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

Catalog No：E-BC-K768-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader (440-460 nm)

Detection range：2.62-42.80 U/L

Elabscience® Glucokinase (GCK) Activity Colorimetric Assay Kit

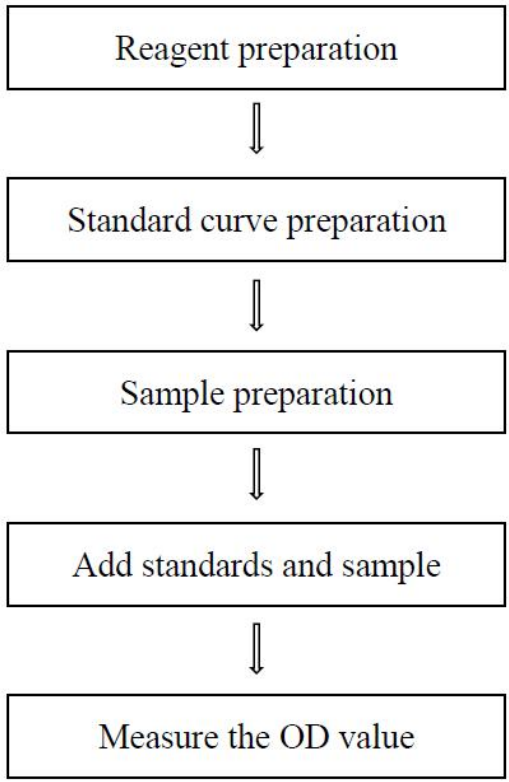
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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 glucokinase (GCK) activity in animal tissue and cell samples.

Detection principle

Glucokinase (GCK) is a key enzyme in the glucose metabolism pathway. It participates in the first step of glycolysis, catalyzes glucose phosphorylation, promotes insulin secretion and glucose metabolism, and effectively controls blood glucose balance in internal. GCK, also known as hexokinase IV, is mainly expressed in hepatocytes and islet β-cell, and has a stronger affinity for glucose than other hexokinases. Therefore, the total hexokinase activity in the sample is measured at high glucose concentration, and the hexokinase activity with low glucose affinity is measured at low glucose concentration. The GCK activity in the sample can be obtained by subtracting the total hexokinase from the total hexokinase.

The detection principle of this kit: GCK catalyzes the substrate reaction to produce NADPH, which reacts with the chromogenic agent. The activity of GCK can be calculated by measuring the generation rate of chromogenic substance at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution A	50 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Extraction Solution B	0.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Buffer Solution	20 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Substrate	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 6	Accelerant	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 7	Chromogenic Agent	3.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 8	Standard	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator(37℃)

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of extraction working solution:

Before testing, please prepare sufficient extraction working solution according to the test wells. For example, prepare 1000 μL of extraction working solution (mix well 990 μL of extraction solution A and 10 μL of extraction solution B). Keep it on ice protected from light and used up within 1 day.

③ The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 1.5 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 5 days protected from light.

④ The preparation of accelerant working solution:

Dissolve one vial of accelerant with 1 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 5 days protected from light.

⑤ The preparation of measuring working solution:

For each well, prepare 100 μL of measuring working solution (mix well 40 μL of buffer solution, 20 μL of substrate, 20 μL of enzyme working solution and 20 μL of accelerant working solution). Keep it on ice protected from light and used up within 1 day.

⑥ The preparation of control substrate:

Before testing, please prepare sufficient control substrate according to the test wells. For example, prepare 1000 μL of control substrate (mix well 995 μL of buffer solution and 5 μL of substrate). Keep it on ice protected from light and used up within 1 day.

⑦ The preparation of control working solution:

For each well, prepare 100 μL of control working solution (mix well 40 μL of buffer solution, 20 μL of control substrate, 20 μL of enzyme working solution and 20 μL of accelerant working solution). Keep it on ice protected from light and used up within 1 day.

⑧ The preparation of 0.5 mmol/L standard solution:

Dissolve one vial of standard with 1.5 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 2 days protected from light.

⑨ The preparation of standard curve:

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

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.35, 0.4, 0.45, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.35**	**0.4**	**0.45**	**0.5**
0.5 mmol/L Standard (μL)	0	40	80	120	140	160	180	200
Double distilled water (μL)	200	160	120	80	60	40	20	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 extraction working solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 3h.

Cell (adherent or suspension) samples:

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

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

③ Homogenize 1×10^6 cells in 200 μL extraction working solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 3h.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse small intestine tissue homogenate	1
1×10^6 Jurkat cells	1
1×10^6 HL-60 cells	1
1×10^6 293T cells	1

Note: The diluent is extraction working solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard with different concentrations to the wells.

