

(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  $\mu$ mol/L**

## **Elabsience<sup>®</sup> 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@elabsience.com](mailto:techsupport@elabsience.com)

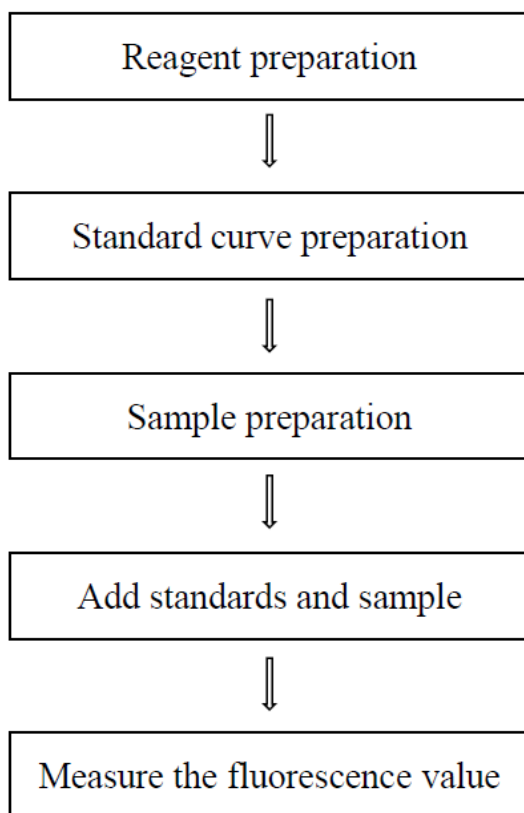
Website: [www.elabsience.com](http://www.elabsience.com)

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  $\alpha$ -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 $\times$ 1 vial   | 40 mL $\times$ 1 vial   | -20 $^{\circ}$ C, 12 months                  |
| Reagent 2 | Enzyme Reagent           | Liquid $\times$ 1 vial  | Liquid $\times$ 1 vial  | -20 $^{\circ}$ C, 12 months<br>shading light |
| Reagent 3 | Probe                    | 0.16 mL $\times$ 1 vial | 0.32 mL $\times$ 1 vial | -20 $^{\circ}$ C, 12 months<br>shading light |
| Reagent 4 | 100 $\mu$ mol/L Standard | 1 mL $\times$ 1 vial    | 2 mL $\times$ 1 vial    | -20 $^{\circ}$ C, 12 months                  |
|           | Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator(37°C)

### 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  $\mu\text{L}$  of chromogenic solution (mix well 1000  $\mu\text{L}$  of buffer solution, 8  $\mu\text{L}$  of enzyme reagent and 15  $\mu\text{L}$  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  $\mu\text{mol/L}$  standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 70, 80, 100  $\mu\text{mol/L}$ . Reference is as follows:

| Item  | ①        | ②         | ③         | ④         | ⑤         | ⑥         | ⑦         | ⑧          |
|---|----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| <b>Concentration (<math>\mu\text{mol/L}</math>)</b>                         | <b>0</b> | <b>20</b> | <b>40</b> | <b>50</b> | <b>60</b> | <b>70</b> | <b>80</b> | <b>100</b> |
| <b>100 <math>\mu\text{mol/L}</math> Standard (<math>\mu\text{L}</math>)</b> | 0        | 40        | 80        | 100       | 120       | 140       | 160       | 200        |
| <b>Double distilled water (<math>\mu\text{L}</math>)</b>                    | 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  $\mu$ L normal saline (0.9% NaCl) with a dounce homogenizer at 4  $^{\circ}$ C.
- ④ Centrifuge at 10000 $\times$ 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 $\times$ 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  $1 \times 10^6$  cells).
- ② Wash cells with normal saline (0.9% NaCl).
- ③ Homogenize  $1 \times 10^6$  cells in 200  $\mu$ L normal saline (0.9% NaCl) with a dounce homogenizer at 4  $^{\circ}$ C.
- ④ Centrifuge at 10000 $\times$ 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 $\times$ 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               |
| $1 \times 10^6$ 293T cell          | 1               |
| $1 \times 10^6$ HL-60 cell         | 1               |
| $1 \times 10^6$ 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  $\mu\text{L}$  of standard with different concentrations into the well.  
Sample well: add 20  $\mu\text{L}$  of sample into the well.
- ② Add 180  $\mu\text{L}$  of chromogenic solution into each well.
- ③ Incubate at 37  $^{\circ}\text{C}$  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 absolved fluorescence value.
3. Plot the standard curve by using absolved 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:

$$\text{G3P content} \left( \frac{\mu\text{mol}}{\text{gprot}} \right) = (\Delta F - b) \div a \div C_{\text{pr}} \times f$$

#### [Note]

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

$f$ : Dilution factor of sample before tested.

