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

Catalog No：E-BC-K610-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-450 nm）

Detection range：5.93-40.11 U/L

Elabscience®Hexokinase (HK) Activity Assay Kit

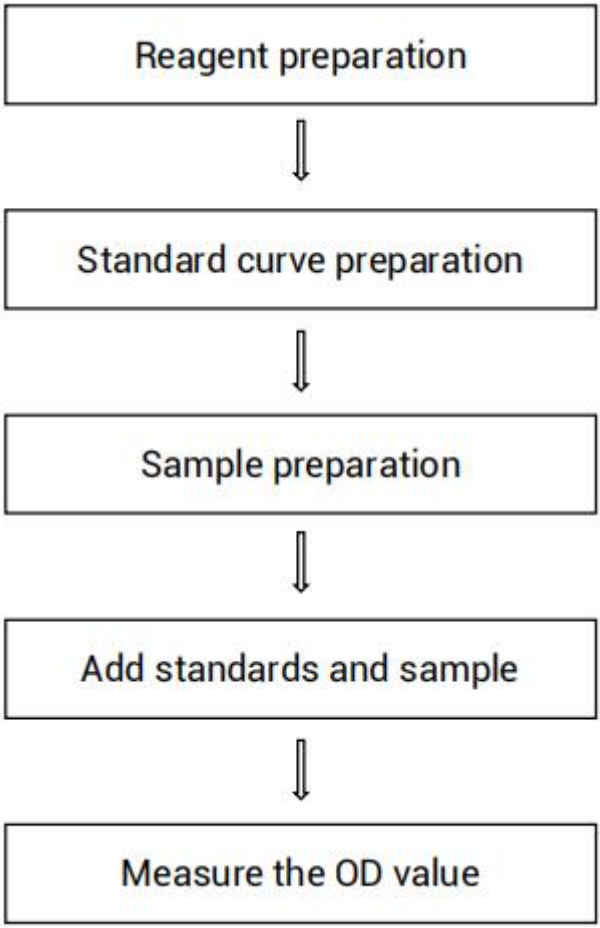
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can measure hexokinase (HK) activity in animal tissue and cell samples.

Detection principle

Hexokinase (HK) is a key enzyme in glucose decomposition, which exists in different species. It is a kind of enzyme that catalyzes the phosphorylation of hexose to generate hexose phosphate, and is also one of the key enzymes in carbohydrate metabolism. HK converts glucose to glucose 6-phosphate, which is catalyzed by the enzyme. Meanwhile, NAD^+ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of HK can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	Powder × 1 vial	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	0.8 mmol/L Standard	3.2 mL × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Centrifuge, 37℃ Incubator, Microplate reader (440-450 nm, optimum wavelength: 450 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate stock solution：
Dissolve one vial of substrate with 12 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 2 weeks protected from light.

③ The preparation of enzyme stock solution：
Dissolve one vial of enzyme reagent with 12 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 2 weeks protected from light.

④ The preparation of enzyme working solution：
Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 300 μL of enzyme working solution (mix well 150 μL of substrate working solution and 150 μL of enzyme stock solution). The enzyme working solution should be prepared on spot. Store at 2-8°C for 12 h protected from light.

⑤ The preparation of sample reaction solution：

For each well, prepare 220 μL of sample reaction solution (mix well 200 μL of enzyme working solution and 20 μL of chromogenic agen). The sample reaction solution should be prepared on spot. Store at 2-8°C for 12 h protected from light.

⑥ The preparation of control reaction solution：

For each well, prepare 220 μL of control reaction solution (mix well 200 μL of double distilled water and 20 μL of chromogenic agent). The control reaction solution should be prepared on spot. Store at 2-8°C for 12 h protected from light.

⑦ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.8 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.16, 0.32, 0.4, 0.48, 0.56, 0.64, 0.8 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.16**	**0.32**	**0.4**	**0.48**	**0.56**	**0.64**	**0.8**
0.8 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 5×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 5×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	2-6
10% Mouse brain tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat liver tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

The powder must be completely dissolved while preparing the working solution.

