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ab273342 cAMP Phosphodiesterase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273342
(use https://www.abcam.cn/ab273342 for china, or
https://www.abcam.co.jp/ab273342 for Japan)

For the determination of cAMP Phosphodiesterase activity in adherent/suspension cells, tissue and purified enzyme preparations.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

cAMP Phosphodiesterase Activity Assay Kit (Fluorometric) (ab273342) provides a rapid, sensitive and straightforward way to measure cAMP Phosphodiesterase (cPDE) activity in various sample types. In this assay, AMP produced by cPDE activity is metabolized by the enzyme mix, developer mix and converter mix to generate an intermediate compound, which reacts with a probe, yielding a fluorescent signal that can be measured at Ex/Em = 535/587 nm.

This assay can detect cPDE activity as low as 0.1 µU per well.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Measure fluorescence in kinetic mode for 30 mins at 37°C.

Determine cAMP Phosphodiesterase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
AMP Standard	100 µL	-20°C
cPDE Assay Buffer	25 ml	-20°C
cPDE Converter Lyophilized	1 vial	-20°C
cPDE Developer Lyophilized	1 vial	-20°C
cPDE Enzyme Mix	200 µL	-20°C
cPDE Positive Control Lyophilized	1 vial	-20°C
cPDE Probe	200 µL	-20°C
cPDE Substrate Lyophilized	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader
- 96-well black opaque plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **AMP Standard:**

Ready to use as supplied. Aliquot and store at -20°C, protected from light.

9.2 **cPDE Assay Buffer:**

Ready to use as supplied. Bring to room temperature before use. Store at +4°C in the dark.

9.3 **cPDE Converter Lyophilized:**

Reconstitute with 220 µl ddH_2O. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles and use within two months.

9.4 **cPDE Substrate Lyophilized:**

Reconstitute with 220 µl ddH2O. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles and use within two months.

9.5 **cPDE Enzyme Mix:**

Ready to use as supplied. Aliquot and store at -20°C, thaw and keep on ice while using.

9.6 **cPDE Positive Control Lyophilized:**

Reconstitute with 100 µl cPDE Assay Buffer, pipet up and down to mix. Aliquot and store at -20°C, protected from light. Use within two months.

9.7 **cPDE Probe:**

Ready to use as supplied. Aliquot and store at -20°C, protected from light.

9.8 **cPDE Developer Lyophilized:**

Reconstitute with 220 µl cPDE Assay Buffer, pipet up and down to mix. Store at -20°C, protected from light. Avoid repeated freeze/thaw cycles and use within two months.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold cPDE Assay Buffer.
10.2 Centrifuge at 10,000 *x g* and 4°C for 15 mins and transfer the supernatant to a fresh tube (keep on ice).
10.3 Add 2-20 µl of sample supernatant to desired well(s) in a black, flat bottom 96-well plate.
10.4 For each test sample, prepare two parallel Sample wells, with one well serving as a Sample Background Control.
10.5 Adjust the volume of all Sample and Sample Background Control wells to 50 µl/well with cPDE Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ Note: For Samples with extremely high cPDE activity, the assay kinetics may only be linear for the first few min. In this case, Samples should be diluted with cPDE Assay Buffer and retested.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute 10 µl AMP Standard with 990 µl cPDE Assay Buffer to generate 0.1 mM AMP Standard.
11.2 Add 0, 2, 4, 6, 8, and 10 µl of the 0.1 mM AMP Standard to a series of wells in a black 96-well plate to generate 0, 200, 400, 600, 800, and 1,000 pmole/well AMP Standard.
11.3 Bring the volume in each well to 50 µl with cPDE Assay Buffer.

Standard #	0.1 mM AMP Standard (µL)	cPDE Assay Buffer (µL)	AMP (pmol/well)
1	0	50	0
2	2	48	200
3	4	46	400
4	6	44	600
5	8	42	800
6	10	40	1,000

12. Assay Procedure

– Keep on ice while in use.

12.1 **cPDE Positive Control:** Add 5-10 µl of the reconstituted cPDE Positive Control to Positive Control well(s). Adjust the volume to 50 µl/well with cPDE Assay Buffer.

