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

Catalog No：E-BC-K790-M

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

Measuring instrument：Microplate reader（430-470 nm）

Detection range：0.49-50.00 U/L

Elabscience® Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) 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.

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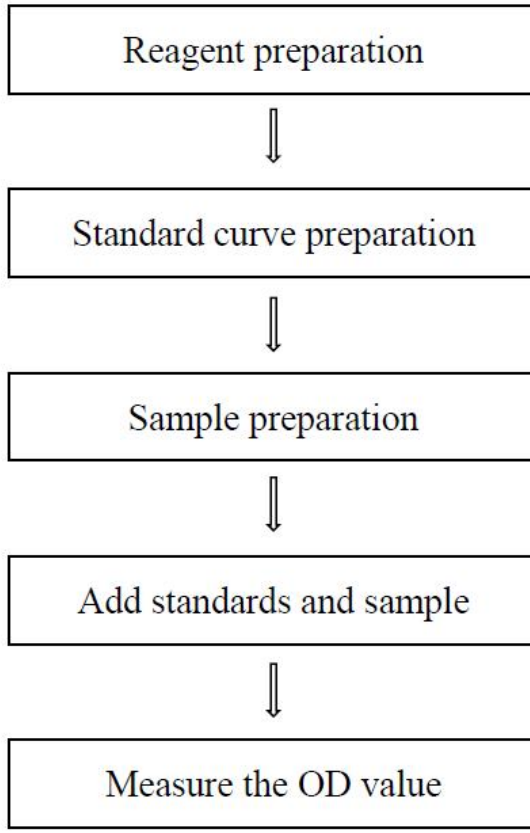
Assay summary



Intended use

This kit can be used to detect the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in tissues and cell samples.

Detection principle

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), a key enzyme in the glycolytic pathway, catalyzes the oxidation of glyceraldehyde-3-phosphate to 1, 3-diphosphoglycerate. This response is closely related to the gluconeogenic pathway, the maintenance of blood glucose concentrations in the body, and the development of diabetes. GAPDH gene is a housekeeping gene, which is expressed at a high level in almost all tissues. Therefore, GAPDH widely exists in tissues and cells and plays an important role in the disorders of glucose, lipid and protein metabolism in the body.

This kit can calculate the enzyme activity of GAPDH by measuring the OD value change at 450 nm per unit time of the product produced by GAPDH catalyzing the oxidation of glyceraldehyde-3-phosphate.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate	Liquid × 2 vials	Liquid × 4 vials	-20°C, 12 months, shading light
Reagent 3	Chromogenic Solution	1.5 mL × 1 vial	3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Standard	Powder × 2 vials	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	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 (430-470 nm, optimum wavelength: 450 nm), Incubator (37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of working solution:
Dissolve one vial of substrate with 2 mL of buffer solution, mix well to dissolve. The solution transfer to the 5 mL EP, then add 1 mL buffer solution, mix well. The working solution should be prepared on spot.

③ The preparation of 1 mmol/L standard solution:
Dissolve one vial of standard with 2 mL of double distilled water, mix well to dissolve. The solution transfer to the 10 mL EP, then add 3 mL double distilled water, mix well. Store at -20°C for 2 weeks protected from light.

④ The preparation of 500 μmol/L standard solution:
Before testing, please prepare sufficient 500 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 500 μmol/L standard solution (mix well 500 μL of 1 mmol/L standard solution and 500 μL of double distilled water). Store at -20°C for 3 days protected from light.

⑤ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 500 μmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 μmol/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:

Tissues 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 (animal:E-BC-K318-M; plant:E-BC-K168-M).

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 normal saline (0.9% NaCl) with a ultrasonic cell disruptor 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 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 lung tissue homogenization	1
10% Spinach tissue homogenization	1
10% Squash tissue homogenization	1
10% Cabbage tissue homogenization	1
1×10^6 HL-60 cells	1
1×10^6 CHO cells	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.

Operating steps

① Standard wells: Add 40 μL of standard to the corresponding wells.
Sample wells: Add 40 μL of sample to the corresponding wells.

② Add 100 μL of working solution to each well. Add 20 μL of chromogenic solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value (A_1) of each wells at 450 nm.

