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

Hexokinase (HK) Activity Assay Kit

Catalog No.: BC00144

Size: 100T

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 (HK) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, cells, tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Microplate reader (optimal detection wavelength 340 nm)

Product Introduction

Hexokinase (HK) (EC 2.7.1.1) is widely found in animals, plants, microorganisms and cultured cells. It is the first key enzyme in the glucose breakdown process, catalyzing the conversion of glucose to glucose-6-phosphate, which is the intersection of glycolysis and the pentose phosphate pathway.

Principle

HK catalyzes the synthesis of glucose-6-phosphate from glucose, and glucose-6-phosphate dehydrogenase further catalyzes the dehydrogenation of glucose-6-phosphate to generate NADPH, which has a characteristic absorption peak at 340 nm.

Components

Components	Size	Storage
Reagent 1	15 mL	2~8℃
Reagent 2	1 bottle of powder	2~8℃
	Add 5mL of distilled water before use, dissolve thoroughly, and store at 4℃.	
Reagent 3	1 tube of powder	-20℃

	Before use, add 0.5 mL of distilled water to one tube of powder to dissolve and set aside. Any unused portion should be repackaged and stored at -20°C to avoid repeated freeze-thaw cycles.	
Consumables 1	Microplate (96 wells) 1 plate	RT
Consumables 2	Plate Sealer 2 pieces	RT

Storage

The kit has a shelf life of 6 months

Preparation before the experiment

Sample processing

1. Tissue: Homogenize tissue at a ratio of 1:5-10 (tissue mass (g): physiological saline (mL) – it is recommended to weigh 0.1g of tissue and add 1mL of physiological saline) on ice. Centrifuge at 8000g at 4℃ for 10min, collect the supernatant and place it on ice for analysis.
2. Serum (plasma): direct sampling and testing
3. Cells or bacteria: Collect cells or bacteria into a centrifuge tube. Centrifuge and discard the supernatant. Resuspend the pellet in normal saline at a ratio of cell/bacterial count (10^4 cells) to saline volume (mL) of 1:500–1000 (recommended: 5×10^6 bacteria or cells per 1 mL normal saline). Sonicate the suspension in an ice bath (power 20% or 200 W; 3 s on, 10 s off, for 30 cycles). Centrifuge at 8000 × g at 4°C for 10 min. Collect the supernatant and place it on ice for subsequent measurement.

Note: Phosphate buffer cannot be used as a homogenizing medium in this experiment.

Operation process

1. Preheat the microplate reader for at least 30 minutes and adjust the temperature to 37℃ (if the microplate reader is not temperature adjustable, a 37℃ water bath or incubator can be prepared as a backup).

2. Before use, preheat reagents 1 and 2 to 37°C (mammals) or 25°C (other species) for 10 minutes, and then mix them in a ratio of 150:50:2 for reagent 1: reagent 2 solution: reagent 3 solution to prepare a working solution. The solution is ready for use.

3. Operation table: (Operating in an orifice plate)

Reagents(μL)	Blank tube	Test tube
Physiological saline	10	--
Sample to be tested	--	10
Working solution	190	190
Start timing while mixing the sample and working solution (and gently shake the plate). Read the initial absorbance A1 at 340nm wavelength on the microplate reader after 20 seconds. Incubate the plate at 37°C. Read the absorbance A2 again at 20 minutes and 20 seconds. Calculate ΔA = A2 - A1 (the reaction time at this time is 20 minutes).		

Note: 1. It is best to take 1-2 sample supernatants for preliminary testing before conducting the formal experiment in order to determine the optimal sampling concentration.

2. The activity levels of HK vary in different samples. If the ΔA value is >0.4, it indicates that the sample activity is too high. The sample should be diluted by a certain factor before measurement (multiply by the corresponding dilution factor in the calculation formula), or the reaction time should be shortened to 10 min or 5 min to make the ΔA value <0.4, thereby improving the detection sensitivity. If the ΔA value is <0.01, it indicates that the sample HK activity is too low. In this case, the sample volume should be increased (the working solution volume remains unchanged, and the variable is substituted into the calculation formula) or the reaction time should be extended (e.g., 30 min or 40 min).

Result calculation

1. Serum (plasma) calculation method:

Unit definition: One enzyme activity unit is defined as 1 nmol of NADPH produced per minute by the catalytic reaction of one milliliter of serum (plasma).

$$\text{HK Activity（nmol/min/mL）} = \frac{\triangle A}{\varepsilon \times d} \times 10^9 \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \div T$$

2. Computational methods for tissues, bacteria, and cells

(1) Calculated based on sample protein concentration

Unit definition: One enzyme activity unit is defined as the amount of NADPH produced by the catalytic reaction of each mg of tissue protein per minute.

$$\text{HK Activity（nmol/min/mg prot）} = \frac{\triangle A}{\varepsilon \times d} \times 10^9 \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \div T \div Cpr$$

(2) Calculated based on sample fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of NADPH produced per gram of tissue per minute through catalytic reaction.

$$\text{HK Activity（nmol/min/g）} = \frac{\triangle A}{\varepsilon \times d} \times 10^9 \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \div T \div \frac{W}{V_{\text{sample total}}}$$

(3) Calculated by bacterial or cell density

Unit definition: One enzyme activity unit is defined as the production of 1 nmol of NADPH per minute by 10,000 bacteria or cells catalyzing a reaction.

$$\text{HK Activity（nmol/min/}10^4\text{ cell）} = \frac{\triangle A}{\varepsilon \times d} \times 10^9 \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \div T \div \text{Cell count}$$

Annotation

$V_{\text{reaction total}}$: total volume of the reaction solution , 0.2×10^{-3} L;

ε: NADPH molar extinction coefficient, 6.22×10^3 L / mol/cm;

d: Cuvette optical path length, 0.55 cm;

10 9 : mol → nmol unit conversion factor;

V_{sample} : Add 0.01 mL of sample volume;

$V_{\text{sample total}}$: The total volume of extract added during homogenization, in mL;

T : Reaction time , 20 min ;

Cpr : Protein concentration in the sample homogenate , mgprot/mL (prot refers to protein);

W : Sample mass , g;

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

For example

Take 0.1g of frozen chicken breast tissue, add 1mL of physiological saline for homogenization, and dilute the resulting supernatant with physiological saline five times. The results show an A1 value of 0.185, an A2 value of 0.295, and a protein concentration of 0.882mg/mL in the 5-fold diluted supernatant. The calculations are as follows:

$$\text{HK Activity（nmol/min/mg prot）} = \frac{0.295 - 0.185}{6.22 \times 10^3 \times 0.55} \times 10^9 \times \frac{0.2 \times 10^{-3}}{0.01} \div 20 \div 0.882$$

$$= 34.80\text{nmol/min/mg prot}$$

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.