Glucose Content Assay Kit (O-toluidine Colorimetry)

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC5875

Size:100T/96S

Components:

Extract Solution: 110 mL×1. stored at 2-8°C.

Reagent I: 2.4 mL×1, stored at 2-8°C. Take 3.6mL reagent II, 18mL acetic acid add reagent I mixed, the final working solution is a homogeneous clarified liquid. The dissolved reagent can be stored at -20°C for 8 weeks. (Note: If Reagent I and Reagent II are mixed first, the solution is a liquid mixture of inorganic and aqueous phases, and the solution will become homogeneous and clarified after adding glacial acetic acid.)

Reagent II:10mL×1, stored at 2-8°C.

Standard: Powder×1. Store at 2-8°C. Add 1 mL of distilled water to fully dissolve the 10 mg/mL glucose standard solution before use; store the inexhaustible reagent at 2-8°C for 4 weeks. Before use, take 30 μL of 10 mg/mL glucose standard solution and add 930 μL of distilled water to make 0.3125 mg/mL glucose standard solution.

Product Description

Glucose is not only the main substrate for cellular energy metabolism, but also its metabolic intermediates are important substrates for biosynthesis. In the case of mammals, glucose is not only the only energy source for the nervous system of the brain, muscles, adipose tissue, etc, but also is closely related to the synthesis of reducing coenzymes, lactose and milk fat.

When glucose and o-toluidine are heated in a strong acid solution, the aldehyde group of glucose condenses with o-toluidine to form glucosylamine, which dehydrates to form Schiff base, which is blue-green in color with an absorption peak at 630nm. **The kit is suitable for the determination of animal tissue, serum (plasma) and cell (bacterial) samples.**

Reagents and Equipment Required but Not Provided.

Visible spectrophotometer/microplate reader, cryogenic centrifuge, analytical balance, micro glass cuvette/ 96-well plate, mortar/homogenizer/cell ultrasonic breaker, adjustable pipette, acetic acid (AR > 98%), 5% trichloroacetic acid (determine if you need to bring your own depending on precautions), isopropyl alcohol (determine if you need to bring your own depending on precautions), ice 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℃ centrifuge for 10 min, take supernatant on standby.

3.Liquid samples such as serum (plasma):

Direct measurement. If the liquid is turbid, centrifuge the supernatant.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30min, adjust the wavelength to 630nm and the spectrophotometer adjust zero with acetic acid.

2. Add the following reagents successively into the 1.5ml centrifuge tube:

Reagent（μL）	Test Tube (A_T)	Standard Tube (A_S)	Blank Tube (A_B)
Sample	50	-	-
Standard	-	50	-
ddH_2O	-	-	50
Reagent I	200	200	200

Vortex mixing, boiling at 100 ℃ for 15min (cover tightly to prevent water loss), cooling to room temperature, take 200μL to micro glass cuvette/ 96-well plate to determine the absorbance value at 630nm, were recorded as At, As, Ab. Calculate ΔAt= At - Ab and ΔAs = As - Ab. The standard and blank tubes should be measured only 1-2 times.

III. Calculation:

1. Calculate by the protein concentration:

Glucose content（mg/mg prot）$=Cs \times \Delta At \div \Delta As \times V_S \div (Cpr \times V_S)$

$=0.3125 \times \Delta At \div \Delta As \div Cpr$

2. Calculate by Sample fresh weight:

Glucose content（mg/g fresh weight)$= Cs \times \Delta At \div \Delta As \times V_E \div W$

$=0.3125 \times \Delta At \div \Delta As \div W$

3. Calculate by volume of liquid

Glucose content（mg/mL)$= Cs \times \Delta At \div \Delta As \times V_S \div V_S$

$=0.3125 \times \Delta At \div \Delta As$

4. Calculate by the number of bacteria or cells

Glucose content（mg/10^4 cell）$= Cs \times \Delta At \div \Delta As \times\times V_E \div N$

$=0.3125 \times \Delta At \div \Delta As \div N$

Cs: glucose solution concentration, 0.3125mg/mL;

Cpr: sample protein concentration, mg/mL;
V_S: the sample volume added, 0.05 mL;
V_E: Volume of pre-treatment extract, 1 mL;
W: sample fresh weight, g;
N: number of bacteria or cells.

Note:

1、Reagent I is corrosive, please wear gloves and mask and operate carefully.

2、If the final reaction solution of the high blood lipid sample is turbid, add 550μL of isopropanol to the reaction solution and mix thoroughly to dissolve the lipids, eliminate the turbidity, and measure the absorbance value. Calculate the absorbance by multiplying the measured absorbance by 1.5.

3、Severe jaundice, hemolysis and other serum samples, it is necessary to prepare protein-free hemofiltrate (it is recommended that 100 μL of sample plus 300 μL of 5% trichloroacetic acid solution, mix thoroughly, equivalent to the sample diluted four times, 3000 rpm 4℃ centrifugation for 10min, take the supernatant to be measured), and then calculate. Note the synchronous modification of the calculation formula.

Experimental example:

1. Take 50 μL of human serum sample, dilute 5 times with distilled water, follow the assay procedure, and measure with a 96-well plate. Calculation: ΔAt= At - Ab=0.450-0.057=0.393, ΔAs = As - Ab=0.708-0.057=0.651 calculated by volume of liquid:

Glucose content (mg/mL) = 0.3125 x ΔAt ÷ ΔAs x 5 = 0.943 mg/mL.

2. Take 0.1g of mouse muscle tissue, operate according to the steps of pre-treatment and assay, measured with 96-well plate. Calculation: ΔAt = At - Ab=0.168-0.057= 0.111, ΔAs = As - Ab=0.708-0.057=0.651, calculated by sample fresh weight:

Glucose content (mg/g) = 0.3125 x ΔAt ÷ ΔAs ÷ W =0.533mg/g.

References:

[1] Yee H Y, Goodwin J F. Evaluation of some factors influencing the o-toluidine reaction with glucose[J]. Analytical Chemistry, 2002, 45(13).DOI:10.1021/ac60335a030.

[2] Dubowski K M. An o-Toluidine Method for Body-Fluid Glucose Determination[J]. Clinical Chemistry, 1962(6) :6.DOI:10.1093/clinchem/8.6.592.

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