Operating steps

① Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.
Control well: Add 10 μL of sample to the corresponding wells
Sample well: Add 10 μL of sample to the corresponding wells.

② Add 220 μL of sample reaction solution to standard well and sample well. Add 220 μL of control reaction solution to control well.

③ Incubate at 37°C for 10 min.

④ Measure the OD value of each well at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue and cells sample:

Definition: The amount of HK in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol glucose-6-phosphate at 37℃ is defined as 1 unit

$$\text{HK activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

[Note]

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{450}: $OD_{Sample} - OD_{Control}$.

T: The time of reaction, 10 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000*: 1 mmol = 1000 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse heart tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.40	25.50	32.00
%CV	3.6	3.2	3.1

Inter-assay Precision

Three mouse heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.40	25.50	32.00
%CV	8.7	9.5	9.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	standard 1	standard 2	standard 3
Expected Conc. (mmol/L)	0.28	0.45	0.62
Observed Conc. (mmol/L)	0.3	0.4	0.6
Recovery rate (%)	101	95	101

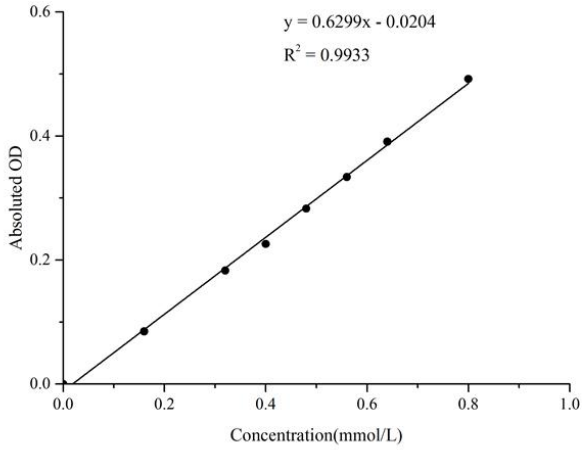
Sensitivity

The analytical sensitivity of the assay is 5.93 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	0.16	0.32	0.4	0.48	0.56	0.64	0.8
Average OD	0.049	0.132	0.232	0.275	0.332	0.383	0.440	0.541
Absoluted OD	0	0.085	0.183	0.226	0.283	0.334	0.391	0.492



Appendix Π Example Analysis

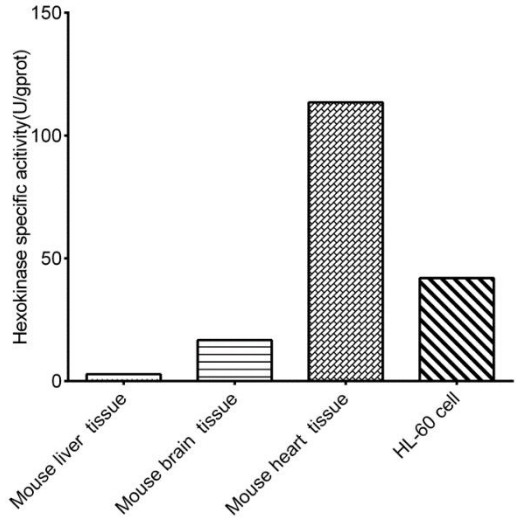
Example analysis：

For mouse liver tissue, take 10 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.6299 x – 0.0204, the average OD value of the control is 0.157, the average OD value of the sample is 0.298, the concentration of protein in sample is 9.45 gprot/L, and the calculation result is:

HK activity (U/gprot) = (0.298 – 0.157 + 0.0204) ÷ 0.6299 ÷ 10 × 1000 ÷ 9.45 = 2.71 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 9.45 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 3.93 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 6.45 gprot/L, dilute for 5 times) and HL-60 cell (the concentration of protein is 0.79 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.