Hexokinase Assay Kit (Spectrophotometry)

Description

Hexokinase (HK; EC 2.7.1.1) is widely present in animals, plants, microorganisms, and cultured cells. It is the first key enzyme in the glucose metabolism pathway and catalyzes the conversion of glucose to glucose-6-phosphate (G6P). Glucose-6-phosphate serves as a metabolic crossroad between glycolysis and the pentose phosphate pathway.

Detection Principle

HK catalyzes the phosphorylation of glucose to form glucose-6-phosphate. Glucose-6-phosphate dehydrogenase further catalyzes the oxidation of glucose-6-phosphate to generate NADPH. NADPH exhibits a characteristic absorbance peak at 340 nm.

Packing

Taking 50T/48S packing for example:

Components	Packing	Storage
CB0088UV-ES	60 mL x 1	4 °C
CB0088UV-A	30 mL x 1	4 °C
CB0088UV-B	1 vial (powder) x 1	4 °C
CB0088UV-C	5 mL×1	4 °C
CB0088UV-D	1 vial (powder) x 1	-20 °C
CB0088UV-E	1 vial (powder) x 1	-20 °C
CB0088UV-F	1 vial (powder) x 1	-20 °C

Note: Before formal measurement, be sure to select 2–3 samples with relatively large expected differences for a preliminary test.

Instructions

I. Required Equipment & Materials:

UV-Vis spectrophotometer, thermostatic water bath, benchtop centrifuge, adjustable micropipette, 1 mL quartz cuvette, mortar and pestle, ice, and distilled water.

II. Reagent Preparation:

CB0088UV-B: Before use, add 30 mL of distilled water and dissolve thoroughly. Any remaining reagent can be stored at 4°C for up to one week.

CB0088UV-D: Before use, add 4 mL of distilled water and dissolve thoroughly. Any remaining reagent can be stored at 4°C for up to one week.

CB0088UV-E: Before use, add 2 mL of distilled water and dissolve thoroughly. Prepare fresh and use immediately.

CB0088UV-F: Before use, add 250 μL of CB0088UV-A and 250 μL of distilled water, then dissolve thoroughly. Any remaining reagent can be stored at 4°C for up to one week.

Sample Preparation:

1. Bacteria or cultured cells:

Collect bacteria or cells into centrifuge tubes, centrifuge, and discard the supernatant. Add CB0088UV-ES according to the ratio of bacteria or cell number (10^4 cells): CB0088UV-ES volume (mL) = 500–1000:1. (It is recommended to add 1 mL CB0088UV-ES per 5 million bacteria or cells.)

Disrupt the bacteria or cells by sonication on ice (power: 20% or 200 W; sonication: 3 s pulse with 10 s intervals; repeat 30 cycles). Centrifuge at 8,000 ×g for 10 min at 4°C. Collect the supernatant and keep it on ice for subsequent analysis.

2. Tissue samples:

Add CB0088UV-ES according to the ratio of tissue weight (g): CB0088UV-ES volume (mL) = 1:5–10. (It is recommended to use approximately 0.1 g tissue with 1 mL CB0088UV-ES.) Homogenize the tissue on ice. Centrifuge at 8,000 ×g for 10 min at 4°C. Collect the supernatant and keep it on ice for subsequent analysis.

3. Serum (plasma) samples:

Samples can be analyzed directly without further preparation.

III. Assay Procedure

1. Preheat the spectrophotometer for 30 min, set the wavelength to 340 nm, and use distilled water to zero the instrument.

2. CB0088UV-A, B, C, D, and E were preheated in a water bath at 25°C (for general species) or 37°C (for mammals) for 10 min.

3. Add the following reagents sequentially

	Sample Tube (μL)
CB0088UV-A	400
CB0088UV-B	400
CB0088UV-C	80
CB0088UV-D	80
CB0088UV-E	40
CB0088UV--F	8
Samples	30
Mix thoroughly. Start timing immediately after adding the sample. Measure the initial absorbance (A1) at 340 nm after 20 seconds. After measurement, quickly place the cuvette containing the reaction mixture into a 37° C water bath or incubator (for mammals) or 25° C water bath or incubator (for other species) and allow the reaction to proceed for exactly 5 minutes. Remove the cuvette promptly, wipe it dry, and measure the absorbance again at 340 nm. Record the absorbance value at 5 minutes and 20 seconds (A2). Calculate the change in absorbance as: △A = A2 − A1.	

IV. Calculation of HK Activity

1. HK Activity in Serum (Plasma)

Definition of Unit: One enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of NADPH per minute per mL of serum (plasma).

HK (U/mL) = [ΔA × V_total reaction ÷ (ε × d) × 10^9] ÷ V_sample ÷ T = 1113 × ΔA

2. HK Activity in Tissues, Bacteria, or Cells

(1) Calculated Based on Sample Protein Concentration

Definition of Unit: One enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of NADPH per minute per mg of tissue protein.

HK (U/mg prot) = [ΔA × V_total reaction ÷ (ε × d) × 10^9] ÷ (V_sample × C_pr) ÷ T = 1113 × ΔA ÷ C_pr

(2) Calculated Based on Sample Fresh Weight

Definition of Unit: One enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of NADPH per minute per g of fresh tissue weight.

HK (U/g fresh weight) = [ΔA × V_total reaction ÷ (ε × d) × 10^9] ÷ (W × V_sample ÷ V_sample total) ÷ T = 1113 × ΔA ÷ W

(3) Calculated Based on Bacterial or Cell Density

Definition of Unit: One enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of NADPH per minute per 10^4 bacteria or cells.

HK (U/10^4 cells) = [ΔA × V_total reaction ÷ (ε × d) × 10^9] ÷ (500 × V_sample ÷ V_sample total) ÷ T = 2.226 × ΔA

Notes:

V_total reaction: Total reaction volume, 1.038 × 10^{-3}L

ε: Molar extinction coefficient of NADPH, 6.22 × 10^3 L/mol/cm

d: Optical path length of cuvette, 1 cm

V_sample: Volume of sample added, 0.03 mL

V_sample total: Total volume of extraction solution added, 1 mL

T: Reaction time, 5 min

C_pr: Protein concentration of sample, mg/mL

W: Sample weight, g

500: Total number of bacteria or cells, 5 million

Precautions

1. If a large number of samples need to be tested at one time, reagents A, B, C, D, E, and F can be mixed together according to the required ratio to prepare a working solution, and pre-incubated for 10 min.
2. HK activity varies among different homogenized tissues. Before performing the formal experiment, it is recommended to conduct a preliminary test using 1–2 samples. If ΔA > 0.5, it indicates that the tissue activity is too high. In this case, the homogenate supernatant should be diluted with extraction buffer to an appropriate concentration (multiply by the corresponding dilution factor in the calculation formula), or the reaction time should be shortened to 2 min to ensure ΔA < 0.5, thereby improving the detection sensitivity.
3. The temperature of the reaction mixture in the cuvette must be maintained at 37°C or 25°C. Prepare a small beaker containing an appropriate amount of distilled water preheated to 37°C or 25°C, and place the beaker in a 37°C or 25°C water bath. During the reaction, keep the cuvette containing the reaction mixture immersed in this beaker to maintain the required temperature. It is also recommended that two people perform this experiment simultaneously: one person performs the colorimetric measurement, while the other keeps track of the reaction time, ensuring the accuracy and reliability of the experimental results.
4. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
5. Please wear a lab coat and disposable gloves.