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

Catalog No：E-BC-F081

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：019-100 μmol/L

Elabscience® Glycerol-3-phosphate (G3P) Fluorometric 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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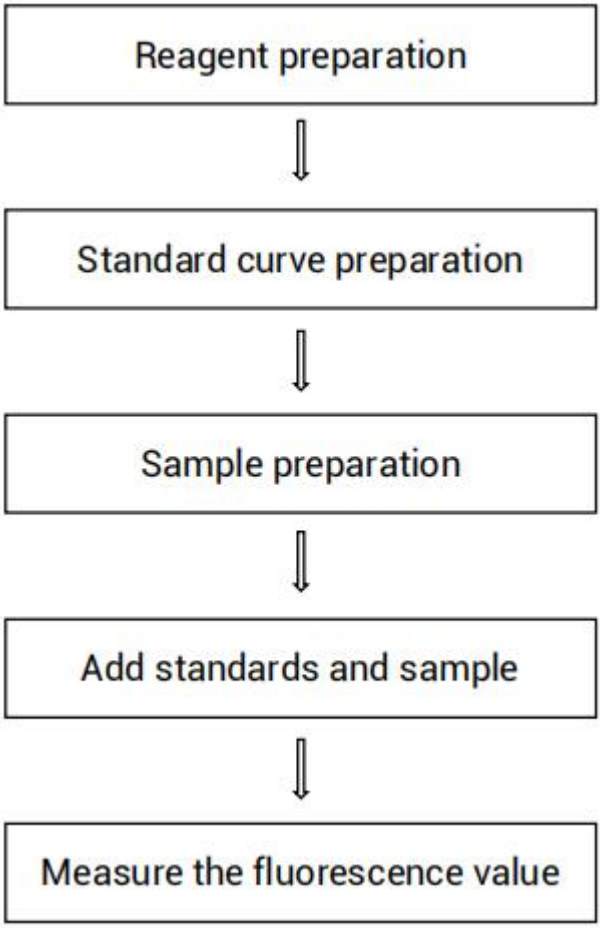
Assay summary



Intended use

This kit can be used to measure glycerol-3-phosphate (G3P) content in tissue and cell samples.

Detection principle

Glycerol 3-phosphate (G3P), also known as 3-glycerophosphoic acid and glycerol α-phosphate, is mainly derived from glycolysis. G3P is an important intermediate in the glycerol-3-phosphate shuttle mechanism and studies have shown that G3P is involved in electron transport, and its metabolic level is related to lipid metabolism, insulin resistance and some cancers. The principle of this kit is that G3P is decomposed by enzymes, and its products generate fluorescent substances with probes under the action of other enzymes. The higher the fluorescence value, the higher the G3P content in the sample.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	40 mL × 1 vial	-20°C, 12 months
Reagent 2	Enzyme Reagent	Liquid × 1 vial	Liquid × 1 vial	-20°C, 12 months shading light
Reagent 3	Probe	0.16 mL × 1 vial	0.32 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	100 μmol/L Standard	1 mL × 1 vial	2 mL × 1 vial	-20°C, 12 months
	Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm),

Incubator(37℃)

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Consumptive material:

3 kD ultrafiltration tube

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of chromogenic solution：
Before testing, please prepare sufficient chromogenic solution according to the test wells. For example, prepare 1023 μL of chromogenic solution (mix well 1000 μL of buffer solution, 8 μL of enzyme reagent and 15 μL of probe). The chromogenic solution should be prepared on spot and used up within 10 min (It is recommended to prepare the chromogenic solution after the sample is added).

③ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 100 μmol/L standard with double distilled water to a serial

concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
100 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

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°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material.

⑤ Collect a part of supernatant and determine the protein concentration (E-BC-K318-M).

⑥ Take another part of supernatant and add it to 3 kD ultrafiltration tube. Centrifuge at 10000×g for 15 min.

⑦ Collect the permeate after ultrafiltration and preserve it on ice for detection.

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 normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material.

⑤ Collect a part of supernatant and determine the protein concentration (E-BC-K318-M).

⑥ Take another part of supernatant and add it to 3 kD ultrafiltration tube. Centrifuge at 10000×g for 15 min.

⑦ Collect the permeate after ultrafiltration and preserve it on ice for detection.

