Glucose Colorimetric Assay Kit (GOD-POD Method)

Introduction

Glucose is a key energy metabolism molecule in living organisms, widely involved in physiological processes such as cellular respiration and glycogen synthesis, and has important clinical significance in the diagnosis and research of diseases such as diabetes and metabolic syndrome. Glucose concentration detection is essential for clinical testing, biological research, and drug development.

Glucose oxidase (GOD) can catalyze the oxidation of glucose to D-gluconic acid and H_2O_2. H_2O_2 can react with phenol and 4-aminoantipyrine under the action of peroxidase (POD) to form quinonimine, a red product. The absorbance of quinonimine at 505 nm is directly proportional to the glucose concentration. The kit is easy to use and highly sensitive, and is suitable for a variety of sample types such as serum, plasma, and tissue. This kit is more sensitive than another common glucose kit (O-toluidine method).

Components and Storage

Components \ Size	48 Assays	96 Assays	Storage
Phenol Solution	10 mL	20 mL	4°C away from light
Enzyme Solution	10 mL	20 mL	4°C away from light
Glucose (50 mmol/L)	1.2 mL	1.2 mL	-20°C
Shipping: Blue ice	**Shelf life: 1 year**		

Protocol

1. Sample preparation

   1) Serum and plasma: Use directly.

   2) Whole blood: Add fresh blood to a heparin anticoagulant tube (heparin concentration: 10-12.5 IU/mL blood) and mix upside down. Then take 0.1 mL to 0.4 mL ddH_2O, mix well for 1 min and let stand for 15 min. The papered 5 times dissolved blood is clear and translucent when observed in light.

   3) Tissue: Homogenize tissue as usual with normal saline, then centrifuge at 10000 g at 4°C for 10 min. Take the supernatant and put it on ice for later use. At the same time, take part of the supernatant for protein concentration determination.

2. Standard dilution: Dilute Glucose (50 mmol/L) with ddH_2O water to different standard concentrations. For

initial use, 0, 2, 5, 10, 15, 20, 25, and 30 mM are recommended as standard curve concentrations.

***Note:** It is recommended to aliquot unused Glucose (50 mmol/L).

3. Preparation of working solution:

   1) According to the needs of the experiment, mix an appropriate amount of Phenol Solution and Enzyme Solution at a ratio of 1:1 to form the working solution. Make a fresh working solution every time.

   2) At the same time, mix an appropriate amount of normal saline and Enzyme Solution at a ratio of 1:1 to form the control working solution. Make a fresh control working solution every time.

***Note:** Use control working solution for whole blood, hemolyzed serum and plasma samples, not for normal serum, plasma and tissue samples.

4. Prepare assay systems in a 96-well plate following the table below. Cover the plate sealer and incubate at 37°C for 15 min.

	Sample well	Control well (optional)	Standard well
Standards	-	-	3 μL
Sample	3 μL	3 μL	-
Working solution	300 μL	-	300 μL
Control working solution	-	300 μL	-

***Note:** Normal serum, plasma and tissue samples do not need to set up the control well.

5. Measure the absorbance (A505) of each well with a microplate reader at 505 nm.

6. Analysis of results:

   The standard curve: y=ax+b

   For normal serum and plasma samples: glucose content (mmol/L) = (ΔA505 - b) ÷ a × d

   For whole blood and hemolysis samples: glucose content (mmol/L) = ($\Delta A'_{505}$ - b) ÷ a × d

   For tissue samples: glucose content (mmol/gprot) = (ΔA505 - b) ÷ a × d ÷ Cpr

***Note:**

y: Standard well A505 - Blank well A505

x: Standard concentration

ΔA505: Sample well A505 - Blank well A505

$\Delta A'_{505}$: Sample well A505 - Control well A505

d: Sample dilution factor

Cpr: sample protein concentration, gprot/L

Note

1. Phenol Solution is highly toxic. Please use it in a fume hood.

2. For your safety and health, please wear lab coats and gloves during the experiment.

3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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