Glucose Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC2500

Size:50T/48S

Components:

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

Reagent II: Liquid 25 mL×1. Storage at 2-8℃.

Reagent III: Liquid 25 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, spectrophotometer, centrifuge, sonicator, 1 mL glass cuvette, transferpettor, mortar/homogenizer and distilled water.

Procedure:

I. Sample Extraction:

1. Tissue treatment:

The tissue mass (g): volume of distilled water (mL) is 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1mL of distilled water), grind into a homogenate, boil in a boiling water bath for 10 min (cover tightly to prevent water loss), cool to room temperature, centrifuge at 8000g for 10 min at 25°C, and remove the supernatant.

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°C centrifuge for

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10 min, take supernatant on standby.

II. Determination procedure:

1. Preheat the spectrophotometer for 30min, adjust the wavelength to 505 nm and adjust zero with distilled water.
2. Add the following reagents successively into the 1.5ml centrifuge tube:

Reagent（μL）	Blank Tube (A_B)	Standard Tube (A_S)	Test Tube (A_T)
Sample			100
Reagent I		100	
ddH_2O	100		
Mixed reagent	900	900	900

Mix thoroughly, incubate at 37°C (mammals) in the water bath for 15 min and read the absorbance of wavelength at 505 nm. Note the light absorption values of blank tube, standard tube and test tube as A_B, A_S and A_T, respectively. Blank and standard tubes only need to be measured 1-2 times.

III. Calculation:

1. Calculate by the protein concentration:

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

$=(A_T-A_B)\div(A_S-A_B)\div Cpr$

2. Calculate by Sample fresh weight:

Glucose content（μmol/g fresh weight)$=C\times(A_T-A_B)\div(A_S-A_B)\times V_S\div(W\div V_{TS}\times V_S)$

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

3. Calculate by the number of bacteria or cells

Glucose content（μmol/10^4 cell）$=C\times(A_T-A_B)\div(A_S-A_B)\times V_S\div(500\div V_{TS}\times V_S)$

$=0.002\times(A_T-A_B)\div(A_S-A_B)$

C: glucose solution concentration, 1 μmol/mL;

Cpr: sample protein concentration, mg/mL;

V_S: the sample volume added, 100 μL=0.1 mL;

V_{TS}: total sample volume, 1 mL;

W: sample fresh weight, g;

500: 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; the supernatant was then diluted 2 times with distilled water and measured according to the procedure, using a glass cuvette to obtain the absorbance value ΔA_T =

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A_T-A_B = 0.758 - 0.006 = 0.752, ΔA_S= A_S-A_B = 0.600 - 0.006 = 0.594. Calculated from the sample mass

Glucose content (μmol/g mass) = $\Delta A_T \div \Delta A_S \div W \times 2$= 0.752 ÷ 0.594 ÷ 0.1 x 2 = 25.320 μmol/ g mass

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. ΔA_T = A_T-A_B = 0.539 - 0.006 = 0.533 and ΔA_S = A_S-A_B = 0.600 - 0.006 = 0.594 measured by glass cuvette.

Glucose content (μmol/g mass) = $\Delta A_T \div \Delta A_S \div W$ = 0.533 ÷ 0.594 ÷ 0.1 = 8.972 μmol/ g mass

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. ΔA_T = A_T-A_B = 0.018 - 0.006 = 0.012, ΔA_S = A_S-A_B = 0.600 - 0.006 = 0.594

Glucose content (μmol/10^6 cell) = 0.2 x $\Delta A_T \div \Delta A_S$ = 0.2 x 0.012 ÷ 0.594 = 4.040 x 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: 0.0078 μmol/mL

Linear range: 0.0625-3 μmol/mL

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