$C_{\text{pr}}$ : 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 ( $\mu\text{mol/L}$ ) | 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 ( $\mu\text{mol/L}$ ) | 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.( $\mu\text{mol/L}$ ) | 30         | 55         | 75         |
| Observed Conc.( $\mu\text{mol/L}$ ) | 28.5       | 56.1       | 78         |
| Recovery rate (%)                   | 95         | 102        | 104        |

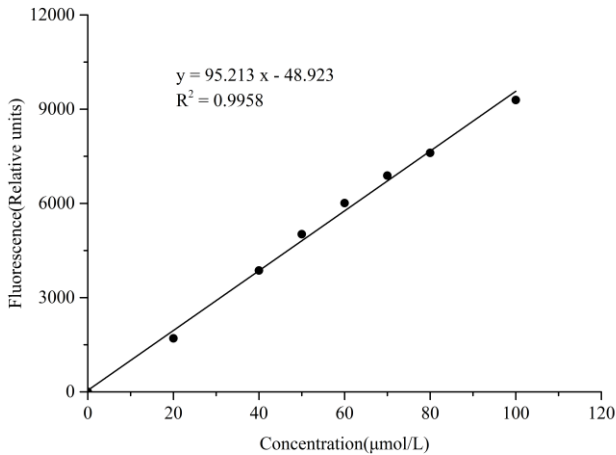
#### Sensitivity

The analytical sensitivity of the assay is 0.19  $\mu\text{mol/L}$ . 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<br>(μmol/L)          | 0   | 20   | 40   | 50   | 60   | 70   | 80   | 100  |
|------------------------------------|-----|------|------|------|------|------|------|------|
| Fluorescence<br>value              | 204 | 1938 | 4118 | 5394 | 6276 | 7253 | 7247 | 9951 |
|                                    | 225 | 1900 | 4039 | 5075 | 6173 | 6947 | 8397 | 9059 |
| Average<br>fluorescence<br>value   | 215 | 1919 | 4079 | 5235 | 6225 | 7100 | 7822 | 9505 |
| Absoluted<br>fluorescence<br>value | 0   | 1704 | 3864 | 5020 | 6010 | 6885 | 7607 | 9290 |



## Appendix II Example Analysis

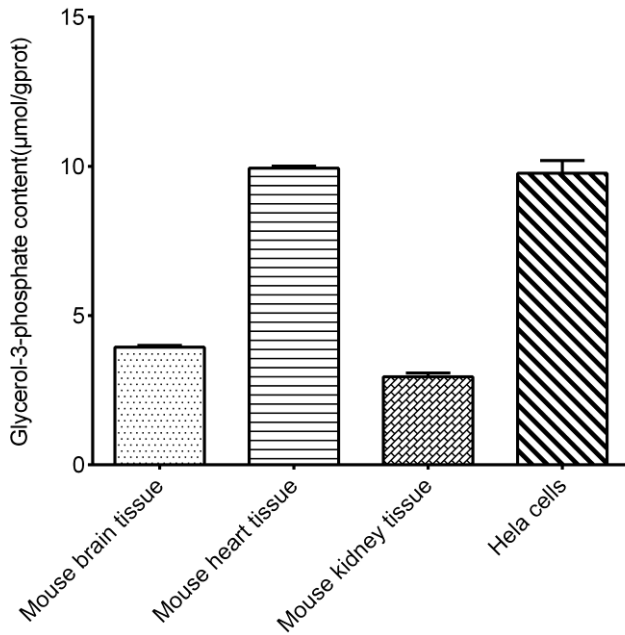
### Example analysis:

Take 20  $\mu\text{L}$  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,  $\Delta F = 3955 - 252 = 3703$ , the concentration of protein in sample is 12.27 gprot/L, and the calculation result is:

$$\text{G3P content } (\mu\text{mol/gprot}) = (3955 - 252 + 119.24) \div 79.083 \div 12.27 = 3.94 \mu\text{mol/gprot}$$

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  $1 \times 10^6$  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.





