

Description

The PDE4B2 Assay Kit is a fluorescence polarization (FP), homogeneous, 96-well assay kit designed for the screening and profiling of PDE4B2 (Phosphodiesterase 4B2) inhibitors. This assay takes advantage of a specific fluorescent phosphate-binding nanoparticle. The kit contains enough purified recombinant PDE4B2, fluorescent probe, PDE assay buffer, Binding Agent, and diluent for 100 reactions.

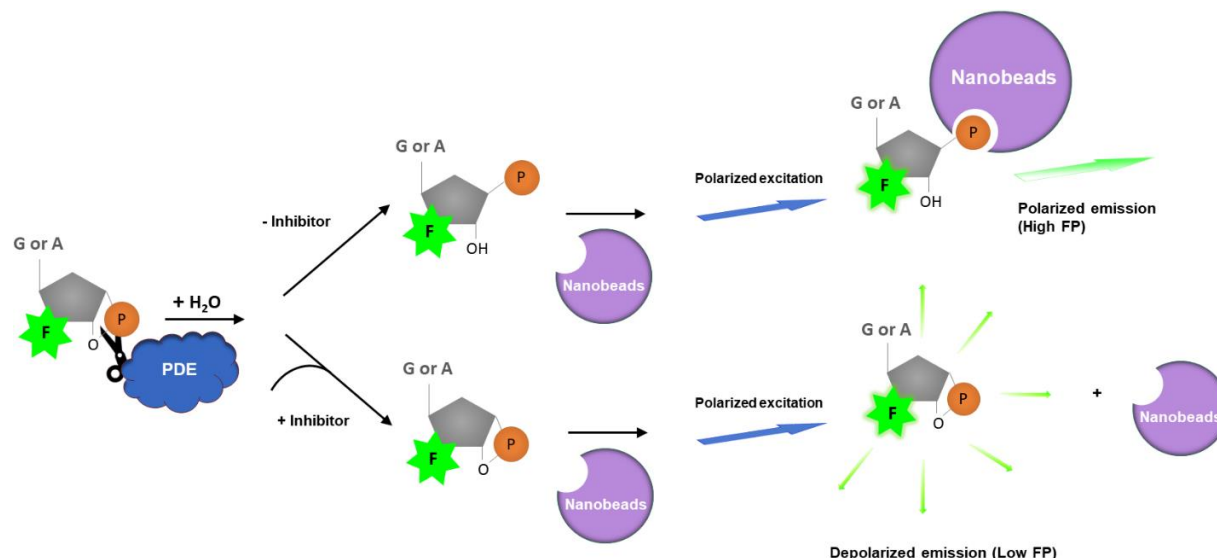


Figure 1: Illustration of the PDE4B2 Assay Kit principle.

The assay uses a fluorescein-labeled cyclic adenosine monophosphate (cAMP-FAM for PDE4B2), in which the phosphate group is engaged within the cyclic nucleotide. This is a very small molecule that rotates fast (low FP). PDE4B2 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 PDE4B2 activity.

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

Note: As of July 2024, this protocol has been re-optimized for performance.

Background

Phosphodiesterases (PDEs) play an important role in the dynamic regulation of the second messengers cAMP (cyclic adenosine monophosphate) and cGMP (cyclic guanosine monophosphate) signaling, by hydrolyzing them. The PDE superfamily is composed of 11 families, with PDE4, 7 and 8 being cAMP-specific hydrolases, and thus regulating positive and negative responses to it. PDE4 is the second most abundant PDE in cardiovascular tissues. PDE4, is a cAMP-specific PDE and the largest PDE subfamily with over 35 different isoforms and as such, the most widely characterized PDE isoenzyme. It is the predominant isoenzyme in the majority of inflammatory cells, and the airways smooth muscle and is implicated in inflammatory airways disease. PDE4 inhibitor roflumilast has been approved for the treatment of chronic obstructive lung disease.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
60042-KC1	PDE4B2, GST-Tag, His-Tag*	>1 µg	-80°C
60200-KC1.2	FAM-Cyclic-3', 5'-AMP**	1.2 nmoles**	-80°C
60393	PDE Assay Buffer (Incomplete)	25 ml	-20°C
60390	PDE Binding Agent	100 µl	4°C
60391	Binding Agent Diluent (cAMP)	10 ml	4°C
82735-KC200	0.5 M DTT	200 µl	-20°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.

** FAM-Cyclic-3', 5'-AMP is provided as a powder. The vial will need to be resuspended in 600 µl of Complete PDE Assay Buffer before use.

