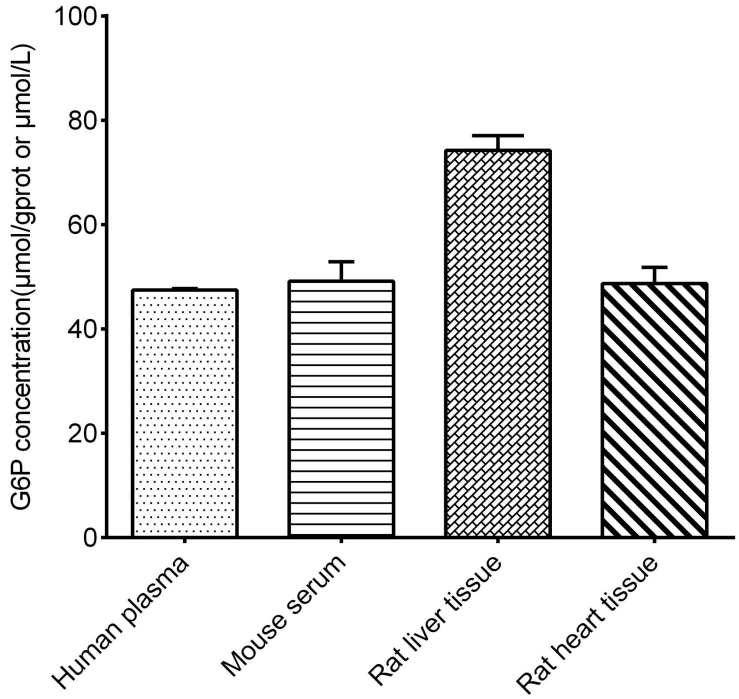
Example analysis:

For rat plasma, 50 µL of sample was added to the sample wells and standard wells and the assay was carried out according to the operating steps. The results are as follows:

Standard curve: y = 0.0027x - 0.0207, the average OD value of control is 0.370, the average OD value of the sample is 0.421, and the calculation result is:

G6P content (µmol/L) = (0.051 + 0.0207) ÷ 0.0027 = 26.56 µmol/L

Human plasma, mouse serum (diluted 2-fold), 10% rat liver tissue homogenate (protein concentration 8.59 g_{prot}/L, diluted 2-fold), and 10% rat heart tissue homogenate (protein concentration 4.53 g_{prot}/L, diluted 2-fold) were detected according to the protocol. The results are as follows:



12. 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.