Description

The PDE2A Assay Kit is a fluorescence polarization (FP), homogeneous, 384-well assay designed for the screening and profiling of PDE2A (Phosphodiesterase 2A) inhibitors. This assay takes advantage of a specific fluorescent phosphate-binding nanoparticle. The kit contains enough purified recombinant PDE2A, fluorescent probe, PDE assay buffer, Binding Agent, and diluent for 400 reactions.

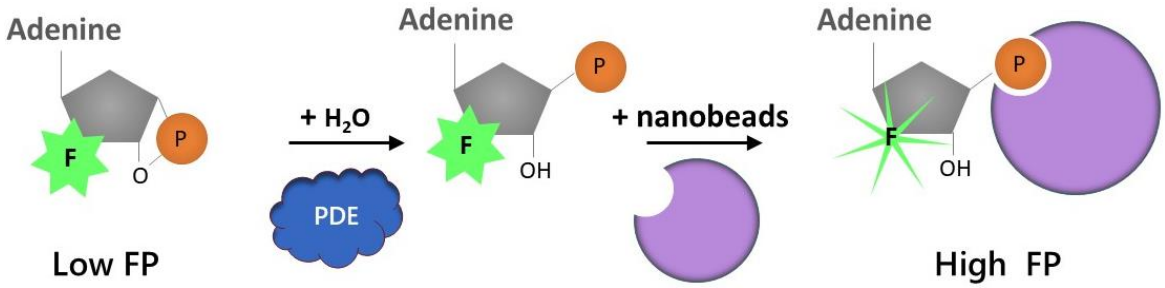

Figure 1: Illustration of the PDE2A assay principle.

The assay uses a fluorescein-labeled cyclic adenine monophosphate (cAMP-FAM), in which the phosphate group is engaged within the cyclic nucleotide. This is a very small molecule that rotates fast (low FP). PDE2A catalyzes the hydrolysis of the phosphodiester bond in the cyclic nucleotide and frees the phosphate group. In a second step the free phosphate group is recognized by a specific phosphate-binding nanobead (Binding Agent) leading to the formation of a large complex, with restricted movement (high FP). FP is proportional to PDE activity.

This assay requires a fluorescent microplate reader ***capable of measuring fluorescence polarization (FP)*** to read the FP signal. For more information FP technology, visit our Tech Note: FP, assay principles and applications.

Background

Phosphodiesterases (PDEs) play an important role in the dynamic regulation of the second messengers cAMP and cGMP signaling, by hydrolyzing them. PDE2A (also known as cGMP-dependent 3',5'-cyclic phosphodiesterase) displays dual specificity for cAMP and cGMP with a bias towards cGMP. It is involved in the regulation of blood pressure and vascular permeability and is a therapeutic target of interest for cardiovascular diseases.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
60020	PDE2A, GST-Tag *	1 µg	-80°C
60200	20 µM FAM-Cyclic-3', 5'-AMP	50 µl	-80°C
60393	PDE Assay Buffer	25 ml	-20°C
60390	Binding Agent	250 µl	4°C
60391	cAMP Binding Agent Diluent	25 ml	4°C
79961	Low binding, black 384-well plate	1	Room Temp.

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Fluorescent plate reader capable of measuring fluorescence polarization.

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Negative Control", "Positive Control" and "Test inhibitor".
- We recommend using inhibitor Bay 60-7550 as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

1. Dilute 20 µM FAM-Cyclic-3', 5'-AMP stock solution 100-fold using PDE Assay Buffer to make a 200 nM dilution (you will need 12.5 µl/well).

2. Add 12.5 µl of diluted FAM-Cyclic-3', 5'-AMP to the "Negative Control", "Positive Control", and "Test Inhibitor" wells.

3. Add 22.5 µl of PDE Assay Buffer to the "Blank" wells.

4. Add 10 µl of PDE Assay Buffer to the "Negative Control" wells.

5. Prepare the Test Inhibitor (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations using PDE Assay Buffer. For the positive and negative controls, use PDE Assay Buffer as Diluent Solution.

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in PDE Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Use 10% DMSO in PDE Assay Buffer (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in PDE Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of the inhibitor serial dilution to the "Test Inhibitor" wells.