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring fluorescence polarization ($\lambda_{ex}=470$ (5 nm bandwidth) and detection at $\lambda_{em}=528$ (10 nm bandwidth))

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%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Reference Control” (minimum FP), “Positive Control” (maximum FP), and “Test Inhibitor” conditions.
- It is recommended all controls are run side by side as they may be necessary for result calculation.
- We recommend using Roflumilast (#27645) 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 IC₅₀ 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 \(bpsbioscience.com\)](https://bpsbioscience.com/protein-faqs/).
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://bpsbioscience.com/serial-dilution-protocol/).

1. Prepare Complete PDE Assay Buffer by adding 20 µl of 0.5M DTT to 10 ml of PDE Assay Buffer (Incomplete).
2. Thaw **PDE4B2** on ice. Briefly spin the tube containing the enzyme to recover its full content.
3. Dilute PDE4B2 with Complete PDE Assay Buffer to 3.8 pg/µl (40 µl/well), by performing a serial dilution. For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).
4. Add 40 µl diluted PDE4B2 to the “Positive Control” and “Test Inhibitor” wells.
5. Add 45 µl of Complete PDE Assay Buffer to the “Blank” wells.
6. Add 40 µl of Complete PDE Assay Buffer to the “Reference Control” wells.
7. 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.

7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in Complete PDE Assay Buffer.

For the positive and negative controls, use Complete PDE Assay Buffer as Diluent Solution.

OR

7.2 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 Complete 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 Complete 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 Complete 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%.

8. Add 5 μ l of the inhibitor serial dilution to the “Test Inhibitor” wells.
9. Add 5 μ l of the Diluent Solution to the “Blank”, “Reference Control”, and “Positive Control” wells.
10. Resuspend one vial of FAM-Cyclic-3', 5'-AMP in 600 μ l of Complete PDE Assay Buffer to make a 2 μ M solution.
11. Initiate the reaction by adding 5 μ l of FAM-Cyclic-3',5'-AMP (2 μ M) to each well designated for the “Reference Control”, “Positive Control”, and “Test Inhibitor”.
12. Incubate at Room Temperature (RT) for 1 hour.

Component	Blank	Reference Control	Positive Control	Test Inhibitor
FAM-Cyclic-3', 5'-AMP	-	5 μ l	5 μ l	5 μ l
Complete PDE Assay Buffer	45 μ l	40 μ l	-	-
Test Inhibitor	-	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	5 μ l	-
Diluted PDE4B2 (3.8 pg/ μ l)	-	-	40 μ l	40 μ l
Total	50 μl	50 μl	50 μl	50 μl

13. Gently mix the tube containing the **PDE Binding Agent** and dilute 100-fold with **Binding Agent Diluent (cAMP)** (100 μ l/well).
14. Add 100 μ l of diluted PDE Binding Agent to each well.
15. Incubate at RT for 30 minutes with gentle agitation (*Note: The signal is stable from 20 to 60 minutes*).
16. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λ_{ex} = 470 (5 nm bandwidth); λ_{em} = 528 (10 nm bandwidth)) **and set to FP**.
17. Subtract the “Blank” value from all other values.

Calculating Results

Fluorescence polarization is a measure of the amount of molecular rotation that takes place in the time between excitation and emission of the fluorescence probe. It can be determined from the measurements of perpendicular (I_{\perp}) and parallel (I_{\parallel}) fluorescence intensity values emitted by the probe relative to the direction of the polarized excitation light (Figure 2).

