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Glucose Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK1037-100T-96S

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent name	Size	Storage
Reagent I	Liquid 20 mL × 1	2-8 ℃
Standard	Liquid 5 mL × 1	2-8 ℃

Solution preparation:

1. Standard: 1 μmol/mL Glucose solution.

Product Description:

Glucose is not only the primary substrate for cellular energy metabolism, but its metabolic intermediates are also crucial substrates for biosynthesis. Plants can produce glucose through photosynthesis. For mammals, glucose serves as the sole energy source for the brain's nervous system, muscles, adipose tissue, and is closely related to the synthesis of reducing coenzymes, lactose, and milk fat.

Glucose Oxidase (GOD) catalyzes the oxidation of glucose into gluconic acid, producing hydrogen peroxide in the process; Peroxidase (POD) catalyzes the oxidation of hydrogen peroxide with 4- aminoantipyrine coupled phenol, generating a colored compound that exhibits a characteristic absorption peak at 505 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water-bath/constant temperature incubator, centrifuge, ultrasonic crusher, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 (wrap with a sealing film to prevent bursting), cool to room temperature, centrifuge at 8000g for 10 min at 25°C, and take the supernatant for later use.

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 (104) : 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 (wrap with a sealing film to prevent bursting), cool to room temperature, centrifuge at 8000g for 10 min at 25°C, and take the supernatant for later use.

I. 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. Sample determination (Add the following reagents successively into the 1.5mL EP tube or 96 well flat-bottom plate):

Reagent (μL)
Blank Tube
Standard Tube
Test Tube
Sample
-
-
20
Standard
-
20
- ddH2O
20
-
-
Reagent I
180
180
180

Mix thoroughly, incubate at 37℃ in the water bath/constant temperature incubator for 15 min, read the absorbance of wavelength at 505 nm. Note the absorbance value of blank tube, standard tube and test tube as AB, AS and AT, respectively. Calculate ΔAT=AT-AB, ΔAS=AS-AB. (Blank tube and standard tube only need to be measured 1-2 times.)

II. Calculation:

1. Calculate by the protein concentration:

Glucose content (μmol/mg prot)=C ×ΔAT÷ΔAS×VS÷(Cpr×VS)= 2×ΔAT÷ΔAS÷Cpr

2. Calculate by Sample fresh mass:

Glucose content (μmol/g fresh mass)=C ×ΔAT÷ΔAS×VS÷(W÷VTS×VS)= 2×ΔAT÷ΔAS ÷W

3. Calculate by the number of bacteria or cells

Glucose content (μmol/106 cell)=C ×ΔAT÷ΔAS×VS÷(N÷VTS×VS)=0.4×ΔAT÷ΔAS÷N

C: standard tube concentration, 2 μmol/mL;

VS: add sample volume, 20 μL=0.02 mL;

VTS: total volume of the sample, 1 mL;

Cpr: sample protein concentration, mg/mL;

W: sample fresh mass, g;

5: number of bacteria or cells, count by 1 million.

Note:

If (AT-AB) 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 1.2, it is sufficient to dilute the supernatant with distilled water. Note the calculation formula multiplied by the dilution factor.