(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Hexokinase Assay Kit

Catalog No.: BC00178

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Hexokinase Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, bacteria
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (340 nm)

Product Introduction

Hexokinase is widely present in animals, plants, microorganisms and cultured cells. As the first key enzyme in glucose catabolism, it catalyzes the conversion of glucose into glucose-6-phosphate, which is the intersection of glycolysis and the pentose phosphate pathway.

Principle

HK catalyzes the conversion of glucose to glucose-6-phosphate. Glucose-6-phosphate dehydrogenase further catalyzes the dehydrogenation of glucose-6-phosphate to produce NADPH, which exhibits a characteristic absorption peak at 340 nm.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	40 mL	Store at 4°C
Reagent 2	Powder	1 vial	Store at 4°C. Add 15 mL of distilled water before use and dissolve completely for later use; store at 4°C.
Reagent 3	Powder	1 vial	Store at 4°C (or at -20°C for long-term storage). Add 1 mL of distilled water to one vial of powder to dissolve before use, and keep the solution for later application. Aliquot any unused portion and store at -20°C to avoid repeated freeze-thaw cycles.

Storage

The kit has a validity period of 6 months.

Preparation

UV spectrophotometer and 1 mL capacity (1 cm path length) quartz cuvettes, thermostatic water bath, desktop centrifuge, vortex mixer, adjustable pipettes, mortar, ice, distilled water, physiological saline, protein assay reagent (for tissue or cell samples, available from our company).

- **Sample handling**

1. Bacteria or cultured cells: First collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant. According to the ratio of bacteria or cell count (10^4): volume of physiological saline (mL) at 500–1000:1 (it is recommended to add 1 mL of physiological saline to 5 million bacteria or cells), disrupt the bacteria or cells by ultrasonication (ice bath, power 20% or 200 W, sonication for 3 s, interval for 10 s, repeat 30 cycles).Centrifuge at 8000 g and 4°C for 10 min, collect the supernatant, place on ice for immediate assay.
2. Tissue: Homogenize on ice at a ratio of tissue weight (g) to physiological saline (mL) of 1:5–10 (recommended: weigh 0.1 g tissue and add 1 mL physiological saline). Centrifuge at 8000 g and 4°C for 10 min, collect the supernatant, and place on ice for testing.
3. Serum (plasma) samples: Take samples directly for detection.

Note: Phosphate-buffered saline must not be used as the homogenization medium in this experiment.

Operation process

Before formal determination, be sure to select 2-3 samples with expected large differences to conduct a preliminary experiment.

1. Preheat the spectrophotometer for at least 30 minutes, set the wavelength to 340 nm, and zero the instrument with distilled water.
2. Preheat Reagent 1 and Reagent 2 at 37 °C (for mammals) or 25 °C (for other species) for 10 minutes before use.
3. Operation table:

	Assay tube
Reagent 1 (μL)	750
Reagent 2 (μL)	250
Reagent 3 (μL)	20
Sample to be tested (μL)	40
Add the above reagents into a 1 mL quartz cuvette in sequence, mix immediately, and start timing when adding the sample. Record the initial absorbance $\mathbf{A_1}$ at 20 seconds and the absorbance $\mathbf{A_2}$ at 20 minutes and 20 seconds at a wavelength of 340 nm. Calculate $\mathbf{\Delta A = A_2 - A_1}$ (the reaction time is 20 minutes).	

Calculation

1. Serum (plasma) HK activity:

Definition: One enzyme activity unit is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per milliliter of serum (plasma).

Calculation formula:

$$\text{HK activity (nmol/min/mL)} = \frac{\Delta A}{\varepsilon \times d} \times 10^9 \times \frac{V_{Total}}{V_{Sample}} \div T$$

2. HK activity in tissues, bacteria or cells:

a. Calculated based on the protein concentration of the sample.

Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per milligram of tissue protein.

Calculation formula:

$$\text{HK activity (nmol/min/mg)} = \frac{\Delta A}{\varepsilon \times d} \times 10^9 \times \frac{V_{Total}}{V_{Sample}} \div T \div Cpr$$

b. Calculated based on sample fresh weight.

Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per gram of tissue.

Calculation formula:

$$\text{HK activity (nmol/min/g)} = \frac{\Delta A}{\varepsilon \times d} \times 10^9 \times \frac{V_{Total}}{V_{Sample}} \div T \div \frac{W}{V_{Extraction\ Solution}}$$

c. Calculated based on bacterial or cell density.

Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per 10,000 bacteria or cells.

Calculation formula:

$$\text{HK activity (nmol/min/}10^4\text{ cells)} = \frac{\Delta A}{\varepsilon \times d} \times 10^9 \times \frac{V_{Total}}{V_{Sample}} \div T \div \text{Cell number}$$

Annotation:

V_{Total}: Total volume of reaction system, 1.06×10^{-3} L

V_{Sample}: Volume of sample added, 0.04 mL

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

d: Cuvette light path length, 1 cm

10^9: Unit conversion factor from mol to nmol

$V_{Extraction\ Solution}$: Total volume of extraction solution added during homogenization, mL

T: Reaction time, 20 min

Cpr: Sample protein concentration, mg/mL

W: Sample mass, g

Cell number: Total number of bacteria or cells, 10^4 cells

Precautions

1. If a large number of samples are to be assayed at one time, Reagent 1, 2 and 3 can be prepared into a working mixture according to the ratio (prepare fresh before use).
2. HK activity varies among different samples. If **△A > 0.5**, it indicates high HK activity in the sample. The sample must be diluted by an appropriate factor before measurement, or the reaction time can be shortened to 10 min or 5 min to make **△A < 0.5**, so as to improve detection sensitivity. If **△A < 0.01**, it indicates low HK activity in the sample. In this case, the sample volume should be increased or the reaction time should be prolonged (e.g., to 30 min or 40 min).
3. It is recommended to use disposable laboratory test tubes for operation.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.