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

**Catalog No：E-BC-K782-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader(400-410 nm)**

**Detection range：0.16-16.64 U/L**

# **Elabscience®** Total Phosphodiesterase (PDEs) 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
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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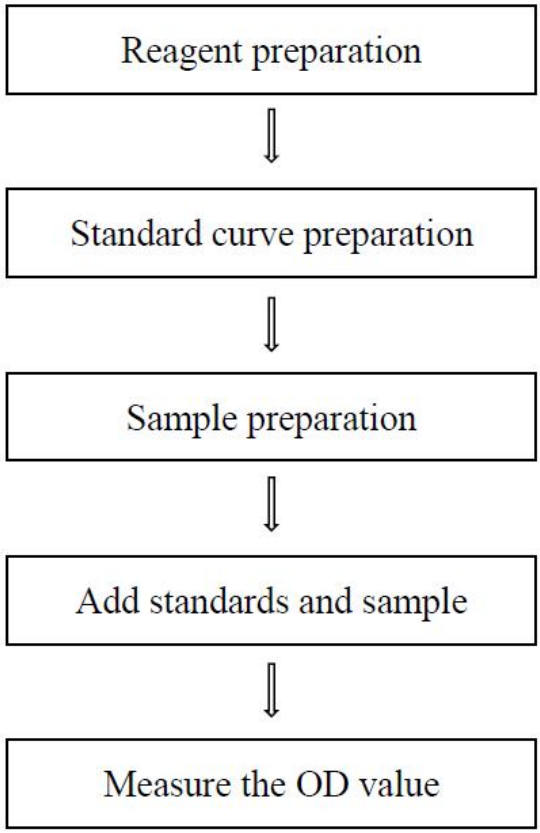
**Assay summary**



## Intended use

This kit can be used to measure total phosphodiesterase (PDEs) activity in serum, plasma, animal tissue and cell samples.

## Detection principle

Phosphodiesterase (PDEs) hydrolyzes the intracellular second messenger (cyclic adenosine phosphate or cyclic guanosine phosphate), thereby ending the biochemical effects of these second messengers.

The principle of this kit is that by hydrolyzing the substrate with total PDEs, the resulting substance has a maximum absorption peak at 405 nm. The OD value at 405 nm and the standard curve were measured to calculate the total PDEs activity in the sample.

## Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | Buffer Solution | 25 mL × 1 vial | -20°C, 12 months |
| Reagent 2 | Substrate | 0.2 mL × 1 vial | -20°C, 12 months, shading light |
| Reagent 3 | Saline Solution | 0.25 mL × 1 vial | -20°C, 12 months, shading light |
| Reagent 4 | 100 mmol/L Standard Solution | 0.2 mL × 1 vial | -20°C, 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 (400-410 nm, optimum wavelength: 405 nm), Incubator

**Reagents:**

Normal saline (0.9% NaCl)

## Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution. For example, prepare 500 μL of substrate working solution (mix well 5 μL of substrate and 495 μL of buffer solution). The substrate working solution should be prepared on spot and used up with the same day.

③ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 500 μL of reaction working solution (mix well 5 μL of saline solution and 495 μL of substrate working solution). The reaction working solution should be prepared on spot and used up within 4 h.

④ The preparation of control working solution:
Before testing, please prepare sufficient control working solution according to the test wells. For example, prepare 500 μL of control working solution (mix well 5 μL of saline solution and 495 μL of buffer solution). The control working solution should be prepared on spot and used up within 4 h.

⑤ The preparation of 1 mmol/L standard solution:
Before testing, please prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 10 μL of 10 mmol/L standard solution and 990 μL of buffer solution). The 1 mmol/L standard solution should be prepared on spot and used up with the same day.

⑥ The preparation of standard curve:

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

Dilute 1 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mmol/L)** | **0** | **0.2** | **0.3** | **0.4** | **0.5** | **0.6** | **0.7** | **1.0** |
| **1 mmol/L Standard (μL)** | 0 | 40 | 60 | 80 | 100 | 120 | 140 | 200 |
| **Buffer solution (μL)** | 200 | 160 | 140 | 120 | 100 | 80 | 60 | 0 |

## Sample preparation

### ① Sample preparation

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

**Animal tissue sample:**

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

② Wash tissue in normal saline (0.9% NaCl).

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 at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. It is recommended to test within 4 hours.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-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 normal saline (0.9% NaCl).

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 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. It is recommended to test within 4 hours.

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 |
|---|---|
| Human plasma | 1 |
| Rabbit serum | 1 |
| 10% Mouse liver tissue homogenate | 1 |
| 10% Mouse kidney tissue homogenate | 1-2 |
| 10% Mouse small intestine tissue homogenate | 1 |
| 10% Mouse heart tissue homogenate | 2-3 |
| 1×10^6 HL-60 cells | 1 |
| 1×10^6 293T cells | 1 |
| 1×10^6 Hela cells | 1 |
| 1×10^6 Molt-4 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.

## The key points of the assay

Equilibrate all reagents to 25°C before use.

## Operating steps

① Standard well: Add 20 μL of different concentrations solution to standard well.

Sample well: Add 20 μL of sample to sample well.

