Glucose Content Assay Kit

Note: The reagents of this product are subject to change. Please note and strictly follow this instruction.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC2505

Size:100T/96S

Components:

Reagent I: Liquid 10 mL×1, 2 μmol/mL Glucose solution. Storage at 2-8℃.

Reagent II: Liquid 10 mL×1. Storage at 2-8℃.

Reagent III: Liquid 10 mL×1. Storage at 2-8℃.

Preparation of mixed reagent: Mix reagent II and reagent III with equal volume 1:1 before use，prepare it fresh.

Product Description

Glucose is not only the main substrate of cell energy metabolism, but also its metabolic intermediate is an important substrate of biosynthesis. Plants produce glucose through photosynthesis. In mammals, glucose is not only the sole source of energy for the nervous system, muscles and adipose tissue of the brain, but also is closely related to the synthesis of reductive coenzymes, lactose and milk fat.

Glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. Peroxidase catalyzes the oxidation of 4-aminoantipyrine bisphenol by hydrogen peroxide to form colored compounds with characteristic absorption peaks at 505 nm.

Reagents and Equipment Required but Not Provided.

Water-bath/constant temperature incubator, transferpettor, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, sonicator, mortar/homogenizer and distilled water.

Procedure:

I. Sample Extraction:

1. Tissue treatment:

The ratio of tissue mass (g): distilled water volume (mL)=1:5 ~10. Suggest that weigh about 0.1 g of sample, add 1 mL distilled water and grind into homogenate. Boil them in a boiling water bath for 10 minutes (cover tightly to prevent water loss). After cooling, centrifuge them at room temperature for 10 min at 8000 g, then take the supernatant on standby.

2. Bacteria or cell treatment:

Collect the bacteria or cells into the centrifuge tube, discard the supernatant after centrifugation; According to the bacteria or cells (10^4): distilled water volume (mL) is according the ratio of 500~1000: 1 (Recommend 1 mL of distilled water is added to 5 million bacteria or cells), ultrasonic broke bacteria or

cells (ice bath, power of 200W, ultrasound for 3s, interval of 10s, repeat 30 times), set in a boiling water bath boil for 10 minutes (tightly closed to prevent moisture loss), after cooling, 8000 g, 25℃ centrifuge for 10 min, take supernatant on standby.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 min, adjust the wavelength to 505nm and set spectrophotometer counter to zero with distilled water.

2. Add the following reagents successively into the 1.5ml centrifuge tube or 96 well flat-bottom plate:

Reagent（μL）	Blank Tube	Standard Tube	Test Tube
Sample			20
Reagent I		20	
ddH_2O	20		
mixed reagent	180	180	180

Mix thoroughly, incubate at 37℃ (mammals) in the water bath/constant temperature incubator or 25℃ (other species) in the water bath for 15 min, read the absorbance of wavelength at 505 nm . Note the light absorption values of blank tube, standard tube and measuring tube as A_B, A_S and A_T, respectively. Make one or two blank tube and one standard tube.

III. Calculation:

1. Calculate by the protein concentration:

Glucose content (μmol/mg prot) $=(C_S \times V1) \times (A_T - A_B) \div (A_S - A_B) \div (V1 \times Cpr)$

$=2 \times (A_T - A_B) \div (A_S - A_B) \div Cpr$。

2. Calculate by Sample fresh weight:

Glucose content（μmol/g fresh weight）$= (C_S \times V1) \times (A_T - A_B) \div (A_S - A_B) \div (W \times V1 \div V2)$

$=2 \times (A_T - A_B) \div (A_S - A_B) \div W$

3. Calculate by the number of bacteria or cells

Glucose content（μmol/10^4 cell）$= (C_S \times V1) \times (A_T - A_B) \div (A_S - A_B) \div (500 \times V1 \div V2)$

$=0.004 \times (A_T - A_B) \div (A_S - A_B)$

C_S: standard tube concentration, 2 μmol/mL;

V1: add sample volume, 20 μL=0.02 mL;

V2: total volume of the sample, 1 mL;

Cpr: sample protein concentration, mg/mL;

W: sample fresh weight, g;

500: total number of bacteria or cells, 5 million.

Note:

If $(A_T - A_B)$ is less than 0.005, it is recommended to increase the extracted sample mass (or cell count) or the amount of sample supernatant added; if (At - Ab) is greater than 1.5, it is sufficient to dilute the supernatant with distilled water. Note the calculation formula multiplied by the dilution factor.

Experimental example:

1. 0.1 g of mouse liver was added to 1 mL of distilled water for the pre-treatment step and the supernatant was centrifuged; and measured according to the procedure, using 96 well flat-bottom plate to obtain the absorbance value $\Delta A_T = A_T-A_B = 0.856-0.051=0.805$, $\Delta As= A_S-A_B =0.724-0.051=0.673$. Calculated from the sample mass

Glucose content (μmol/g mass) = $\Delta A_T \div \Delta A_S \div W \times 2=2\times0.805\div0.673\div0.1= 23.923$ μmol/g

2. 0.1 g of green leaf was added to 1 mL of distilled water for the pre-treatment step, the supernatant was centrifuged and measured according to the determination procedure. $\Delta A_T = A_T-A_B =0.318-0.051=0.267$ and $\Delta A_S = A_S-A_B =0.724-0.051=0.673$ measured by 96 well flat-bottom plate.

Glucose content (μmol/g mass) =$2\times \Delta A_T \div \Delta A_S \div W =2\times0.267\div0.673\div0.1=7.935$ μmol/g

3. 5 million Jurkat cell samples were added to 1 mL of distilled water for the pre-treatment step, the supernatant was centrifuged and determined according to the assay procedure. $\Delta A_T = A_T-A_B=0.057-0.051=0.007$, $\Delta A_S = A_S-A_B =0.724-0.051=0.673$

Glucose content (μmol/10^6 cell) = $0.4 \times \Delta A_T \div \Delta A_S =0.4\times0.007\div0.673=4.16 \times 10^{-3}$ μmol/10^6 cell

Recent Product Citations:

[1] Meixi Peng, Dan Yang, Yixuan Hou,et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKP and CS enhances hypoxic breast cancer cell invasion. Cell Death and Disease. March 2019; (IF5.959)

[2] Jing Li,Yabing Duan,Chuanhong Bian,et al. Effects of validamycin in controlling Fusarium head blight caused by Fusarium graminearum: Inhibition of DON biosynthesis and induction of host resistance. Pesticide Biochemistry and Physiology. January 2019; 153:152-160. (IF2.87)

References:

[1] Basagni U, Bonicolini F. Ready to use liquid reagent for determining the glucose content in blood: U.S. Patent 5,077,199[P]. 1991-12-31.

[2] Kabasakalian P, Kalliney S, Westcott A. Enzymatic blood glucose determination by colorimetry of N, N-diethylaniline-4-aminoantipyrine[J]. Clinical chemistry, 1974, 20(5): 606-607.

Related Products:

BC0340/BC0345	Glucogen Content Assay Kit
BC2540/BC2545	Cellulase(CL) Activity Assay Kit
BC0330/BC0335	Trehalose Content Assay Kit
BC2490/BC2495	Blood Glucose Content Assay Kit

Technical Specifications:

The detection limit: 0.0188 μmol/mL

Linear range: 0.125-8 μmol/mL

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