TECHNICAL MANUAL

Glucose (Glu) Colorimetric Assay Kit (GOD-POD)

- **SKU CODE:** MAES0154
- **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 sample
- Incubate at 37°C for 15 min
- Measure the OD value at 505 nm

2. Intended use

This kit can be used to measure glucose (Glu) content in whole blood, serum, plasma, and tissue samples.

3. Detection principle

Glucose oxidase catalyzes the oxidation of glucose to gluconic acid, producing hydrogen peroxide. In the presence of chromogenic oxygen receptors, peroxidase catalyzes hydrogen peroxide and oxidizes pigment sources to form colored substances. The OD value is measured at 505 nm, and glucose content can be calculated indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Phenol Solution	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8°C, 12 months, shading light
Reagent 2	Enzyme Solution	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8°C, 12 months, shading light
Reagent 3	50 mmol/L Glucose Standard	1.2 mL x 1 vial	1.2 mL x 1 vial	6 mL x 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells		No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
	Plate Sealer	2 pieces	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (500-510 nm), Vortex mixer, Micropipettor, Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** For each well, prepare 300 µL of enzyme working solution by thoroughly mixing 150 µL of phenol solution and 150 µL of enzyme solution. Store at 2-8°C for 24 hours, protected from light.
3. **Preparation of control working solution:** For each well, prepare 300 µL of control working solution by thoroughly mixing 150 µL of normal saline and 150 µL of enzyme solution. Store at 2-8°C for 24 hours, protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 mmol/L glucose standard with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 2, 5, 10, 15, 20, 25, 30 mmol/L.

7. Sample preparation

1. **Sample preparation:** Serum, plasma and whole blood: detect directly. If not detected 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 normal saline (0.9% NaCl). 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 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.

2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): Human serum: 1 Mouse serum: 1 Rat serum: 1 Human plasma: 1 Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

- Control wells must be set for whole blood samples as well as hemolyzed serum and plasma samples. Control wells are not required for normal serum, plasma, and tissue samples.
- Enzyme solution should be aliquoted before use to avoid contamination.

9. Operating steps

1. Standard well: Take 3 μL of standard solution with different concentrations to the wells. Sample well: Take 3 μL of sample to the wells. Control well: Take 3 μL of sample to the wells.
2. Add 300 μL of enzyme working solution into the standard and sample wells. Add 300 μL of control working solution into the control wells.
3. Cover with plate sealer and incubate at 37°C for 15 minutes.
4. Measure the OD value of each well with microplate reader at 505 nm. Note: Set control wells for whole blood, hemolysis serum and plasma samples, but not for normal serum, plasma and tissue samples.

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:

1. Normal serum (plasma) sample:

Glu content (mmol/L) = (ΔA_505 - b) ÷ a × f

2. Whole blood and hemolysis sample:

Glu content (mmol/L) = (ΔA' - b) ÷ a × f

3. Tissue sample:

Glu content (mmol/g_{prot}) = (ΔA_505 - b) ÷ a × f ÷ C_{pr}

[Note]

ΔA_505: OD_{Sample} - OD_{Blank}.

ΔA': OD_{Sample} - $OD_{Control}$.

f: Dilution factor of sample before testing.

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

11. 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)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	13.40	25.50
%CV	2.4	2.1	1.2

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 (mmol/L)	1.50	13.40	25.50
%CV	2.5	1.7	2.7

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 100%.

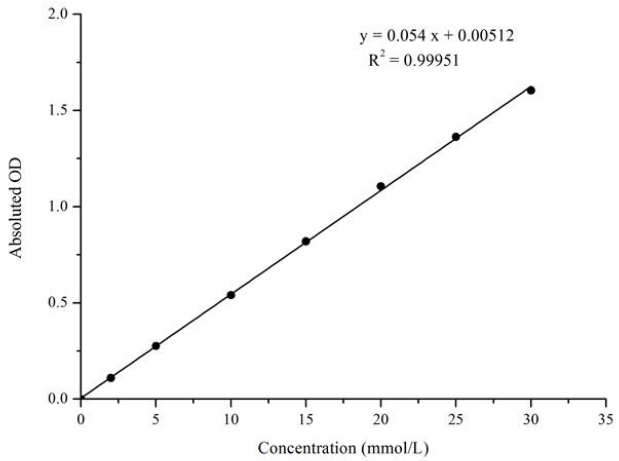
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	3.6	11.5	24.5
Observed Conc. (mmol/L)	3.6	11.4	24.5
Recovery rate (%)	101	99	100

Sensitivity

The analytical sensitivity of the assay is 0.04 mmol/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 actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Concentration (mmol/L)	Average OD	Absolute OD
0	0.043	0
2	0.152	0.110
5	0.318	0.276
10	0.583	0.540
15	0.863	0.820
20	1.148	1.106
25	1.406	1.363
30	1.647	1.604

12. Appendix II Example Analysis

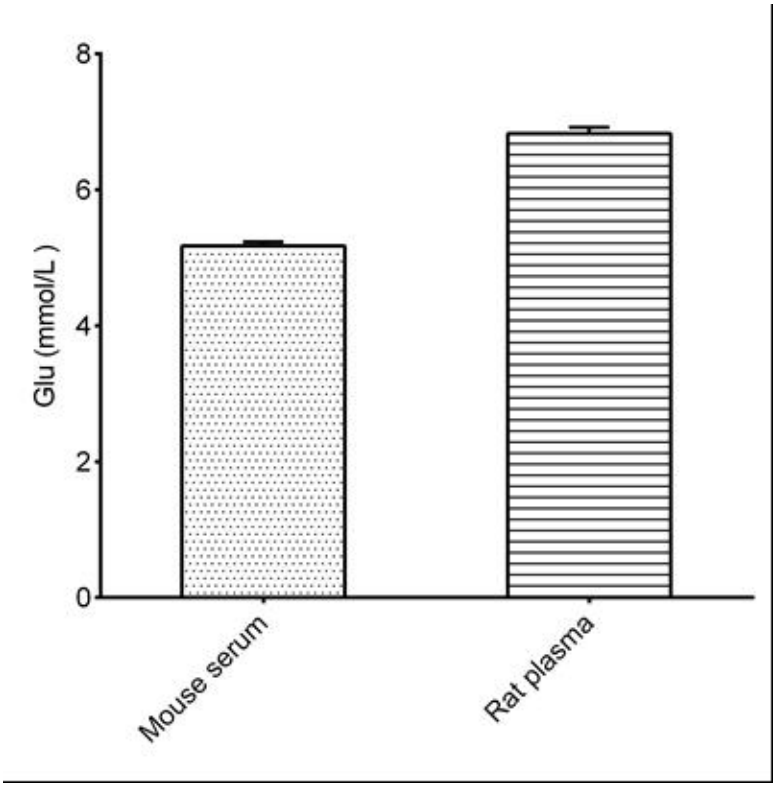
Example analysis:

Take 3 µL of mouse serum, perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.054x + 0.00512, the average OD value of the sample is 0.327, the average OD value of the blank is 0.043, and the calculation result is:

Glu content (mmol/L) = (0.327 - 0.043 - 0.00512) ÷ 0.054 = 5.17 mmol/L

Detect mouse serum and rat plasma according to the protocol, the results are 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.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.