Glucose Content Assay Kit

Cat. No. KITG-076 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Glucose is the most widely distributed monosaccharide in nature. It is not only the principal substrate of cellular energy metabolism, but its metabolic intermediates are also important substrates for biosynthesis. Glucose has an important role in biology and is widely used in the food industry, medicine, and other fields. Glucose oxidase (GOD) oxidizes glucose to gluconic acid and releases H_2O_2. Peroxidase (POD) catalyzes the H_2O_2-mediated oxidation of 4-aminoantipyrine coupled with phenol to form a red quinone compound. The product has a characteristic absorption peak at 505 nm, and glucose content can therefore be quantitatively determined from the change in absorbance.
Description	Method: Visible spectrophotometry. Assay Format: GOPOD. Pack Size: 70 tests / 50 samples. Storage Temperature: 2-8°C. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Quantitative determination of glucose content in tissue, bacterial or cell, and liquid samples. Glucose is a major substrate of cellular energy metabolism, and its metabolic intermediates are important substrates for biosynthesis. The assay has research value in biology and applications in the food industry and medicine.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer; 1 mL glass cuvette with a 10 mm optical path, 3 mm slit, and 1.05 mL volume; mortar/homogenizer; adjustable pipettes; benchtop centrifuge; thermostatic water

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bath/incubator; and distilled water. 1. Preparation of Samples to Be Tested The sample amount and ratio may be adjusted appropriately according to preliminary experimental results. 1) Tissue Process the sample at a ratio of tissue mass (g) to distilled water volume (mL) of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL distilled water. Grind to form a homogenate. Treat at 95°C for 10 min in a sealed container to prevent water loss. Cool to room temperature. Centrifuge at 8,000 × g at room temperature for 10 min. Collect the supernatant as the sample to be tested. 2) Bacteria or Cells Collect bacteria or cells in a centrifuge tube by centrifugation. Process the sample at a ratio of bacterial or cell number (10^4) to distilled water volume (mL) of (500-1000):1. It is recommended to add 1 mL distilled water to 5 million bacteria or cells. Disrupt the sample by sonication in an ice bath at 200 W, with 3 s sonication and 10 s intervals, repeated 30 times. Treat at 95°C for 10 min in a sealed container to prevent water loss. Cool to room temperature. Centrifuge at 8,000 × g at room temperature for 10 min. Collect the supernatant as the sample to be tested. 3) Liquid Samples Assay directly or dilute appropriately before the assay. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min, set the wavelength to 505 nm, and zero the instrument with distilled water. 2) Preparation of Detection Working Solution (prepare immediately before use) Before use, prepare the amount required by mixing Reagent 1 and Reagent 2 at a volume ratio of 1:1. 3) Preparation of Standard Dilutions (prepare immediately before use) Dilute the 10 mg/mL glucose standard solution with distilled water to prepare standard dilutions of 0.4, 0.3, 0.2, 0.1, 0.05, and 0.025 mg/mL as follows: A: Mix 100 µL of the 10 mg/mL standard solution with 900 µL distilled water to obtain 1.0 mg/mL. 1: Mix 200 µL of the 1.0 mg/mL solution with 300 µL distilled water to obtain 0.4 mg/mL. 2: Mix 150 µL of the 1.0 mg/mL solution with 350 µL distilled water to obtain 0.3 mg/mL. 3: Mix 100 µL of the 1.0 mg/mL solution with 400 µL distilled water to obtain 0.2 mg/mL. 4: Mix 50 µL of the 1.0 mg/mL solution with 450 µL distilled water to obtain 0.1 mg/mL. 5: Mix 200

μL of the 0.1 mg/mL solution with 200 μL distilled water to obtain 0.05 mg/mL. 6: Mix 200 μL of the 0.05 mg/mL solution with 200 μL distilled water to obtain 0.025 mg/mL. 4) Reaction Setup Add the following reagents sequentially to centrifuge tubes: Test tube: 100 μL sample and 900 μL detection working solution. Standard tube: 100 μL standard dilution and 900 μL detection working solution. Blank tube: 100 μL distilled water and 900 μL detection working solution. Mix thoroughly and react at 37°C for 15 min. 5) Absorbance Measurement Transfer the reaction solution to a 1 mL glass cuvette and measure absorbance at 505 nm. Record the values as A_test, A_standard, and A_blank. Calculate ΔA_test = A_test - A_blank and ΔA_standard = A_standard - A_blank. The standard tube at each concentration and the blank tube only need to be measured 1-2 times. 6) Establishment of the Standard Curve Use 0.4, 0.3, 0.2, 0.1, 0.05, and 0.025 mg/mL as the x-axis values and the corresponding ΔA_standard values as the y-axis values. Plot the standard curve and obtain the standard equation y = kx + b. Substitute ΔA_test into the equation to obtain x in mg/mL. 3. Calculation of Glucose Content 1) Based on Tissue Protein Concentration Glucose content (μg/mg prot) = [x × V_total × 1000 × D] / (Cpr × V_total) = (x × 1000 × D) / Cpr. 2) Based on Tissue Sample Mass Glucose content (μg/g) = [x × V_total × 1000 × D] / W = (x × 1000 × D) / W. 3) Based on Bacterial or Cell Number Glucose content (μg/10^4 cells) = [x × V_total × 1000 × D] / bacterial or cell number = (x × 1000 × D) / bacterial or cell number. 4) Based on Liquid Sample Volume Glucose content (μg/mL) = 1000 × x × D. Variable Definitions: V_total: total volume of the sample to be tested, 1 mL. Cpr: protein concentration of the sample to be tested, mg/mL. W: sample mass, g. Bacterial or cell number: expressed in units of 10^4. For 5 million bacteria or cells, enter 500. 1000: unit conversion factor; 1 mg/mL = 1000 μg/mL. D: dilution factor of the sample to be tested; use 1 when the sample is not diluted. 4. Precautions 1) Avoid skin contact with Reagent 1 and Reagent 2. If either reagent is accidentally spilled, rinse the contacted area with plenty of water. 2) If A_test or ΔA_test is outside the linear absorbance range of the standards, proceed as follows: when the value is higher than the maximum, dilute the sample appropriately

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	with distilled water and repeat the assay; when the value is lower than the minimum, prepare a more concentrated sample and repeat the assay. Modify the calculation accordingly. 3) To ensure accurate results and avoid reagent loss, carefully read the instructions before the assay, confirm that reagent storage and preparation are adequate, and ensure that the operating steps are clear. Perform a preliminary assay with 2-3 samples expected to show relatively large differences. Obtain technical assistance promptly if problems arise during the procedure.
Storage	Kit Components and Storage: The kit is stored at 2-8°C. Reagent 1: liquid, 35 mL × 1 bottle; store at 4°C. Reagent 2: liquid, 35 mL × 1 bottle; store at 4°C protected from light. Standard Solution: liquid, 1 mL × 1 tube; 10 mg/mL glucose standard solution; store at 4°C.
Reconstitution	Reagent Preparation: Detection Working Solution: Prepare immediately before use by mixing Reagent 1 and Reagent 2 at a volume ratio of 1:1 according to the amount required.
Reaction conditions	React at 37°C for 15 min. Measure absorbance at 505 nm in a 1 mL glass cuvette.

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