Description

The Rat PDE4B Assay Kit is a fluorescence polarization (FP), homogeneous 96-well assay designed for the screening and profiling of rat PDE4B inhibitors. The kit contains enough purified recombinant rat PDE4B, fluorescent probe (cAMP), PDE Assay Buffer, Binding Agent, and diluent for 100 reactions. This assay requires a fluorescent microplate reader *capable of measuring fluorescence polarization (FP)* to read the FP signal. For more information on the principles of FP, visit fp_assays.pdf (bpsbioscience.com).

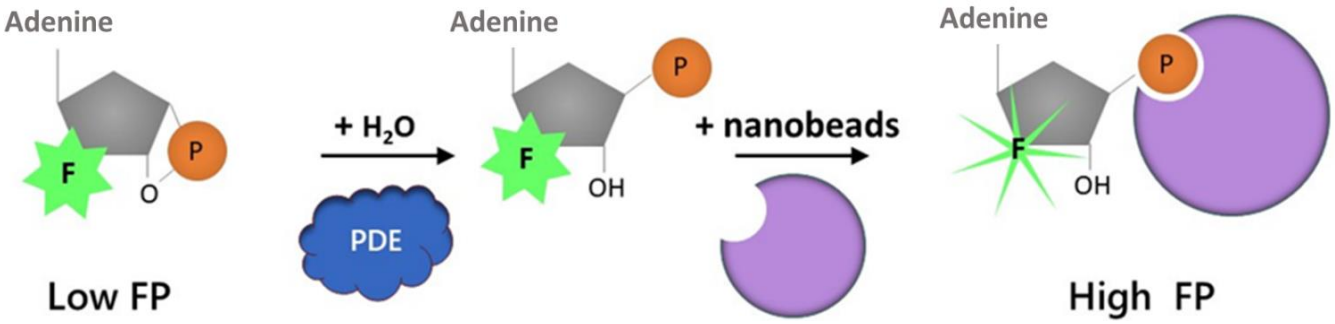

Figure 1: Illustration of the PDE4B assay principle.

The assay uses a fluorescein-labeled cyclic adenosine monophosphate (cAMP-FAM), in which the phosphate group is engaged within the cyclic nucleotide. This is a very small molecule that can rotate fast (low FP). PDE4B 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 large complex, with restricted movement (high FP). FP values are proportional to PDE activity.

Background

PDE4B, also known as cAMP-specific 3'.5'-cyclic phosphodiesterase 4B, is a protein of the PDE (cyclic nucleotide phosphodiesterase) family. cGMP and cAMP are second messengers in responses to several signals, such as hormones and neurotransmitters. By hydrolyzing cyclic nucleotides, PDEs regulate multiple pathways. PDE4B hydrolizes cAMP, and its dysfunction is involved in schizophrenia, bipolar disease, and psoriasis. It is also involved in neutrophil and microvascular obstruction in acute myocardial infarction. Inhibitors of PDE4B have been shown to have antipsychotic effects and protect against myocardial ischemia reperfusion (MI/R) injury.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
60049	Rat PDE4B, 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	100 µl	4°C
60391	cAMP Binding Agent Diluent	10 ml	4°C
	0.5 M DTT	100 µl	-80°C
79685	Low binding, black 96-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.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Substrate Control", "Positive Control" and "Test inhibitor".
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Dilute 20 µM FAM-Cyclic-3',5'-AMP stock solution 100-fold with PDE Assay Buffer to make a 200 nM solution (you will need 25 µl/well).

2. Dilute 0.5 M DTT stock solution 250-fold in the diluted FAM-Cyclic-3',5'-AMP.

3. Add 25 µl of diluted FAM-Cyclic-3',5'-AMP with DTT to the "Substrate Control", "Positive Control", and "Test Inhibitor" wells.

4. Add 45 µl of PDE Assay Buffer to the "Blank" wells.

5. Add 20 µl of PDE Assay Buffer to the "Substrate Control" wells.

6. Prepare the Test Inhibitor (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 50 µl.

    a) 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.

    b) 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%.

7. Add 5 µl of the inhibitor dilution to the "Test Inhibitor" wells.

8. Add 5 µl of the Diluent Solution to the "Blank", "Substrate Control" and "Positive Control" wells.

9. Thaw **rat PDE4B** on ice. Briefly spin the tube containing the enzyme to recover its full content.

    Note: The concentration of protein is lot-specific and will be indicated on the tube containing the protein. Rat PDE4B is very sensitive to freeze/thaw cycles. Do not re-use the diluted enzyme.

10. Dilute rat PDE4B in PDE Assay Buffer to 1 pg/µl (20 µl/well).

11. Initiate the reaction by adding 20 µl of diluted PDE4B to the "Positive Control" and "Test Inhibitor" wells. Discard any remaining diluted enzyme after use.

Component	Blank	Substrate Control	Positive Control	Test Inhibitor
Diluted FAM-Cyclic-3',5'-AMP with DTT	-	25 µl	25 µl	25 µl
PDE Assay Buffer	45 µl	20 µl	-	-
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
Diluted PDE4B (1 pg/µl)	-	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

13. Gently mix the tube containing the **Binding Agent** and dilute 1:100 with the **cAMP Binding Agent Diluent**.

14. Add 100 µl of diluted Binding Agent to each well.

15. Incubate at RT for 30 minutes with gentle agitation.

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

17. 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:
   ΔmP = (mP value of the sample) – (mP 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 Results

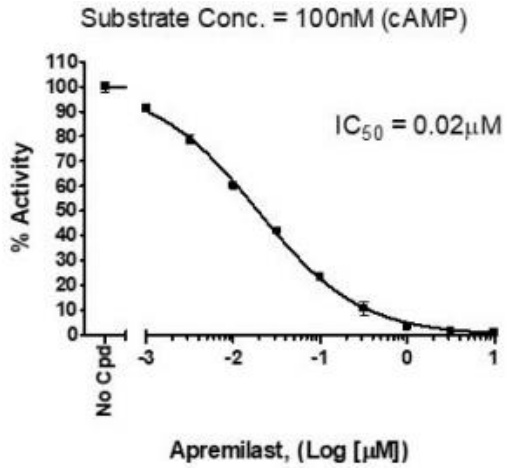

Figure 2: Inhibition of rat PDE4B by Apremilast.
Rat PDE4B was incubated with increasing concentrations of Apremilast (Cayman Chemicals #18502) in the presence of 100 nM FAM-Cyclic-3',5'-AMP substrate. Fluorescence Polarization was measured using a Tecan M1000 fluorescent microplate reader. Results are expressed in percent of FP control, where FP in the absence of inhibitor 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

Related Products

Products	*Catalog #*	*Size*
PDED4A1A, GST-Tag, Recombinant	60040	10 µg
PDE4B2, GST-Tag, His-Tag Recombinant	60042	5 µg
PDE4B1, GST-Tag Recombinant	60041	10 µg
PDE4B (Dog), GST-tag Recombinant	60055	5 µg
PDE4B Cell-Based Activity Assay Kit	79526	500 reactions