Glucose Assay Kit (Spectrophotometry)

Description

Glucose is not only the primary substrate for cellular energy metabolism, but its metabolic intermediates also serve as important substrates for biosynthesis. Plants can produce glucose through photosynthesis. In mammals, glucose is not only the sole energy source for the brain and nervous system, muscles, and adipose tissue, but is also closely involved in the synthesis of reducing coenzymes, lactose, and milk fat.

Glucose in mammalian blood is referred to as blood glucose and represents the main transport form of carbohydrates in the body. Blood glucose concentration is regulated by the nervous system and hormones and is maintained at a relatively stable level. When this regulation is disrupted, hyperglycemia or hypoglycemia may occur. Diabetes mellitus, increased intracranial pressure, and dehydration can all lead to hyperglycemia; physiological hyperglycemia may also occur after meals or during mental stress. Conversely, hypoglycemia may be observed in conditions such as hyperplasia or tumors of pancreatic β cells, hypofunction of the pituitary, adrenal cortex, and thyroid gland, as well as in patients with severe liver disease. In addition, fasting and strenuous exercise can cause transient hypoglycemia.

Detection Principle

Glucose oxidase catalyzes the oxidation of glucose to gluconic acid, producing hydrogen peroxide. Peroxidase then catalyzes the oxidation of 4-aminoantipyrine coupled with phenol by hydrogen peroxide to form a colored compound, which has a characteristic absorption peak at 505 nm. The absorbance intensity is directly proportional to the original glucose concentration.

Packing

Taking 50T/48S packing for example:

Components	Packing	Storage
CB0082S-A	Glucose Solution (2 μmol/mL), 10 mL per vial	4 ℃
CB0082S-B	Liquid, 25 mL per vial	4 ℃
CB0082S-C	Liquid, 25 mL per vial	4 ℃
Before use, mix CB0082S-B and CB0082S-C at a 1:1 ratio (equal volumes). Prepare only the amount required for immediate use.		

Before the formal assay, be sure to select 2–3 samples with relatively large expected differences for a preliminary test.

Instructions

I. Required Materials (to be prepared by the user)

Visible spectrophotometer, 1 mL glass cuvette, water bath, analytical balance, centrifuge, adjustable micropipettes, distilled water.

II. Glucose Extraction

1. Tissue samples

Homogenize the tissue with distilled water at a ratio of tissue weight (g) : distilled water volume (mL) = 1 : 5–10 (recommended: weigh ~0.1 g tissue and add 1 mL distilled water).

Boil the homogenate in a boiling water bath for 10 minutes (cap tightly to prevent water loss).

After cooling, centrifuge at 8000 × g for 10 minutes at room temperature.

Collect the supernatant for analysis.

2. Bacterial or cell samples

Collect bacteria or cells into centrifuge tubes and centrifuge; discard the supernatant.

Add distilled water according to the ratio of bacterial or cell number (10^4 cells) : distilled water volume (mL) = 500~1000 : 1 (recommended: add 1 mL distilled water to 5 × 10^6 bacteria or cells).

Disrupt bacteria or cells by ultrasonication (ice bath, 20% power or 200 W; sonicate for 3 s with 10 s intervals, repeat 30 times).

Boil in a boiling water bath for 10 minutes (cap tightly to prevent water loss).

After cooling, centrifuge at 8000 × g for 10 minutes at 25 °C.

Collect the supernatant for analysis.

3. Serum (plasma): Measure directly.

III. Assay Procedure:

1. Preheat the spectrophotometer for at least 30 minutes, set the wavelength to 505 nm, and zero with distilled water.
2. Preparation of working solution: Mix equal volumes of CB0082S-B and CB0082S-C before use; prepare only the amount needed.
3. Add the following reagents into a 1.5 mL centrifuge tube:

	Blank Tube (μL)	Control Tube (μL)	Sample Tube (μL)
Sample			100
CB0082S-A		100	
Distilled Water	100		
Working Solution	900	900	900
Mix well and incubate in a water bath at 37 °C (for mammals) or 25 °C (for other species) for 15 minutes. Measure the absorbance A at 505 nm. Record the absorbance of the blank, standard, and test tubes as A1, A2, and A3, respectively. **Note:** Only one tube is needed for the blank and the standard.			

IV. Calculation of Glucose / Blood Glucose Content:

1. Based on sample protein concentration

Glucose content (μmol/mg protein)=(Cstd×V1)×(A3-A1)÷(A2-A1)÷(V1×Cpr)=2×(A3-A1)÷(A2-A1)÷Cpr

2. Based on sample mass

Glucose content (μmol/g fresh weight)= (Cstd×V1)×(A3-A1)÷(A2-A1)÷(W×V1÷V2)=2×(A3-A1)÷(A2-A1)÷W

3. Based on bacterial or cell density

Glucose content (μmol/10^4 cells)=(Cstd×V1)×(A3-A1)÷(A2-A1)÷(500×V1÷V2) = 0.004×(A3-A1)÷(A2-A1)

4. Blood glucose content

Blood glucose (μmol/mL)=Cstd×(A3-A1)÷(A2-A1) = 2×(A3-A1)÷(A2-A1)

Note: Cstd：2μmol/mL；V1：Volume of sample added, 100μL=0.1mL；V2：Volume of the sample extraction，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight，g；500：total number of cells or bacteria, (5 × 10^6)

Precautions

1. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
2. Please wear a lab coat and disposable gloves.

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