Sample well: Add 20 μL of sample to the wells.

Control well: Add 20 μL of sample to the wells.

② Add 100 μL of measuring working solution to standard and sample wells. Add 100 μL of control working solution to control wells.

③ Add 30 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 5 s and measure the OD value of each well at 450 nm with microplate reader, as A_1. Incubate at 37°C for 5 min, measure the OD value of each well at 450 nm with microplate reader, as A_2. (The standard curve is fitted to the standard well in A_2 value.)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean A_2 value of the blank (Standard #①) from all standard readings. This is the absoluted A_2 value.
3. Plot the standard curve by using absoluted A_2 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:

For tissue and cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that consume 1 μmol NADPH at 37°C is defined as 1 unit.

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

[Note]

ΔA_{450}: $\Delta A_{450} = \Delta A_{sample} - \Delta A_{control}$, $A = A_1 - A_2$.

T: Reaction time, 5 min.

1000: 1 mmol/L = 1000 μmol/L.

f: Dilution factor of sample before test.

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

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)	12.00	25.00	35.00
%CV	2.9	3.5	6.6

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)	12.00	25.00	35.00
%CV	1.3	3.9	7.4

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	12.00	25.00	35.00
Observed Conc. (U/L)	11.5	25.0	38.5
Recovery rate (%)	96	100	110

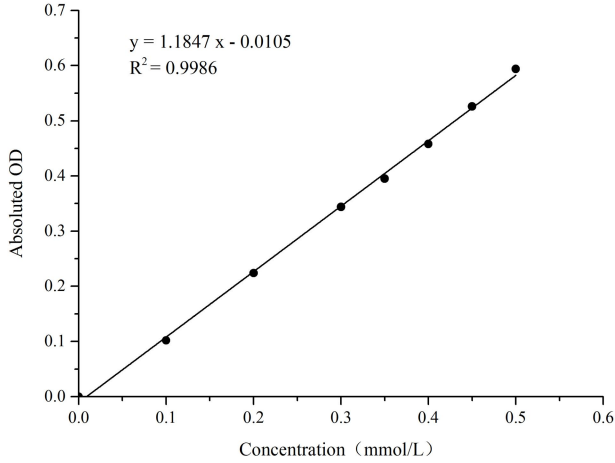
Sensitivity

The analytical sensitivity of the assay is 2.62 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**	**0.1**	**0.2**	**0.3**	**0.35**	**0.4**	**0.45**	**0.5**
OD Value	0.059	0.158	0.278	0.398	0.438	0.510	0.575	0.648
	0.062	0.167	0.291	0.410	0.473	0.526	0.597	0.660
Average OD	0.061	0.163	0.285	0.404	0.456	0.518	0.586	0.654
Absoluted OD	0	0.102	0.224	0.344	0.395	0.458	0.526	0.594



Appendix II Example Analysis

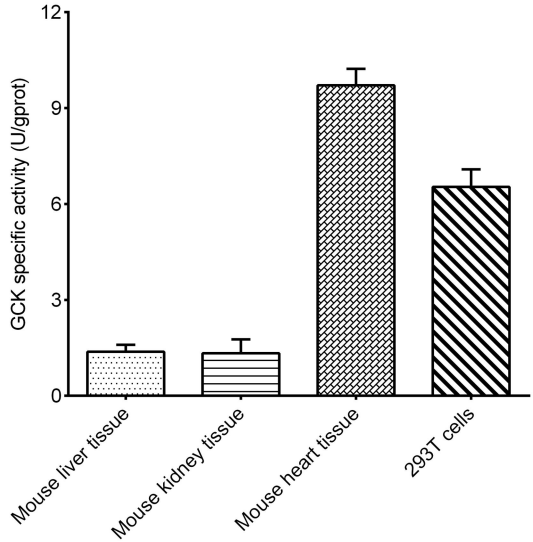
Example analysis:

Take 20 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.1847 x - 0.0105, The A_1 value of the sample is 0.394, the A_2 value of the sample is 0.887, $\Delta A_{sample} = 0.887 - 0.394 = 0.493$; the A_1 value of the control is 0.312, the A_2 value of the control is 0.746, $\Delta A_{control} = 0.746 - 0.312 = 0.434$, $\Delta A_{450} = \Delta A_{sample} - \Delta A_{control} = 0.493 - 0.434 = 0.059$, the concentration of protein is 8.65 gprot/L, and the calculation result is:

$$\text{GCK activity (U/gprot)} = (0.059 + 0.0105) \div 1.1847 \div 5 \times 1000 \div 8.65 = 1.36 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 8.65 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 8.13 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 8.27 gprot/L) and 1×10^6 293T cells (the concentration of protein is 0.484 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.