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

Catalog No：E-BC-K791-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (545-565 nm)

Detection range：9.28-170.00 U/L

Elabscience® Glycerol Kinase (GK) Acitivity 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.

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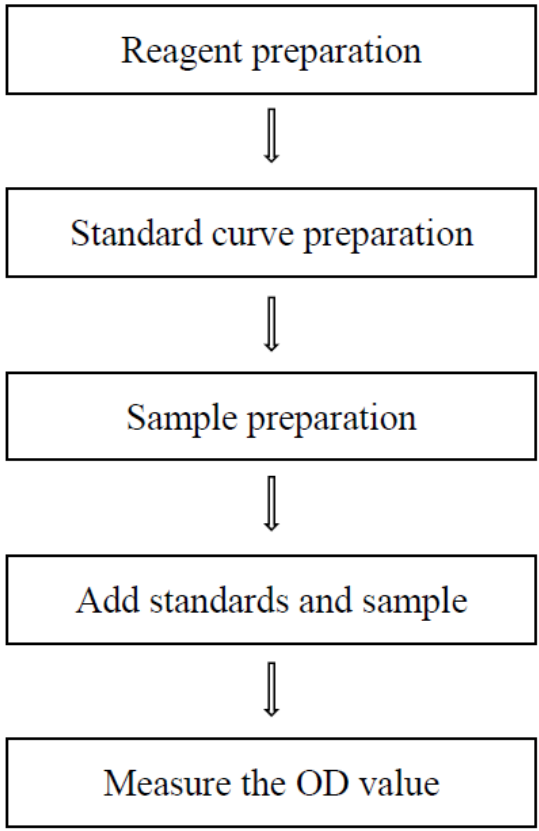
Assay summary



Intended use

This kit can be used to detect the glycerol kinase (GK) acitivity in animal tissue samples.

Detection principle

Glycerol kinase (GK) is a rate-limiting enzyme in glycerol metabolism, and the lack of this enzyme directly results in the inability of cells to utilize glycerol.
This kit produces hydrogen peroxide under the action of glycerophosphate oxidase. The reaction between hydrogen peroxide and the chromogenic agent has the maximum absorption peak at 555 nm. The enzyme activity of GK can be determined by measuring the increase rate at 555 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	6 mL ×1 vial	12 mL ×1 vial	-20 ℃, 12months, shading light
Reagent 2	Stop Solution	6 mL ×1 vial	12 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 3	Standard	Powder ×1 vial	Powder ×1 vial	-20 ℃, 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 (545-656 nm, optimum wavelength: 555 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use. The working solution can be aliquoted storage at -20 ℃ for 6 months protected from light.

② The preparation of 4.25 mmol/L standard solution：

Dissolve one vial of standard with 5 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 1 month.

③ The preparation of standard curve：

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

Dilute 4.25 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.85, 1.28, 1.70, 2.13, 2.98, 3.40, 4.25 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.85**	**1.28**	**1.70**	**2.13**	**2.98**	**3.40**	**4.25**
4.25 mmol/L Standard (μL)	0	40	60	80	100	140	160	200
Double distilled water (μL)	200	160	140	120	100	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 min 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).

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 homogenization	1
10% Mouse kidney tissue homogenization	1
10% Mouse small intestine tissue homogenization	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

When using the pipetting gun to take 4.25 mmol/L standard solution, carefully suction to avoid bubbles.

Operating steps

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

Sample well: Add 10 μL of sample to the corresponding wells.

② Add 100 μL of working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of sample well at 555 nm, as A_1.

④ Incubate at 37 ℃ for 25 min protected from light. Add 100 μL of stop solution to each well. Measure the OD value of each well at 555 nm, 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 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 sample:

Definition: The amount of enzyme in 1 g tissue protein per 1 min that produce 1 μmol glycerin 3-phosphate at 37 ℃ is defined as 1 unit.

$$\underset{\text{(U/gprot)}}{\text{GK activity}} = (\Delta A - b) \div a \div C_{pr} \times f \div T \times 1000^{*}$$

[Note]

ΔA: $\Delta A = A_2 - A_1$.

