TECHNICAL MANUAL

Glucose (GLU) Fluorometric Assay Kit

- **SKU CODE:** MAES0008
- **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
- Measure the fluorescence intensity

2. Intended use

This kit can be used to measure glucose (GLU) content in serum, plasma, urine, saliva, milk, and cell samples.

3. Detection principle

Glucose oxidase catalyzes the oxidation of glucose into gluconic acid and produces hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with a non-fluorescent substance to form a fluorescent product. The glucose content can be calculated indirectly by measuring the fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 590 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL x 1 vial	50 mL x 2 vials	-20 °C, 12 months
Reagent 2	Enzyme Reagent	Powder x 1 vial	Powder x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Chromogenic Agent	0.125 mL x 1 vial	0.25 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 4	5 mmol/L Standard	0.5 mL x 1 vial	0.5 mL x 1 vial	-20 °C, 12 months
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/590 nm), Micropipettor, Incubator, Vortex mixer, Water bath, Centrifuge

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 250 µL of buffer solution and mix well. Store at -20 °C for 1 month, protected from light.
3. **Preparation of chromogenic agent working solution:** For each well, prepare 50 µL of chromogenic agent working solution by mixing 46 µL of buffer solution, 2 µL of enzyme working solution, and 2 µL of chromogenic agent. Mix well. Prepare the working solution immediately before use and protect from light.
4. **Preparation of 50 µmol/L glucose standard:** Dilute 7 µL of 5 mmol/L Standard with 693 µL of buffer solution and mix well. Store at 2-8 °C for 7 days.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 µmol/L glucose standard with buffer solution to serial concentrations. The recommended dilution gradient is: 0, 2, 4, 6, 8, 10, 15, 20 µmol/L.

7. Sample preparation

Sample preparation:

Serum, plasma, and other liquid samples: If the sample is cloudy, centrifuge at 10,000 x g for 10 min at 4 °C. Collect the supernatant and preserve on ice for detection. If not analyzed on the same day, samples can be stored at -80 °C for one month.

Cell samples (adherent or suspension):

1. Harvest the required number of cells (initial recommendation: 2 x 10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 2 x 10^6 cells in 200 µL buffer solution using an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect the supernatant and keep on ice for detection.

5. Determine the protein concentration of the supernatant.

Dilution of samples:

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

Human plasma: 300-600

Human serum: 300-600

Chicken serum: 600-1000

Human urine: 1

Human milk: 400-600

Saliva: 3-5

Note: Use buffer solution as the diluent. For other sample types, perform a pre-test to confirm the appropriate dilution factor.

8. The key points of the assay

Avoid repeated freezing and thawing of the enzyme working solution. It is recommended to aliquot the enzyme working solution into smaller quantities and store at -20 °C.

9. Operating steps

1. Standard wells: Add 50 µL of standards with different concentrations into the wells. Sample wells: Add 50 µL of sample into the wells.
2. Add 50 µL of chromogenic agent working solution and mix thoroughly.
3. Mix thoroughly using a microplate reader for 10 s and incubate at 37 °C for 15 min, protected from light.
4. Measure the fluorescence intensity of each well at an excitation wavelength of 535 nm and an emission wavelength of 590 nm.

10. Calculation

Standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This gives the absolute fluorescence value.

3. Plot the standard curve using the absolute fluorescence value of standards as the y-axis and the corresponding concentration as the x-axis. Create the standard curve (y = ax + b) using graph software or Excel.

Sample calculation:

1. Serum, plasma, and other liquid samples:

GLU content (μmol/L) = (deltaF - b) / a x f

2. Cell samples:

GLU content (μmol/gprot) = (deltaF - b) / a x f / C_{pr}

Where:

deltaF: Absolute fluorescence intensity of sample (F_{sample} - F_{blank})

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample (gprot/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 (μmol/L)	2.50	15.00	25.00
%CV	2.0	1.5	1.6

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	2.50	15.00	25.00
%CV	3.2	2.5	2.7

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 99%.