12.2 **Reaction Mix:**
Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Reaction mix:

	Reaction mix (µl)	Background Control mix (µl)
cPDE Assay Buffer	41	43
cPDE Substrate	2	---
cPDE Enzyme Mix	2	2
cPDE Converter	2	2
cPDE Developer	2	2
cPDE Probe	1	1

12.3 Add 50 µl of the Reaction Mix to all Sample, Positive Control (if applicable) and AMP Standard curve wells.
12.4 Add 50 µl of the Background Control Mix to all Sample Background well(s).
12.5 Measure the fluorescence of all wells at Ex/Em = 535/587 nm in kinetic mode for 30 mins at 37°C. The AMP Standard curve should reach the endpoint (maximal signal) within 10 mins.

Δ Note: Measurement time for the linear phase of the reaction depends on the cPDE activity in Samples. Measure the fluorescence in kinetic mode and choose any two time points (T1 and T2) in the linear range to calculate the cPDE activity of the Sample(s). There may be a brief lag phase at the start of the reaction due to temperature equilibration. In our experience, the linear phase begins roughly 2-5 mins after the initiation of the reaction.

13. Calculations

13.1 For the AMP Standard Curve, subtract the 0 pmol/well Standard reading from all Standard readings.

13.2 Plot the background subtracted values and calculate the slope of the Standard Curve.

13.3 For each Sample reaction well (and paired Sample Background well), choose two time points (T1 and T2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU1 and RFU2) and determine the change in fluorescence over the time interval: ΔF = RFU2 – RFU1.

13.4 Calculate the corrected fluorescence generated by cAMP Phosphodiesterase activity (denoted by CF) by subtracting the Sample Background Control (ΔFBC) from the corresponding Test Sample (ΔFS): CF = ΔFS – ΔFBC.

13.5 Apply the CF value to the Standard Curve to get B pmoles of AMP generated during the reaction time ($\Delta T = T2 - T1$).

13.6 Sample cAMP Phosphodiesterase Activity is calculated as:

$$cPDE\ activity\ = \frac{B}{(\Delta T\ x\ V)} X\ D = pmol/min/ml$$

B = AMP amount from the Standard Curve (pmol)
ΔT = Reaction time ($T_2 – T_1$) (mins)
V = Sample volume added to reaction well (m)
D = Dilution Factor (For Undiluted Samples, D=1)

pmol/min/ml = µU/ml.

cPDE Unit Definition: One unit of cPDE is the amount of enzyme that generates 1.0 µmole of AMP per min at pH 7.0 at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

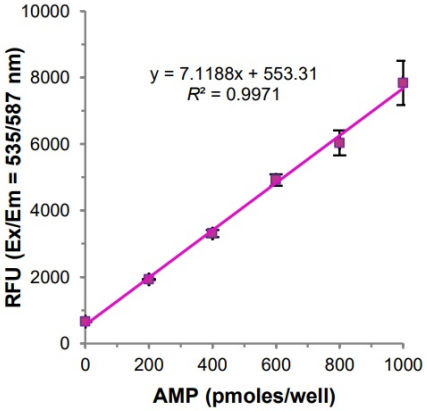

Figure 1. AMP Standard Curve.

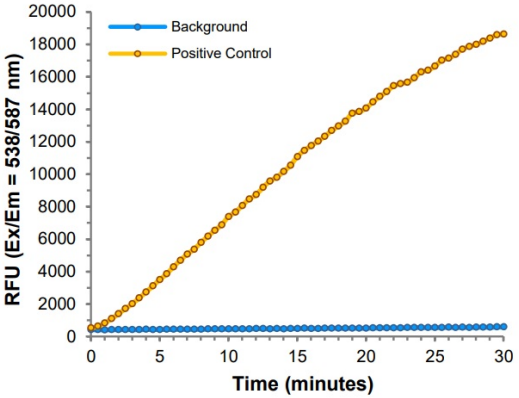

Figure 2. Reaction kinetics of cPDE Positive Control.

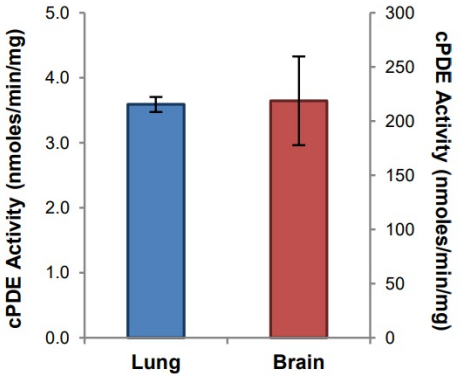

Figure 3. cPDE activity in Rat lung and brain lysate. Rat tissue was prepared according to the described protocol. For activity determination, experiments were run in duplicates, with 0.9-3.6 µg protein (lung) or 0.1-0.4 µg protein (brain) loaded per well.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)