Technical Manual

Glucose (Glu) Fluorometric Assay Kit

- **Catalogue Code: MAES0008**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.1-20 mmol/L

Sensitivity:

0.1 mmol/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Glucose is a monosaccharide with the molecular formula $C_6H_{12}O_6$. Glucose can be produced by breaking down glycogen or be synthesized in the liver and kidneys through gluconeogenesis. As a blood sugar, glucose circulates in the blood of animals. glucose is the main bioenergy substance in the body, which is used to produce energy. Blood sugar balance is achieved through a complex interaction between several organs and hormones.

3. Intended Use

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

4. Detection Principle

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

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	50 mL × 2 vials	-20°C, 6 months
Enzyme Reagent	Powder × 1 vial	-20°C, 6 months, shading light
Chromogenic Agent	0.25 mL × 1 vial	-20°C, 6 months, shading light
Standard (5 mmol/L)	0.5 mL × 1 vial	-20°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/590 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **enzyme working solution:** Dissolve a vial of enzyme reagent with 250 μL of buffer solution and mix fully. The unused solution can be stored at -20°C for 1 month with shading light.

3. Preparation of **chromogenic agent working solution:** Mix the buffer solution, enzyme working solution and chromogenic agent at a ratio of 46:2:2. Prepare the fresh solution before use and stored with shading light.

4. Preparation of **glucose standard (50 μmol/L):** Mix the standard (5 mmol/L) and buffer solution at a ratio of 1:99. Prepare the fresh solution before use. The unused solution can be stored at 2-8°C for 7 days.

8. Sample Preparation

1. Serum (Plasma) sample:
Detect directly. Serum or plasma should be isolated from the sample tube as soon as possible without hemolysis. Serum or plasma can be stored at 2~8°C for 24h.

2. Urine:
Collect the fresh urine and centrifuge the sample at 10000 g for 15 min at 4°C. Take the supernatant for detection.

3. Saliva:
Collect the fresh saliva and centrifuge the sample at 10000 g for 15 min at 4°C. Take the supernatant for detection.

4. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): buffer solution (μL) =1: 100. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.1-20 mmol/L).

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

Sample Type:	Dilution Factor
Human plasma	300-600
Human serum	300-600
Chicken serum	600-1000
Human urine	1
Human milk	400-600
Saliva	3-5

Note: The diluent is buffer solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/590 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute glucose standard with buffer solution (50 μmo/L) to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 4, 6, 8, 10, 15, 20 μmol/L.

The measurement of samples

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

2. Add 50 μL of chromogenic agent working solution and mix fully.

3. Mix fully with microplate reader for 10 s and incubate at 37°C for 15 min with shading light.

4. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

Operation Table

	Standard well	Sample well
Standards with different concentrations (μL)	50	
Sample (μL)		50
Chromogenic agent working solution (μL)	50	50
Mix fully with microplate reader for 10 s and incubate at 37°C for 15 min with shading light. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.		

11. Calculations

Plot the standard curve by using fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample. The standard curve is: y= ax + b.

1. Liquid sample:

$$\text{Glu content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Cell sample:

$$\text{Glu content (μmol/gprot)} = (\Delta F - b) \div a \times f \div C_{pr}$$

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)
x: The concentration of standard
a: The slope of standard curve
b: The intercept of standard curve
ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$)
C_{pr}: The concentration of protein in sample, gprot/L
f: Dilution factor of sample before tested

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12. Performance Characteristics

Detection Range	0.1-20 mmol/L
Sensitivity	0.1 mmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	2.8
Average intra-assay CV (%)	1.7

Analysis

For human plasma, dilute for 300 times with buffer solution, take 50 µL of diluted sample according to the instructions, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 162.43 x – 29.055, the average fluorescence value of the sample is 2741, the average fluorescence value of the blank is 80, and the calculation result is:

$$\text{GLU content (µmol/L)} = (2741 - 80 + 29.055) \div 162.43 \times 300$$
$$= 4968.40 \text{ µmol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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