Recovery

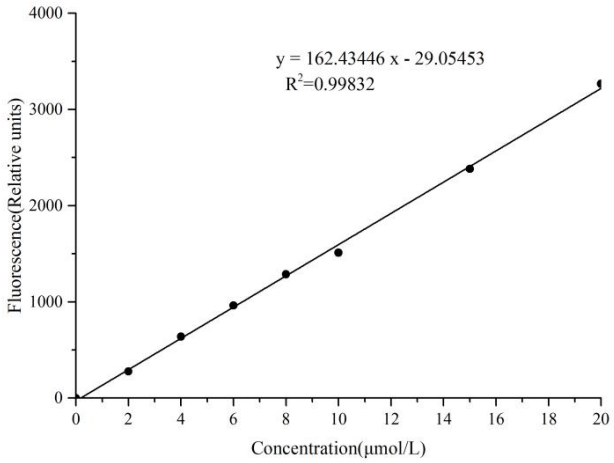
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	3.5	7	12
Observed Conc. (µmol/L)	3.5	6.9	11.9
Recovery rate (%)	100	98	99

Sensitivity

The analytical sensitivity of the assay is 0.1 µmol/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Standard curve

As the fluorescence values 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 below are provided for reference only:



Standard curve data

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	147	148	148	0
2	427	422	424	276
4	780	791	786	638
6	1104	1117	1110	962
8	1446	1423	1434	1286
10	1639	1678	1658	1510
15	2515	2546	2530	2382
20	3400	3429	3414	3266

12. Appendix II Example Analysis

Example analysis:

For human plasma, dilute 300-fold with buffer solution. Take 50 μL of diluted sample according to the instructions and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 162.43x - 29.055

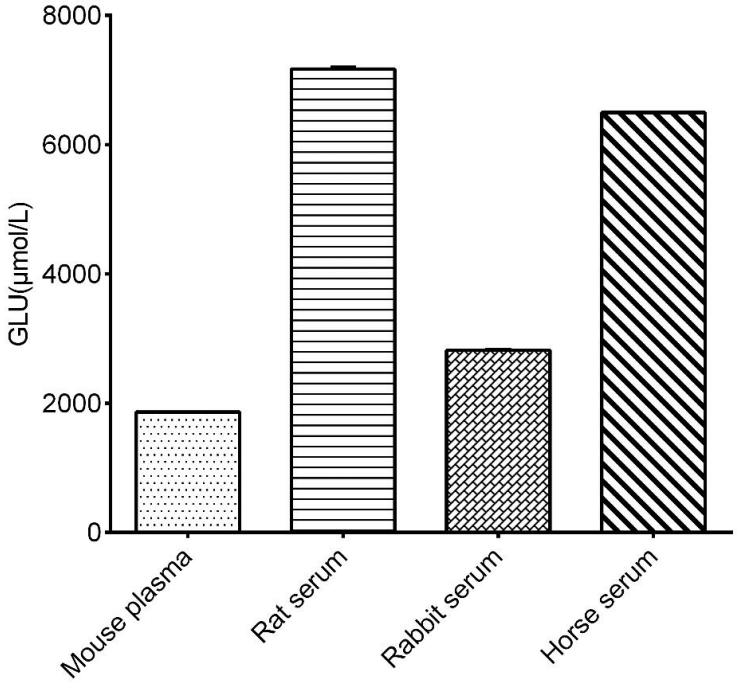
Average fluorescence value of sample: 2741

Average fluorescence value of blank: 80

Calculation result:

GLU content (μmol/L) = (2741 - 80 + 29.055) / 162.43 x 300 = 4968.40 μmol/L

Detect mouse serum (diluted 200-fold), rat serum (diluted 600-fold), rabbit serum (diluted 200-fold), and horse serum (diluted 600-fold) 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 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.