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ISO 13485:2016 ISO 9001:2015

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Glucose Content Assay Kit

BC12001-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Glucose is not only the main substrate of cell energy metabolism, but also its metabolic intermediate is an important substrate of biosynthesis.

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 coloured compounds with characteristic absorption peaks at 505 nm.

Kit components

Components	Volume	Storage
Reagent I	10mL × 1 1μmol/mL Glucose solution	2-8°C
Reagent II	25mL × 1	2-8°C
Reagent III	25mL × 1	2-8°C
Mixed reagent: Mix Reagent II and Reagent III at equal volume 1:1 before use, prepare fresh for each experiment.		

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer, 1mL glass cuvette and distilled water.

Protocol

I. Sample Preparation

Tissue: 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 minutes (cover tightly to prevent water loss), cool to room temperature, centrifuge at 8000g for 10 minutes at 25°C, collect the supernatant for the assay.

Bacteria or cells: 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 3seconds, interval of 10seconds, 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 10 minutes, take supernatant on standby.

Serum or Plasma

Directly use for the assay

II. Assay procedure

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

Reagent(μL)	Blank tube (A _B)	Standard tube (A _S)	Test Tube (A _T)
Reagent I	-	100μL	-
Sample	-	-	100μL
Distilled water	100μL	-	-
Mixed reagent	900μL	900μL	900μL

- Mix thoroughly, incubate at 37°C (mammals) or 25°C (other species) in the water bath for 15 minutes and read the absorbance of wavelength at 505 nm. Record A_T , A_B and A_S

Calculations

1. Protein concentration

$$\begin{aligned}\text{Glucose content } (\mu\text{mol/mg protein}) &= C \times (A_T - A_B) \div (A_S - A_B) \times V_S \div (C_{pr} \times V_S) \\ &= (A_T - A_B) \div (A_S - A_B) \div C_{pr}\end{aligned}$$

2. Sample weight

$$\begin{aligned}\text{Glucose content } (\mu\text{mol/g weight}) &= C \times (A_T - A_B) \div (A_S - A_B) \times V_S \div (W \div V_{TS} \times V_S) \\ &= (A_T - A_B) \div (A_S - A_B) \div W\end{aligned}$$

3. Number of cells or bacteria

$$\begin{aligned}\text{Glucose content } (\mu\text{mol}/10^4 \text{ cells}) &= C \times (A_T - A_B) \div (A_S - A_B) \times V_S \div (500 \div V_{TS} \times V_S) \\ &= 0.002 \times (A_T - A_B) \div (A_S - A_B)\end{aligned}$$

C : Glucose solution concentration, 1 $\mu\text{mol/mL}$

C_{pr} : Sample protein concentration, mg/mL

V_S : 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 $(A_T - A_B)$ is greater than 1.5, it is sufficient to dilute the supernatant with distilled water. Note the calculation formula multiplied by the dilution factor.