④ Incubate at 37°C for 10 min protected from light and measure the OD value (A_2) of each well at 450 nm.(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 (A_2) 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 cell samples:

Definition: The amount of enzyme in 1 g tissue or cell protein that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\text{GAPDH activity (U/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f \div T$$

[Note]

ΔA: $\Delta A = A_2 - A_1$.

f: Dilution factor of sample before test.

T: Reaction time, 10 min.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse kidney 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)	5.00	15.00	20.00
%CV	3.2	4.3	5.0

Inter-assay Precision

Three mouse kidney 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)	5.00	15.00	20.00
%CV	3.2	6.7	8.6

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	150	280	370
Observed Conc. (μmol/L)	142.5	277.2	373.7
Recovery rate (%)	95	99	101

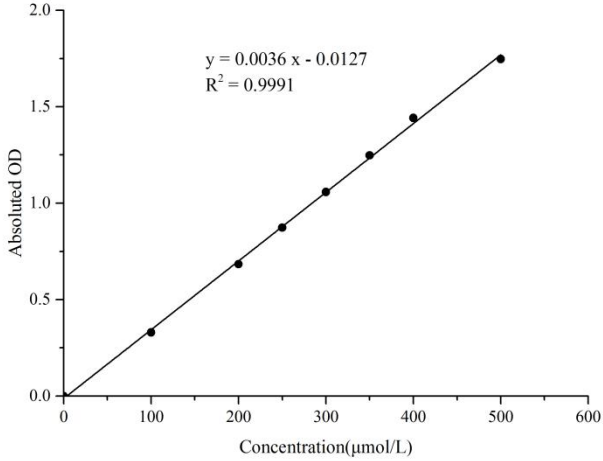
Sensitivity

The analytical sensitivity of the assay is 0.49 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 (μmol/L)	0	100	200	250	300	350	400	500
OD value	0.05	0.378	0737	0.922	1.1	1.298	1.498	1.811
	0.05	0.382	0.731	0.924	1.117	1.298	1.486	1.784
Average OD	0.05	0.38	0.734	0.923	1.109	1.298	1.492	1.798
Absoluted OD	0	0.33	0.684	0.873	1.059	1.248	1.442	1.748



Appendix II Example Analysis

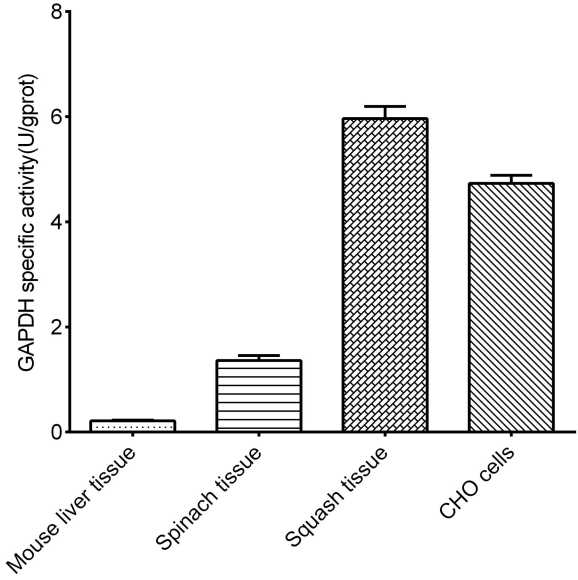
Example analysis：

Take 40 μL of 10% mouse liver tissue homogenization and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0036 x - 0.0127, the average A_1 of the sample is 0.138, the average A_2 of the sample is 0.236, the concentration of protein in sample is 14.06 gprot/L and the calculation result is:

GAPDH activity (U/gprot) = (0.236 – 0.138 + 0.0127) ÷ 0.0036 ÷ 14.06 ÷ 10 = 0.22 U/gprot

Detect 10% mouse liver tissue homogenization (the concentration of protein in sample is 14.06 gprot/L), 10% spinach tissue homogenization (the concentration of protein in sample is 2.00 gprot/L), 10% squash tissue homogenization (the concentration of protein in sample is 0.08 gprot/L) and 1×10^6 CHO cells (the concentration of protein in sample is 0.59 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.