Glucose Colorimetric Assay Kit (O-toluidine 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.

This kit is based on the O-toluidine colorimetric method, where glucose reacts with o-toluidine under acidic conditions to form a blue-green product. The absorbance of this product is directly proportional to the glucose concentration and can be quantitatively measured at 630 nm. This kit is easy to use and highly sensitive, and suitable for various sample types such as serum, plasma, and cells. Compared with another common glucose detection method (GOD-POD method), the O-toluidine method only develops color with aldose and is not affected by other reducing substances, making it very suitable for the detection of glucose in serum, urine and other solutions.

Components and Storage

Size / Components	200 Assays	1000 Assays	Storage
Glucose Assay Reagent	40 mL	200 mL	-20°C away from light
Glucose (200 mg/mL)	0.3 mL	1.5 mL	-20°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. Sample preparation:

   1) Liquid samples such as plasma/serum/urine: can be tested directly.

   2) Cells: Remove the medium and wash 2 times with PBS. For a 6-well plate, seed 0.5-1 x 10^6 cells per well, add 100-200 μL of suitable lysis buffer per well. Pipette several times to fully lysis, then centrifuge at 12000 g for 5 min. Take the supernatant for the detection.

***Note:** You can use Cell lysis buffer for WB and IP (Cat. No: K1123).

   3) Tissue: Take an appropriate amount of fresh or frozen tissue, add lysis buffer at a ratio of 1:10 (mg/μL) and homogenize on ice. After homogenization, centrifuge at 12000 g for 5 min. Take the supernatant for

the detection.

2. Standard preparation: Dilute Glucose (200 mg/mL, i.e., 20,000 mg/dl) with PBS, saline, or distilled water to the desired concentration for the standard curve. For first assay, the recommended standard concentrations are 0, 5, 10, 20, 200, 400, 800, 1200, 1600, and 2000 mg/dl.
3. Reagent preparation: Warm the Glucose Assay Reagent to room temperature in advance.
4. Sample detection:

1) Take 5 μL of sample or standards into PCR tubes, PCR strips or 96-well PCR plates.

***Note:** The sample addition volume can be adjusted depending on the experiment. If the glucose concentration in the sample is low, 20 μL of sample can be added. If the sample is precious but at a high concentration, 1-2 μL of sample can be taken.

2) Add 185 μL of Glucose Assay Reagent to a final volume of 190 μL.

***Note:** If the sample volume is adjusted in the previous step, the Glucose Assay Reagent should also be adjusted to make the final volume 190 μL.

3) After vortex mixing, centrifuge at 5000 g for a few seconds to allow the liquid to accumulate at the bottom of the tubes.

4) Put PCR tubes in a thermal cycler. Heat tubes at 95°C for 8 min, and then cool down to 4°C.

5) Transfer 180 μL of liquid per tube to a new 96-well plate.

***Note:** Be careful not to create air bubbles.

6) Measure absorbance at 630 nm (or 620-650 nm). It is suggested to complete the detection within 30 min after heating.

7) Calculate the glucose concentration in the sample according to the standard curve. For cell or tissue samples, it is usually necessary to detect protein concentration for calculating the glucose concentration per unit protein sample. Typically, 1 mg/dl of glucose concentration is equivalent to 55.5 μM, 0.001%, or 10 ppm.

***Note:** If the sample detection value is outside the standard curve range, dilute the sample. If the detection value is too low when the sample volume is increased to 20 μL, the sample is not suitable for testing with this kit.

Notes

1. Glucose Assay Reagent is light brown. If the color changes significantly, it may need to be discarded.
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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