TECHNICAL MANUAL

Total Phosphodiesterase (PDEs) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0444
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 60 min
- Measure OD value at 405 nm

2. Intended use

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

3. Detection principle

Phosphodiesterase (PDEs) hydrolyzes the intracellular second messengers (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 are measured to calculate the total PDEs activity in the sample.

4. 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, protect from light
Reagent 3	Saline Solution	0.25 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 4	100 mmol/L Standard Solution	0.2 mL × 1 vial	-20°C, 12 months, protect from light
	Microplate	96 wells	No requirement

Item	Component	Size (96 T)	Storage
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (400-410 nm, optimum wavelength: 405 nm), Incubator

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of substrate working solution:** Before testing, 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 fresh and used the same day.
3. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the number of 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 fresh and used within 4 hours.
4. **Preparation of control working solution:** Before testing, prepare sufficient control working solution according to the number of 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 fresh and used within 4 hours.
5. **Preparation of 1 mmol/L standard solution:** Before testing, prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 µL of 1 mmol/L standard solution (mix well 10 µL of 100 mmol/L standard solution and 990 µL of buffer solution). The 1 mmol/L standard solution should be prepared fresh and used the same day.
6. **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.

7. Sample preparation

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 10,000×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 (MAES0177).

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 an ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10,000×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 (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

Equilibrate all reagents to 25°C before use.

9. Operating steps

1. Standard well: Add 20 µL of different concentration solutions to standard wells. Sample well: Add 20 µL of sample to sample wells. Control well: Add 20 µL of sample to control wells.
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. Incubate at 37°C for 60 min protected from light, then measure the OD value of each well at 405 nm with microplate reader.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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 produces 1 µmol p-nitrophenol at 37°C is defined as 1 unit.

total PDEs activity (U/L) = (ΔA - b) ÷ a × f ÷ T × 1000*

2. Tissue and cell samples:

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

total PDEs activity (U/gprot) = (ΔA - b) ÷ a ÷ C_{pr} × f ÷ T × 1000*

[Note]

ΔA: ΔA = 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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human plasma samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	3.40	10.00
%CV	4.1	5.6	4.6

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 99.3%.

Recovery

	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

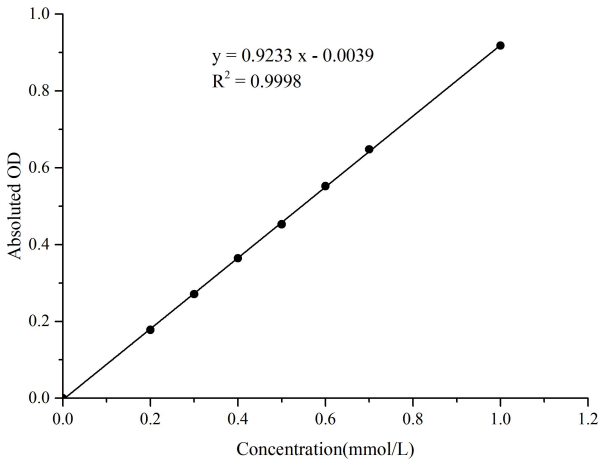
Sensitivity

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.

Standard curve

(It is not necessary to prepare the standard curve for this kit; 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), the standard curve and data are provided below for reference only.



Standard curve

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

12. Appendix II Example Analysis

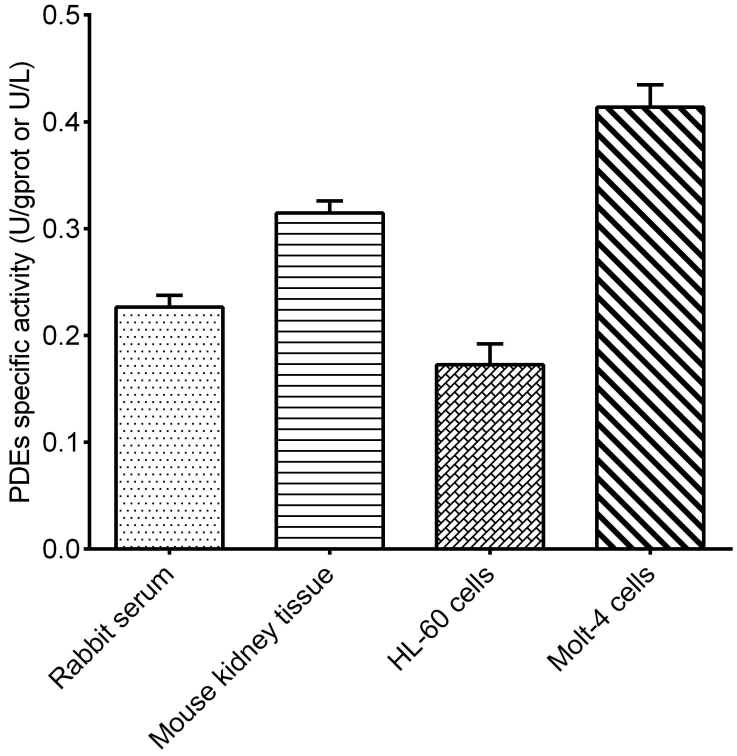
Example analysis:

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

Standard curve: y = 0.9233x - 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:

total PDEs activity (U/gprot) = (0.094 - 0.074 + 0.0039) ÷ 0.9233 ÷ 0.97 ÷ 60 × 1000 = 0.44 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 results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.