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

**Catalog No：E-BC-K778-M**

**Specification：48T(46 samples)/96T(94 samples)**

**Measuring instrument：Microplate reader(340 nm)**

**Detection range：3.22-391.32 U/L**

# **Elabscience**® Phosphoglycerate Kinase (PGK) 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
Tel: 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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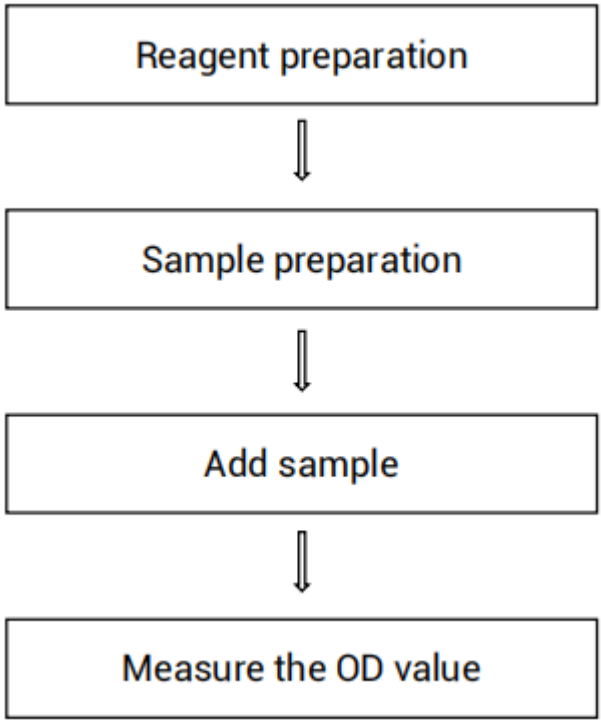
## Assay summary



## Intended use

This kit can be used to measure phosphoglycerate kinase (PGK) activity in serum, plasma, animal tissue and cell samples.

## Detection principle

Phosphoglycerate kinase (PGK) is an enzyme that plays a key role in glycolysis and operates in the second stage of the glycolytic pathway. Catalyze the reversible transfer of phosphate groups from 1, 3-diphosphoglycerate (1, 3-DPG) to ADP, thereby generating 3-phosphoglycerate (3-PGA) and ATP. PGK is an indispensable key enzyme in living organisms, and its protein kinase activity plays an important role in maintaining cellular homeostasis.
The determination principle of this kit: PGK catalyzes the substrate reaction and consumes the reducing agent. The reducing agent has the maximum absorbance at 340 nm. The enzyme activity of PGK is calculated by calculating the amount of reducing agent consumed per unit time of the reaction.

## Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Buffer Solution | 25 mL × 1 vial | 50 mL × 1 vial | -20°C, 12 months shading light |
| Reagent 2 | Cofactors | 0.5 mL × 1 vial | 1 mL × 1 vial | -20°C, 12 months shading light |
| Reagent 3 | Substrate | 0.3 mL × 1 vial | 0.6 mL × 1 vial | -20°C, 12 months shading light |
| Reagent 4 | Enzyme Reagent | 0.6 mL × 1 vial | 1.2 mL × 1 vial | -20°C, 12 months shading light |
| Reagent 5 | Reducing Reagent | Powder × 1 vial | Powder × 2 vials | -20°C, 12 months shading light |
| | UV-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:**

Microplate reader (340 nm), Incubator

**Reagents:**

Normal saline (0.9%NaCl)

## Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of reducing working solution：
Dissolve one vial of reducing reagent with 1.5 mL of double distilled water, mix well to dissolve. Keep it on ice during use and store at -20°C for 7 days protected from light.

③ The preparation of determination working solution：
Before testing, please prepare sufficient determination working solution according to the test wells. For example, prepare 200 μL of determination working solution (mix well 169 μL of buffer solution, 8 μL of cofactors, 5 μL of substrate, 10 μL of enzyme reagent and 8 μL of reducing working solution). The determination working solution should be prepared on spot protected from light and used up within 8 h.

## Sample preparation

**① Sample preparation**

**Serum or plasma samples:** detect directly.

**Tissue samples:**

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL normal saline (0.9%NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of animal supernatant (E-BC-K318-M).

**Cell samples:**

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

② Homogenize 1×10^6 cells in 200 μL normal saline (0.9%NaCl) with a ultrasonic cell disruptor at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ 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 |
|---|---|
| Rat plasma | 1 |
| Mouse serum | 1 |
| Human serum | 1 |
| 10% Mouse liver tissue homogenate | 20-40 |
| 10% Mouse kidney tissue homogenate | 20-40 |
| 10% Mouse muscle tissue homogenate | 20-40 |
| 1×10^6 HL-60 cells | 1-3 |
| 1×10^6 293T cells | 1-5 |
| 1×10^6 Jurkat cells | 1-3 |

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

If the measured absorbance $A_2$ is lower than 0.25, the sample needs to be diluted.

