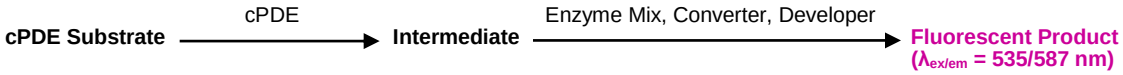
# cAMP Phosphodiesterase Activity Assay Kit (Fluorometric)

*09/19*

**(Catalog # K2013-100; 100 assays; Store at -20°C)**

## I. Introduction:

Cyclic adenosine-3',5'-monophosphate (cyclic AMP or cAMP) is a vital second messenger molecule in eukaryotes as well as a relevant signaling effector in bacteria. Intracellular cAMP-mediated signaling cascades control many biological pathways such as glucose and lipid metabolism via activation of protein kinase A and cyclic nucleotide gated ion channels. Intracellular cAMP is synthesized from ATP by the enzyme adenylyl cyclase and is inactivated by hydrolytic cyclic-AMP phosphodiesterase enzymes (cPDEs; EC 3.1.4.53). Inhibition of cPDEs has been shown to impact many biological pathways including cognition, neuronal signaling, inflammation and vascular smooth muscle contractility. Eleven superfamilies of PDEs with varying selectivity for cAMP or other cyclic nucleotides have been identified in mammals. The predominant phosphodiesterase isoforms involved in the degradation of cAMP are phosphodiesterase 4 (PDE4) family members. There are many unique isoforms of the enzyme, expressed either as products of different genes or through alternative splicing, and their expression levels are cell-type dependent. **BioVision's cAMP Phosphodiesterase Activity Assay Kit** provides a rapid, sensitive and straightforward way to measure 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.




## II. Applications:

- Measurement of Cyclic-AMP Phosphodiesterase activity in various tissues or cells

## III. Sample Type:

- Animal tissues: liver, lung, intestine, *etc*.
- Purified enzyme preparations
- Adherent or suspension cultured cells

## IV. Kit Contents:

| Components | K2013-100 | Cap Code | Part Number |
|---|---|---|---|
| cPDE Assay Buffer | 25 ml | WM | K2013-100-1 |
| cPDE Substrate | 1 vial | Orange | K2013-100-2 |
| cPDE Enzyme Mix | 200 µl | Blue | K2013-100-3 |
| cPDE Converter | 1 vial | Amber | K2013-100-4 |
| cPDE Developer | 1 vial | Green | K2013-100-5 |
| cPDE Probe | 200 µl | Red | K2013-100-6 |
| cPDE Positive Control | 1 vial | Violet | K2013-100-7 |
| AMP Standard | 100 µl | Clear | K2013-100-8 |

## V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multiwell fluorescence microplate reader

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **cPDE Assay Buffer:** Warm to room temperature (RT) before use. When not in use, store at 4ºC, protected from light.
- **cPDE Enzyme Mix:** Aliquot and store at -20°C, thaw and keep on ice while using.
- **cPDE Developer:** 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
- **cPDE Substrate** and **cPDE Converter:** 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.
- **cPDE Positive Control:** 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.
- **cPDE Probe & AMP Standard (10 mM):** Aliquot and store at -20°C, protected from light.

## VII. cAMP Phosphodiesterase Activity Assay Protocol:

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

   **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.

   **Note:** For Unknown Samples, we suggest testing several sample dilutions to ensure the readings are within the Standard Curve range. 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.

2. **AMP Standard Curve:** Dilute 10 µl AMP Standard with 990 µl cPDE Assay Buffer to generate 0.1 mM AMP Standard. 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 1000 pmole/well AMP Standard. Bring the volume in each well to 50 µl with cPDE Assay Buffer.
3. **Reaction Mix Preparation:** Prepare Reaction Mix and Background Control Mix according to the table below. Make a sufficient amount of each type of mix to add 50 µl to all assay wells of that type.

| | Reaction Mix | Background Control Mix |
|---|---|---|
| cPDE Assay Buffer | 41 µl | 43 µl |
| cPDE Substrate | 2 µl | — |
| cPDE Enzyme Mix | 2 µl | 2 µl |
| cPDE Converter | 2 µl | 2 µl |
| cPDE Developer | 2 µl | 2 µl |
| cPDE Probe | 1 µl | 1 µl |

Mix well. Add 50 µl of the Reaction Mix to all Sample, Positive Control (if applicable) and AMP Standard curve wells. Add 50 µl of the Background Control Mix to all Sample Background well(s).