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
1 × 10^6 293T cell	1
1 × 10^6 HL-60 cell	1
1 × 10^6 Hela cell	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

① The chromogenic solution should be prepared on the spot and used within 10 minutes, otherwise the background fluorescence value is too high, which will affect the experimental results.

② The sample concentration can be appropriately increased when the sample fluorescence value is too low, and the sample can be appropriately diluted when the sample fluorescence value is too high.

③ Cell and tissue samples need to be ultrafiltration.

④ Before incubation, the microplate can be shaken slightly to mix the reaction reagent fully.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the well.
Sample well: add 20 μL of sample into the well.
② Add 180 μL of chromogenic solution into each well.
③ Incubate at 37°C for 10 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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:

$$\text{G3P content (μmol/gprot)} = (\Delta F - b) \div a \div C_{pr} \times f$$

[Note]

ΔF: The absolute fluorescence value of sample, $F_{sample} - F_{blank}$ (F_{blank} is the fluorescence value when the standard concentration is 0).

f: Dilution factor of sample before tested.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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 (μmol/L)	30.00	55.00	75.00
%CV	0.7	1.6	2.8

Inter -assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	30.00	55.00	75.00
%CV	4.6	5.6	10.0

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	30	55	75
Observed Conc.(μmol/L)	28.5	56.1	78
Recovery rate (%)	95	102	104

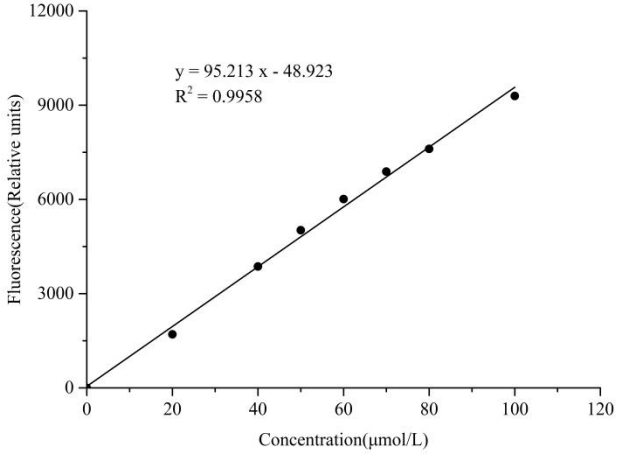
Sensitivity

The analytical sensitivity of the assay is 0.19 μmol/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 fluorescence 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	20	40	50	60	70	80	100
Fluorescence value	204	1938	4118	5394	6276	7253	7247	9951
	225	1900	4039	5075	6173	6947	8397	9059
Average fluorescence value	215	1919	4079	5235	6225	7100	7822	9505
Absoluted fluorescence value	0	1704	3864	5020	6010	6885	7607	9290



Appendix Π Example Analysis

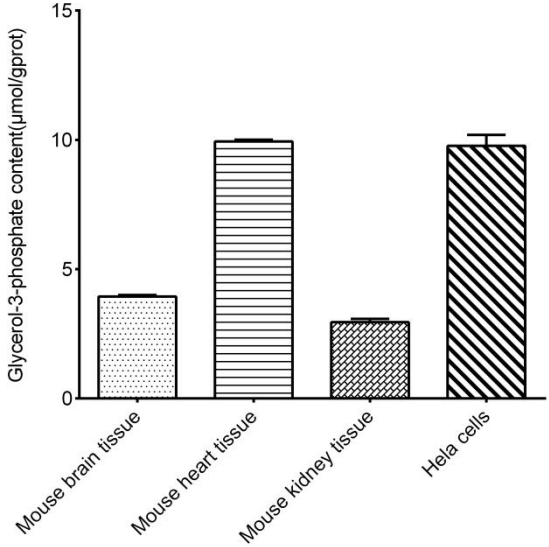
Example analysis：

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

standard curve: y = 79.083x - 119.24, the average fluorescence value of the sample is 3955, the average fluorescence value of the blank is 252, ΔF = 3955 - 252 = 3703, the concentration of protein in sample is 12.27 gprot/L, and the calculation result is:

G3P content (μmol/gprot) = (3955 - 252 + 119.24) ÷ 79.083 ÷ 12.27 = 3.94 μmol/gprot

Detect 10% mouse brain tissue homogenate (the concentration of protein is 12.2 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 8.64 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 5.85 gprot/L) and 1×10^6 Hela cells (the concentration of protein is 0.40 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.