## Operating steps

① Blank well: add 20 μL of normal saline (0.9%NaCl) into blank wells. Sample well: add 20 μL of sample into sample wells.

② Add 180 μL of determination working solution into each well.

③ Mix well for 5 s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, as $A_1$. Incubate at 37°C for 5 min and measure the OD value of each well at 340 nm with microplate reader, as $A_2$.

## Calculation

**The sample**:

**1. Serum or plasma samples:**

**Definition:** The amount of 1 L serum or plasma per 1 min that produce 1 μmol of production at 37 °C is defined as 1 unit.

$$\text{PGK activity (U/L)} = \frac{\Delta A_{340} \times V_1 \times f}{\varepsilon \times d \times V_2 \times T}$$

**2. Tissue or cell samples:**

**Definition:** The amount of 1 g tissue or cell per 1 min that produce 1 μmol of production at 37 °C is defined as 1 unit.

$$\text{PGK activity (U/gprot)} = \frac{\Delta A_{340} \times V_1 \times f}{\varepsilon \times d \times V_2 \times T \times C_{pr}}$$

**[Note]:**

$\Delta A_{blank}$: $\Delta A_{blank} = A_1 - A_2$.

$\Delta A_{sample}$: $\Delta A_{sample} = A_1 - A_2$.

$\Delta A_{340}$: $\Delta A_{340} = \Delta A_{sample} - \Delta A_{blank}$

ε: The molar extinction coefficient at 340 nm, 6.22×10^-3 L/μmol/cm.

d: Optical path, 0.60 cm.

$V_1$: The volume of reaction system, 0.2 mL.

$V_2$: The volume of sample added to the reaction system, 0.02 mL.

T: Reaction time, 5 min.

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

f: Dilution factor of sample before test.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three mouse serum 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)** | 50.00 | 100.00 | 200.00 |
| **%CV** | 1.6 | 2.0 | 2.2 |

### Inter-assay Precision

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (U/L)** | 50.00 | 100.00 | 200.00 |
| **%CV** | 2.5 | 5.3 | 6.5 |

### 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%.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Expected Conc. (U/L)** | 50.00 | 100.00 | 200.00 |
| **Observed Conc. (U/L )** | 49.5 | 96.0 | 210.0 |
| **Recovery rate (%)** | 99.0 | 96.0 | 105.0 |

### Sensitivity

The analytical sensitivity of the assay is 3.22 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.

## Appendix Π Example Analysis

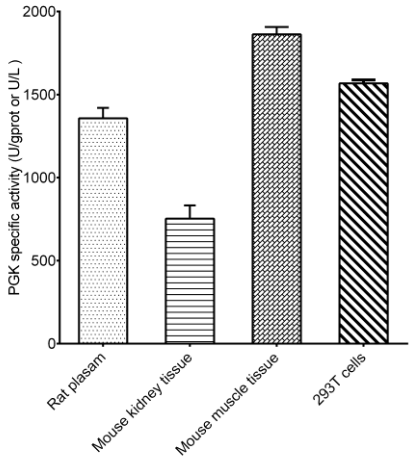
**Example analysis：**

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

The $A_1$ of the sample well is 1.098, the $A_2$ of the sample well is 0.550, $\Delta A_{sample}$ = 1.098 – 0.550 = 0.548. The $A_1$ of the blank well is 1.214, the $A_2$ of the sample well is 1.195, $\Delta A_{blank}$ = 1.214 – 1.195 = 0.019, $\Delta A_{340}$ = 0.548 – 0.019 = 0.529, the concentration of protein is 3.70 gprot/L, and the calculation result is:

$$\text{PGK activity (U/gprot)} = \frac{0.529 \times 0.2 \times 20}{(6.22 \times 10\text{^-3}) \times 0.6 \times 0.02 \times 5 \times 3.70} = 1837.53 \text{ U/gprot}$$

Detect rat plasma, 10% mouse kidney tissue homogenate (the concentration of protein is 8.32 gprot/L, dilute for 20 times), 10% mouse muscle tissue homogenate (the concentration of protein is 3.70 gprot/L, dilute for 20 times) and 1×10^6 293T cells (the concentration of protein is 1.31 gprot/L, dilute for 4 times), 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.