Control well: Add 20 μL of sample to control well.

② Add 180 μL of reaction working solution to standard and sample wells. Add 180 μL of control working solution to control wells

③ Mix fully with microplate reader for 5 s. Incubated at 37°C for 60 min protected from light, measure the OD value of each well at 405 nm with microplate reader.

## 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**:

**1. Serum (plasma) samples:**

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

$$\text{total PDEs activity (U/L)} = (\Delta A - b) \div a \times f \div T \times 1000^*$$

**2. Tissue and cell samples:**

**Definition:** The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol p-nitrophenol at 37 °C is defined as 1 unit.

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

**[Note]**

ΔA: $\Delta A = OD_{sample} - OD_{control}$.

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

T: Reaction time, 60 min.

1000*: 1 mmol/L=1000 μmol/L

f: Dilution factor of sample before test.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three human plasma 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)** | 2.50 | 3.40 | 10.00 |
| **%CV** | 1.2 | 3.0 | 2.9 |

### Inter-assay Precision

Three human plasma 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)** | 2.50 | 3.40 | 10.00 |
| **%CV** | 4.1 | 5.6 | 4.6 |

### 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 99.3%.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Expected Conc. (U/L)** | 2.50 | 3.40 | 10.00 |
| **Observed Conc. (U/L)** | 2.475 | 3.400 | 9.900 |
| **Recovery rate (%)** | 99 | 100 | 99 |

### Sensitivity

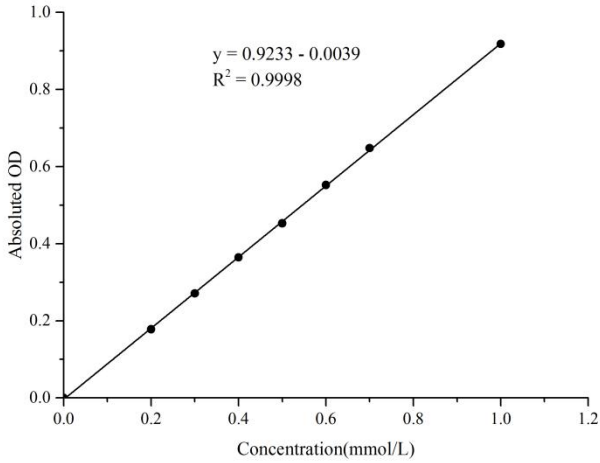
The analytical sensitivity of the assay is 0.16 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## 2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 1.0 |
|---|---|---|---|---|---|---|---|---|
| OD value | 0.055 | 0.235 | 0.328 | 0.421 | 0.507 | 0.612 | 0.704 | 0.979 |
| | 0.055 | 0.231 | 0.324 | 0.418 | 0.509 | 0.602 | 0.702 | 0.967 |
| Average OD value | 0.055 | 0.233 | 0.326 | 0.420 | 0.508 | 0.607 | 0.703 | 0.973 |
| Absoluted OD value | 0 | 0.178 | 0.271 | 0.365 | 0.453 | 0.552 | 0.648 | 0.918 |



# Appendix II Example Analysis

**Example analysis：**

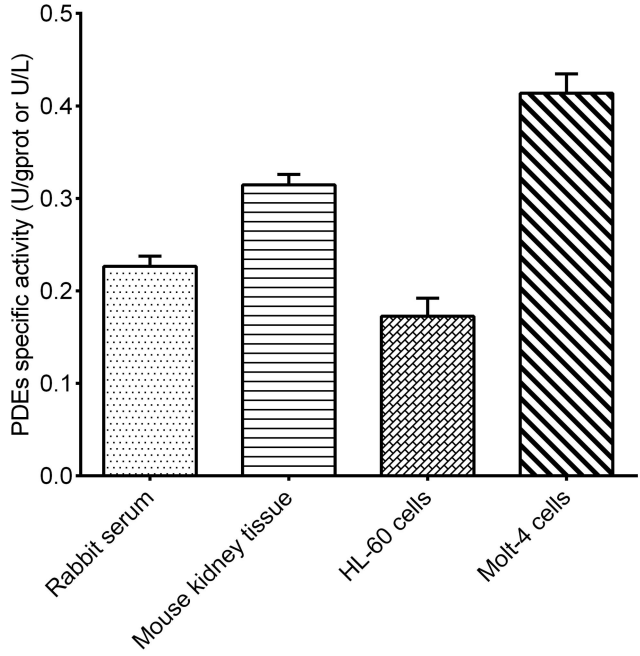
Take 20 μL of 1×10^6 HL-60 cells homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.9233 x - 0.0039. the average OD value of the sample well is 0.094, the average OD value of the control well is 0.074, the concentration of protein is 0.97 gprot/L, and the calculation result is:

$$\text{total PDEs activity (U/gprot)} = (0.094 - 0.074 + 0.0039) \div 0.9233 \div 0.97 \div 60 \times 1000$$

$$= 0.44 \text{ U/gprot}$$

Detect 10% fish muscle tissue homogenate, 10% mouse heart tissue homogenate, 10% mouse brain tissue homogenate and 10% mouse kidney tissue homogenate 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.