7. Add 2.5 µl of the Diluent Solution to the "Blank", "Negative Control", and "Positive Control" wells.

8. Thaw **PDE2A** on ice. Briefly spin the tube containing the enzyme to recover its full content.

9. Dilute PDE2A with PDE Assay Buffer to 25 pg/µl (10 µl/well).

10. Initiate the reaction by adding 10 µl of diluted PDE2A to the "Positive Control" and "Test Inhibitor" wells.

11. Incubate at Room Temperature (RT) for 1 hour.

Component	Blank	Negative Control	Positive Control	Test Inhibitor
FAM-Cyclic-3', 5'-AMP (200 nM)	-	12.5 µl	12.5 µl	12.5 µl
PDE Assay Buffer	22.5 µl	10 µl	-	-
Test Inhibitor	-	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	2.5 µl	-
Diluted PDE2A (100 pg/µl)	-	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

12. Gently mix the tube containing the **Binding Agent** and dilute 100-fold with **cAMP Binding Agent Diluent** (100 µl/well).

13. Add 50 µl of diluted Binding Agent to each well.

14. Incubate at RT for 20 minutes with gentle agitation.

    Note: the signal is stable from 20 to 60 minutes.

15. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λexc = 485 nm; λem = 528 nm) **and set to FP**.

16. Subtract the "Blank" value from all other values.

Calculating Results

Users may ignore the G-factor when all experiments are performed using the same instrument since the G-factor is instrument-dependent.

If desired, the G-factor is set before measurements are performed. It needs to be determined by the investigator when not clearly indicated by the manufacturer. The instrument manual will contain information about how to establish the G-factor. For example, BPS Bioscience's scientists use a Tecan M1000 fluorescent plate reader which has a G-factor set to 22 mP.

Instruments provide measurement in milli-Polarization = mP.

Results are calculated as follows.

1. Subtract the "Blank" value from all other values.

2. Calculate ΔmP for all samples:

$$\Delta mP = (mP \text{ value of the sample}) - (mP \text{ of the Reference control})$$

Where mP refers to milli-Polarization values provided by the instrument and Reference control is the mP value obtained in the condition containing only the fluorescent probe (a condition in which the probe is in free state).

Example of Assay Results

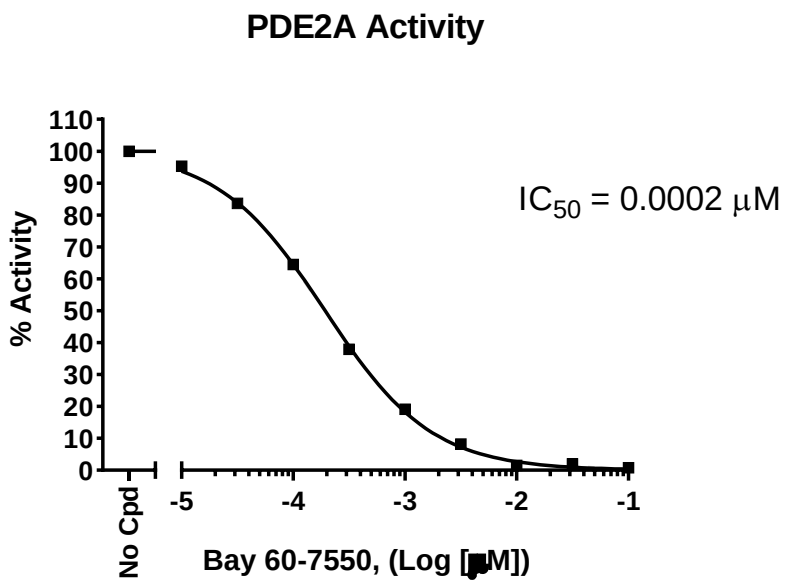

Figure 2: Inhibition of PDE2A by the inhibitor Bay 60-7550.
PDE2A was incubated with increasing concentrations of Bay 60-7550 (Cayman Chemicals #10011135) in the presence of 100 nM FAM-Cyclic-3', 5'-AMP substrate. Fluorescence Polarization was measured using a Tecan M1000 fluorescent microplate reader. Results are expressed in % activity, in which FP in the absence of inhibitor (positive control) is set to 100%.

Data shown is representative. For lot-specific information, contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, email support@bpsbioscience.com

References
Maurice D.H., 2005 *Front. Biosci.* 10: 1221-1228.

Related Products

Products	*Catalog #*	*Size*
PDE2A Assay Kit	60320	96 reactions
PDE2A (Mouse) Assay Kit	79648	96 reactions
PDE7A-HEK293 Recombinant Cell Line	60407	2 vials

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