TECHNICAL MANUAL

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

- **SKU CODE:** MAES0414
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples to enzyme working solution
- Incubate at 37°C for 25 minutes
- Measure absorbance at 505 nm

2. Intended use

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

3. Detection principle

Glucose oxidase (GOD, EC 1.1.3.4) catalyzes the oxidation of glucose to gluconic acid, producing hydrogen peroxide. In the presence of a chromogenic substrate, peroxidase catalyzes the reaction of hydrogen peroxide to generate a colored product. Within a certain range, the color intensity is proportional to the glucose concentration and can be measured at 505 nm.

4. Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Phenol Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months, protected from light
Reagent 2	Enzyme Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months, protected from light
Reagent 3	5 mmol/L Glucose Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (505 nm), vortex mixer, magnetic stirrers, micropipettor, 37°C 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 tube, prepare 2000 µL of enzyme working solution by mixing 1000 µL of phenol solution and 1000 µL of enzyme solution. Mix well and store at 2-8°C for up to 24 hours, protected from light.

7. Sample preparation

Sample preparation:

Serum and plasma: 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×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 (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Rat/Human plasma: 1

Mouse/Human serum: 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.

8. The key points of the assay

1. Separate serum or plasma from red blood cells immediately after blood collection to avoid glycolysis.
2. Serum and plasma samples must be clarified.

9. Operating steps

1. Add 2000 µL of enzyme working solution to each 5 mL EP tube (standard tube, sample tube, and blank tube).
2. Add to each tube: Blank tube - 20 µL double distilled water; Standard tube - 20 µL of 5 mmol/L glucose standard; Sample tube - 20 µL of sample.
3. Mix well and incubate at 37°C for 25 minutes.
4. Set to zero with double distilled water and measure the OD value of each tube using a 1 cm optical path cuvette at 505 nm.

10. Calculation

Serum (plasma) sample:

Glu content (mmol/L) = (ΔA_1/ΔA_2) × c × f

2. Tissue sample:

Glu content (mmol/g_{prot}) = (ΔA_1/ΔA_2) × c × f ÷ C_{pr}

[Note]

ΔA_1: OD_{Sample} - OD_{Blank}

ΔA_2: $OD_{Standard}$ - OD_{Blank}

c: Concentration of standard (5 mmol/L)

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)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.60	18.40	25.70
%CV	1.5	1.3	0.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.60	18.40	25.70
%CV	1.0	1.4	1.5

Recovery

Three samples of high, middle, and low concentrations were tested six times in parallel for each concentration to obtain an average recovery rate of 101%.

Recovery

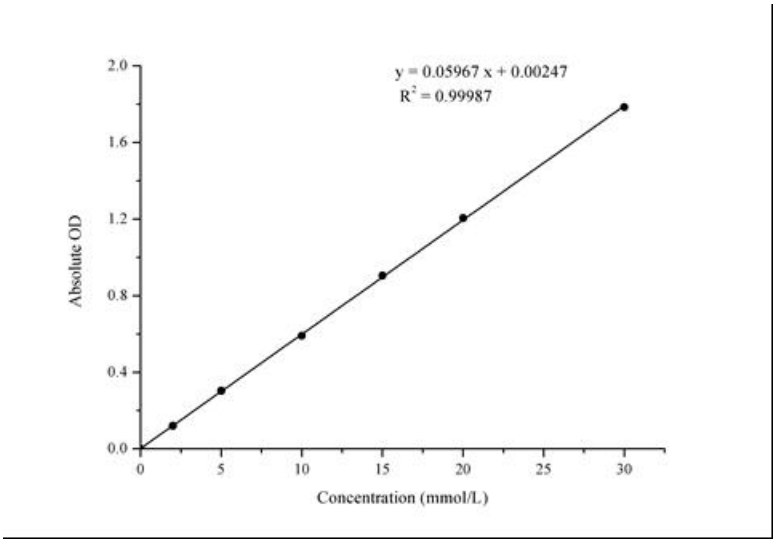
Sample 1	Sample 2	Sample 3
4.5	15	22
4.5	15.5	22.2
99	103	101

Sensitivity

The analytical sensitivity of the assay is 0.05 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 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:



Standard curve

Concentration (mmol/L)	Average OD	Absolute OD
0	0.007	0
2	0.128	0.121
5	0.310	0.303
10	0.598	0.591
15	0.912	0.905
20	1.212	1.205
30	1.792	1.785

12. Appendix II Example Analysis

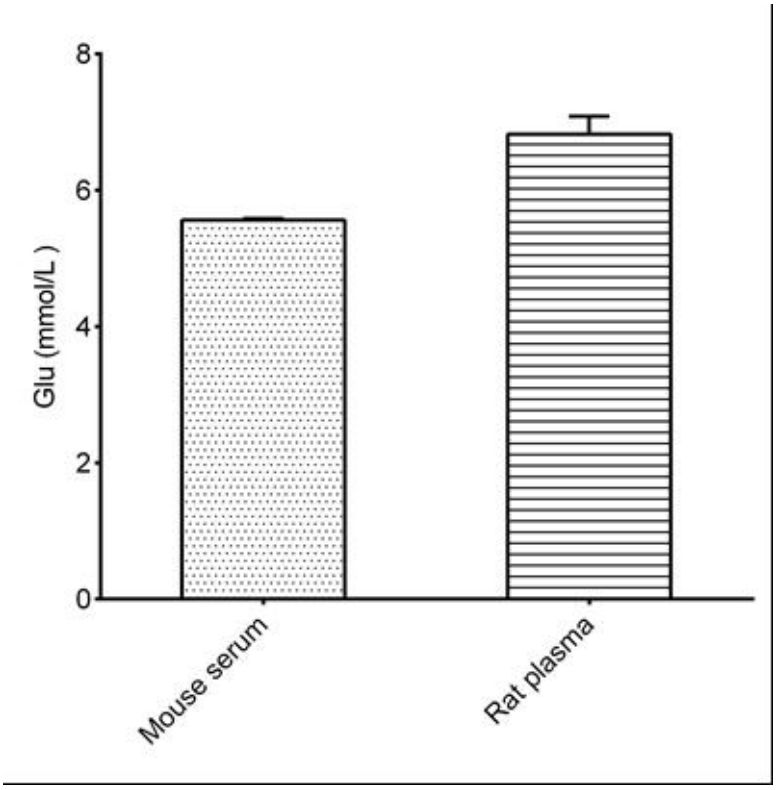
Example analysis:

Take 0.02 mL of mouse serum and perform the assay according to the operating steps. The results are as follows:

The average OD value of the standard is 0.308, the average OD value of the sample is 0.343, the average OD value of the blank well is 0.009, and the calculation result is:

Glu content (mmol/L) = (0.343 - 0.009)/(0.308 - 0.009) × 5 = 5.56 mmol/L

Detection of mouse serum and rat plasma according to the protocol shows the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.
2. Please read the instructions carefully and calibrate the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protective measures must be taken by wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, an additional dilution or concentration should be performed on the sample.
5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to the condition of reagents, operations, environment, and other factors. Assay Genie will guarantee the quality

of the kits only and will NOT be responsible for sample consumption caused by using the assay kits. It is advisable to calculate the possible usage of samples and reserve sufficient samples before use.

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.