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

f: Dilution factor of sample before test.

T: Reaction time, 25 min.

1000*: Unit conversion, 1 mmol/L= 1000 μmol/L.

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Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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)	25	45	120
%CV	3.0	3.2	3.6

Inter-assay Precision

Three mouse liver 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)	25	45	120
%CV	6.5	7.8	8.0

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	25	45	120
Observed Conc. (U/L)	24	45	117.6
Recovery rate (%)	96	100	98

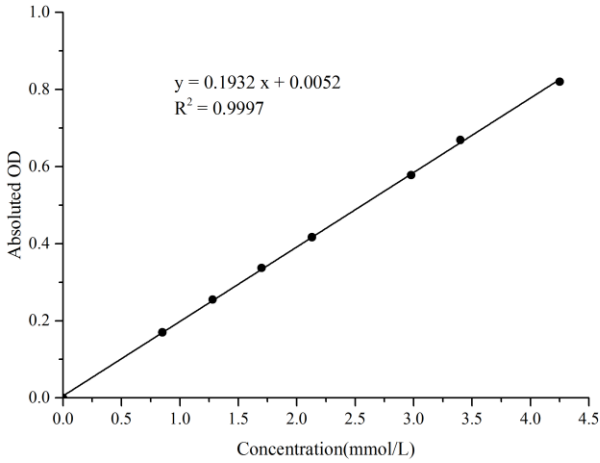
Sensitivity

The analytical sensitivity of the assay is 9.28 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**	**0.85**	**1.28**	**1.70**	**2.13**	**2.98**	**3.40**	**4.25**
OD value	0.046	0.215	0.300	0.383	0.463	0.621	0.712	0.865
	0.046	0.216	0.302	0.383	0.462	0.626	0.718	0.866
Average OD	0.046	0.216	0.301	0.383	0.463	0.624	0.715	0.866
Absoluted OD	0	0.170	0.255	0.337	0.417	0.578	0.669	0.820



Appendix II Example Analysis

Example analysis:

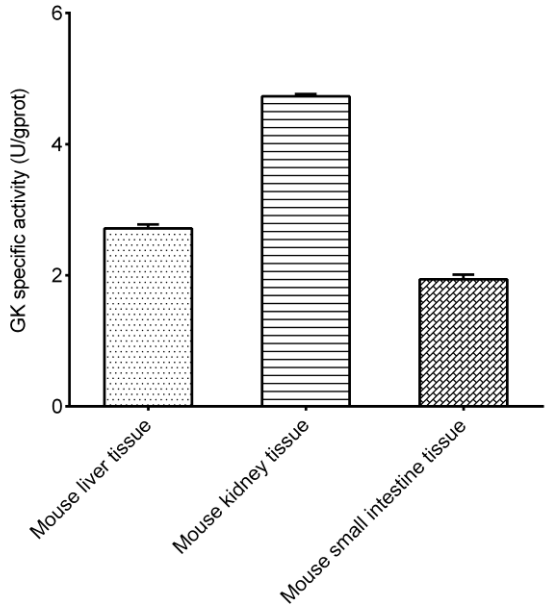
Take 10 μL of 10% mouse liver tissue homogenization and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.1932 x + 0.0052. The A_1 of sample is 0.060, the A_2 of sample is 0.240, the concentration of protein in sample is 13.20 gprot/L, and the calculation result is:

$$\text{GK activity (U/gprot)} = (0.240 - 0.060 - 0.0052) \div 0.1932 \div 13.20 \div 25 \times 1000$$

$$= 2.74 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenization (the concentration of protein is 13.20 gprot/L), 10% mouse kidney tissue homogenization (the concentration of protein is 10.55 gprot/L) and 10% mouse small intestine tissue homogenization (the concentration of protein is 7.75 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.