4. **Measurement:** Measure the fluorescence of all wells at Ex/Em = 535/587 nm in kinetic mode for 30 min at 37°C. The AMP Standard curve should reach the endpoint (maximal signal) within 10 min.

**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 ($T_1$ and $T_2$) 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.

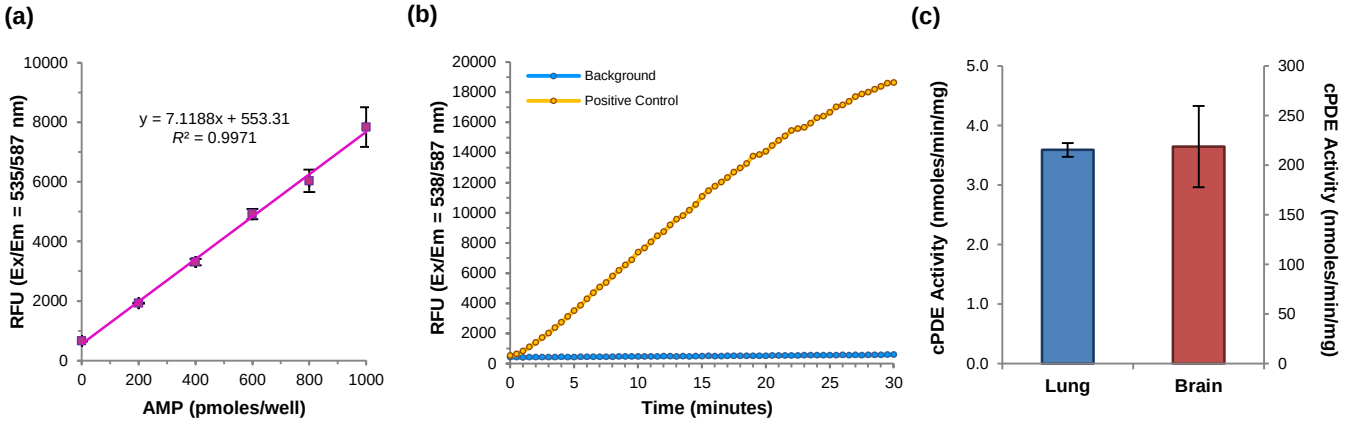
5. **Calculation:** For the AMP Standard Curve, subtract the 0 pmol/well Standard reading from all Standard readings. Plot the background-subtracted values and calculate the slope of the Standard Curve. For each Sample reaction well (and paired Sample Background well), choose two time points ($T_1$ and $T_2$) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points ($RFU_1$ and $RFU_2$) and determine the change in fluorescence over the time interval: $\Delta F = RFU_2 - RFU_1$. Calculate the corrected fluorescence generated by cAMP Phosphodiesterase activity (denoted by $C_F$) by subtracting the Sample Background Control ($\Delta F_{BC}$) from the corresponding Test Sample ($\Delta F_S$): $C_F = \Delta F_S - \Delta F_{BC}$. Apply the $C_F$ value to the Standard Curve to get $B$ pmoles of AMP generated during the reaction time ($\Delta T = T_2 - T_1$). Sample cAMP Phosphodiesterase Activity is calculated as:

$$\textbf{Sample Cyclic AMP Phosphodiesterase Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T \times V}} \times \boldsymbol{D} = \textbf{pmol/min/ml} \equiv \textbf{µU/ml}$$

Where: $\boldsymbol{B}$ = AMP amount from the Standard Curve (in pmoles)
$\Delta\boldsymbol{T}$ = linear phase reaction time $T_2 - T_1$ (in min)
$\boldsymbol{V}$ = Sample volume added into the reaction well (in ml)
$\boldsymbol{D}$ = Dilution Factor (For Undiluted Samples, $D$=1)

**Note:** To express cPDE activity in terms of sample protein content (pmol/min/mg), measure sample protein concentration using the Bradford reagent (BioVision Cat. #K810) or an equivalent protein assay.

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




**Figures:** (a) AMP Standard Curve. (b) Reaction kinetics of cPDE Positive Control. (c) 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.

## VIII. Related Products:

Total Phosphodiesterase Activity Assay Kit (K927)
cAMP ELISA Kit (E4715)
(R,S)-Rolipram (2010)
cAMP Direct Immunoassay Kit (K371)
AMP Assay Kit (K229)
Roflumilast (2675)

***FOR RESEARCH USE ONLY! Not to be used on humans.***