Glucose Assay Kit (GOD-POD Microplate Method)

G1373336

Storage: -20℃. Protect from light.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

Glucose, considered one of the most widely distributed and important monosaccharides in nature, can be directly absorbed into the bloodstream. It is an essential nutrient for human energy metabolism and life maintenance, playing a critical role in sustaining metabolism and homeostasis. Naturally occurring glucose exists exclusively in the dextrorotatory (D) form, namely D-Glucose. Glucose serves as a major energy source and a key metabolic intermediate in living organisms. On one hand, it is a primary product of photosynthesis; on the other, it is a major substrate in respiration. During respiration, glucose is oxidized through a series of enzymatic reactions to carbon dioxide and water, while generating the crucial energy molecule ATP. Due to its central role in energy metabolism, blood glucose levels have become an important diagnostic marker for various metabolic diseases. Pathological hyperglycemia is associated with conditions such as diabetes mellitus, hyperthyroidism, and pituitary or adrenal hyperactivity, whereas pathological hypoglycemia is commonly observed in tumors related to insulin secretion, myxedema, and pituitary or adrenal insufficiency. Additionally, intracellular glucose levels serve as an important indicator for monitoring cellular metabolic status.

The detection principle of this kit is as follows: Glucose is oxidized by glucose oxidase (GOD) in the presence of oxygen to produce D-gluconic acid and H_2O_2. The generated H_2O_2 then reacts with phenol and 4-aminoantipyrine under the catalysis of peroxidase (POD) to form a red quinoneimine chromophore. The absorbance of the red quinoneimine at 510 nm is measured to determine the glucose concentration in the sample. The absorbance is directly proportional to the amount of glucose present.

This assay kit demonstrates high detection sensitivity, a wide linear range, and low sample consumption. With a sample volume of 40 μL, it can detect glucose concentrations as low as 0.01 mg/mL and exhibits a strong linear relationship within the range of 0.01–0.4 mg/mL (equivalent to 0.056–2.22 mM).

Kit Contents

G1373336	Component	100 T	500 T	Storage conditions
G1373336A	Lysis Buffer	20 mL	50 mL×2	-20℃.
G1373336B	Assay Buffer	30 mL	75 mL×2	-20℃.
G1373336C	Assay Reagent A	0.8 mL	4 mL	-20℃.Store in the dark.
G1373336D	Assay Reagent B	1.8 mL	9 mL	-20℃.Store in the dark.
G1373336E	Enzyme Solution	0.2 mL	1 mL	-20℃.Store in the dark.
G1373336F	Glucose Standard (4 mg/mL)	2 mL	5 mL×2	-20℃.Store in the dark.

Usage Protocol

1. Sample Preparation

1) Preparation of Blood Samples:

For serum samples: Allow whole blood to clot at room temperature for 30 minutes to 2 hours. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant.

For plasma samples: Collect whole blood using heparin or EDTA as an anticoagulant. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant.

2) Preparation of Cell Samples:

For cultured adherent cells: Wash the cells once with PBS; For cultured suspension cells: Centrifuge to collect the cells and wash them once with PBS. Add Lysis Buffer at a ratio of 200 μL per 1 million cells. Pipette appropriately to mix and incubate on ice for 10 minutes for complete lysis. Centrifuge at 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

3) Preparation of Tissue Samples:

Add Lysis Buffer at a ratio of 100 μL per 10 mg of tissue. Homogenize the tissue using a homogenizer on ice. Centrifuge at approximately 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

4) Preparation of Cell Culture Supernatant Samples:

For adherent cells: Directly collect the culture medium; For suspension cells: Centrifuge the culture and collect the supernatant.

All the above procedures must be performed at 4°C or on ice. Prepared cell or tissue samples can be stored at -20°C or -80°C if not assayed immediately.

2. Establishment of Standard Curve

Take 30 μL of the Glucose Standard (4 mg/mL) and add it to 270 μL of lysis buffer or assay buffer (use lysis buffer for lysed cell or tissue samples prepared with lysis buffer; use assay buffer for samples such as blood or supernatant that do not require lysis treatment), to prepare

a 0.4 mg/mL glucose standard solution. Then pipette 0, 1, 2.5, 5, 10, 20, 30, and 40 μL of the 0.4 mg/mL glucose standard solution into the standard wells of a 96-well plate, and bring the volume in each well to 40 μL by adding the corresponding lysis buffer or assay buffer. The resulting standard curve concentrations are 0, 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL, respectively.

3. Preparation of Chromogenic Solution

Prepare the Chromogenic Solution based on a volume of 160 μL per reaction. Prepare an appropriate amount according to the number of samples to be tested (including standards). For specific preparation methods, refer to the table below. It is recommended to prepare the working solution freshly before use.

Samples	1	10	20	50
Assay Buffer	132 μL	1320 μL	2640 μL	6600 μL
Enzyme Solution	2 μL	20 μL	40 μL	100 μL
Assay Reagent A	8 μL	80 μL	160 μL	400 μL
Assay Reagent B	18 μL	180 μL	360 μL	900 μL
Total volume	160 μL	1600 μL	3200 μL	8000 μL

Note: The presence of H_2O_2 may interfere with the glucose detection. If the sample contains H_2O_2, a sample background control well must be set up in parallel. Add the Chromogenic Solution without the enzyme solution (replace the 2 μL of enzyme solution with Assay Buffer). When calculating results, subtract the absorbance reading of the sample background control well from that of the sample well.

4. Sample Assay

1) Add 1–40 μL of sample or diluted sample to the sample wells of a 96-well plate. Then add the corresponding Lysis Buffer or Assay Buffer to bring the volume to 40 μL. At the same time, set up wells containing only Lysis Buffer or Assay Buffer as blank control wells (use Lysis Buffer if detecting cell or tissue samples that require lysis; use Assay Buffer if detecting samples such as blood or supernatant that do not require lysis).
2) Add 160 μL of Chromogenic Solution to each well and mix. Incubate at 37 °C in the dark for 15 minutes.
3) After the reaction, measure the absorbance at 510 nm (A_{510}).

5. Calculation of Results

Establish the standard curve and calculate the glucose concentration in the sample (A). The formula for calculating glucose concentration is as follows: C (mg/mL) = A × n

Note: A is the glucose concentration (mg/mL) determined from the standard curve; n is the total dilution factor of the sample.

The molar concentration of glucose in the sample (mM) can be calculated using the molecular weight of glucose (180): Glucose molar concentration (mM) = C / 0.18.

Precautions

1. It is recommended to prepare the Color Developer Working Solution freshly before use whenever possible.
2. The presence of H_2O_2 may interfere with glucose detection. If the sample contains H_2O_2, a sample background control well must be set up. Add the Color Developer Working Solution prepared without the enzyme solution (replace the 2 μL of enzyme solution with Assay Buffer). When calculating results, subtract the absorbance reading of the sample background control well from that of the sample well.
3. If serum or similar samples are stored at 4 °C, the storage time should not exceed two weeks; otherwise, the accuracy of the detection results may be affected. Generally, serum samples are recommended to be stored at –20 °C, and storage at –80 °C is even better.