Technical Manual

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

- Catalogue Code: MAES0154
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.04-30 mmol/L

Sensitivity:

0.04 mmol/L

Storage:

2-8°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

It is very important for diagnosis of hyperglycemia to accurate determination of glucose. Usually, there is also a variety of inhibition test and determination of glucose tolerance test at the same time with glucose measuring during finding the cause of these conditions. Glucose level increases seen in diabetes mellitus, glucose intake, cushing syndrome and cerebrovascular accident. Glucose content decreases seen in insulinoma, insulin overdose and congenital carbohydrate metabolism disorder.

3. Intended Use

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

4. Detection Principle

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

5. Kit components & storage

Item	Specification	Storage
Phenol Solution	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme Solution	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Glucose Standard (50 mmol/L)	1.2 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (500-510 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

1. Set control wells for whole blood, hemolysis serum and plasma samples.

2. To prevent contamination, do not put the pipette directly into the reagent bottle when taking enzyme solution.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **enzyme working solution:** Mix the phenol solution and enzyme solution at a ratio of 1:1. Prepare fresh solution before use. It can be stored at 2-8°C for 24 hours avoiding direct sunlight.

3. Preparation of **control working solution:** Mix the normal saline and enzyme solution at a ratio of 1:1. Prepare fresh solution before use. It can be stored at 2-8°C for 24 hours avoiding direct sunlight.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at-80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant and it is 10-12.5 IU of Heparin into 1 mL blood), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at-80°C for a month.

3. Whole blood:
Take fresh blood to the tube containing heparin anticoagulant and mix it upside and down, then take 0.1 mL of the whole blood and add 0.4 mL of pre-cooled double distilled water. Mix fully for 1 min and stand for 15 min until the prepared hemolysis is transparent when observing under light.

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 (0.04-30 mmol/L).

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

Sample Type:	Dilution Factor
Human serum	1
Mouse serum	1
Rat serum	1
Human plasma	1

Note: The diluent is normal saline (0.9% NaCl);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 505 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 (50 mmol/L) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 5, 10, 15, 20, 25, 30 mmol/L.

The measurement of samples

1. **Standard well:** Take 3 μL of standard solution with different concentration 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 well.

3. Add 300 μL of control working solution into the control well.

4. Cover the plate sealer and incubate at 37 °C for 15 min.

5. 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 and plasma.

Operation Table

	Standard well	Sample well	Control well
Glucose standard with different concentration (μL)	3		
Sample (μL)		3	3
Enzyme working solution (μL)	300	300	
Control working solution (μL)			300
Cover the plate sealer and incubate at 37 °C for 15 min. Measure the OD value of each well with microplate reader at 505 nm.			

11. Calculations

1. Nomal serum (plasma):

Glu content（mmol/L）= (ΔA_{505} - b) ÷ a × f

2. Whole blood and hemolysis samples:

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

ΔA_{505}: OD_{sample}-OD_{blank}

ΔA': OD_{sample}-$OD_{Control}$

y: OD Standard – OD Blank (OD Blank is the OD value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

f: Dilution factor of sample before test

12. Performance Characteristics

Detection Range	0.04-30 mmol/L
Sensitivity	0.04 mmol/L
Average recovery rate (%)	100
Average inter-assay CV (%)	2.3
Average intra-assay CV (%)	1.9

Analysis

Take 3 μL of mouse serum, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.054 x + 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)

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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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