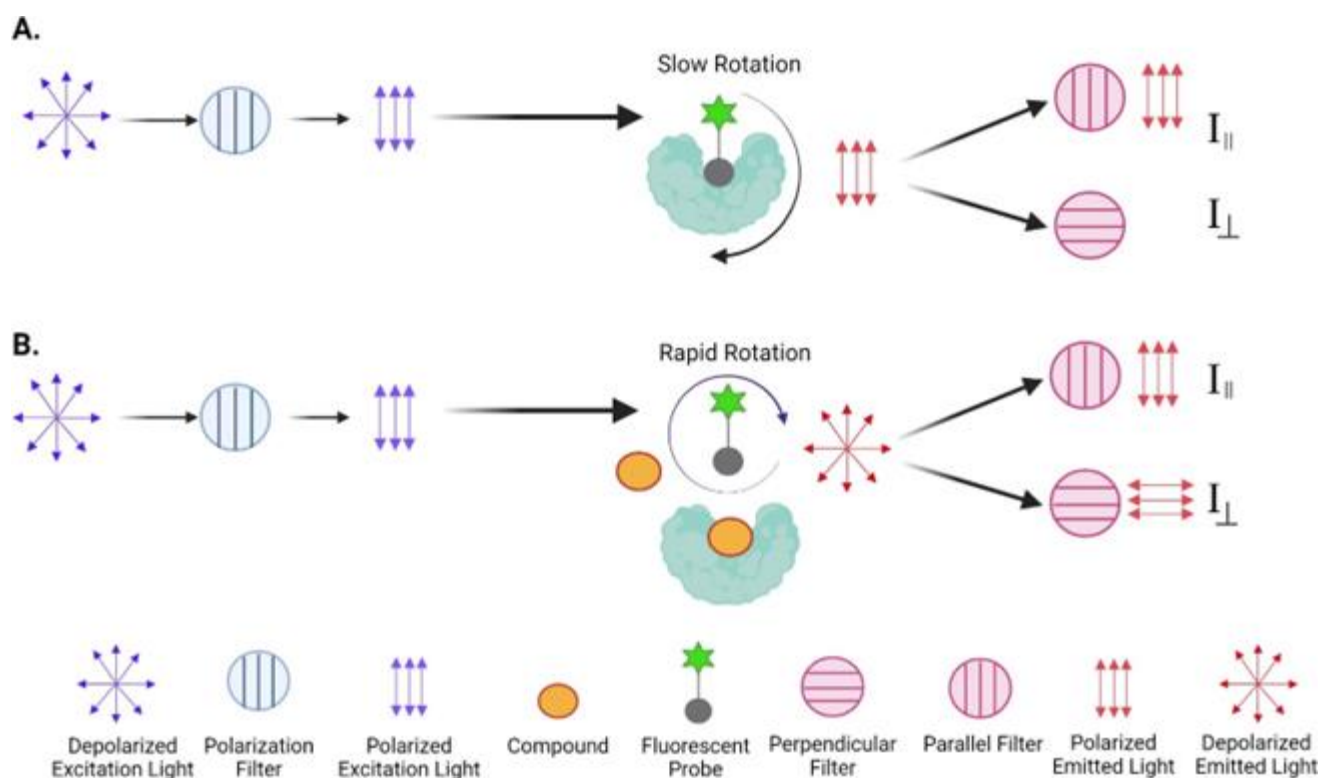


Figure 2: Fluorescence polarization principle.

A. When the fluorescently labeled probe binds to a larger protein it creates a complex of a big molecular weight that has a slow rotation ability. In this state the probe has a reduced rotational diffusion so when it is excited by polarized light, it still emits highly polarized light with a degree of polarization that is inversely proportional to the rate of molecular rotation.

B. In the presence of a compound that has affinity for the protein, the fluorescent probe remains in solution and can rotate rapidly. Unbound probe has a high rotational diffusion so when it is excited by the polarized light it emits light in orientations that can be detected by both the perpendicular and parallel filters.

Polarization is defined as the difference between the emission intensities of parallel fluorescence ($I_{||}$) and perpendicular fluorescence (I_{\perp}), divided by the total fluorescence emission intensity. The polarization value (P) being a ratio of light intensities, is a dimensionless number, often expressed in milli P units where 1 P unit = 1000 mP units. To calculate P one has to take into consideration that light is not transmitted equally well through both parallel and perpendicular channels and therefore a correction must be made. This correction factor is called the "G Factor" (G) and it is specific to the instrument used. mP can thus be calculated using the following formula:

$$mP = \left(\frac{I_{||} - G(I_{\perp})}{I_{||} + G(I_{\perp})} \right) \times 1000$$

Modern instruments usually have the G factor pre-calculated and can automatically calculate fluorescence polarization for your experiments. If you need to determine, set up or calculate the G factor please refer to your instrument manual (the instrument manual should contain information about how to establish the G-factor) or check our FAQ section ([FAQs \(bpsbioscience.com\)](https://www.bpsbioscience.com/FAQs)).

For accurate calculations it is necessary to provide the correct plate schematic when setting up your instrument, with defined positions for the “Blank” and “Reference” (also known as Substrate Control) wells, and to ensure that the emission intensities from the “Blank” wells are subtracted from all other wells prior to further data analysis.

We encourage you to analyze raw data and if appropriate to exclude those “Blank” or “Reference” wells that show aberrant readouts prior to mP determination.

The % of Activity can be calculated as follows:

$$\% \text{ of Activity} = \frac{(\text{mP value from Test Inhibitor} - \text{mP value from Reference Control})}{(\text{mP value from Positive Control} - \text{mP value from Reference Control})} \times 100$$

Example of Assay Results

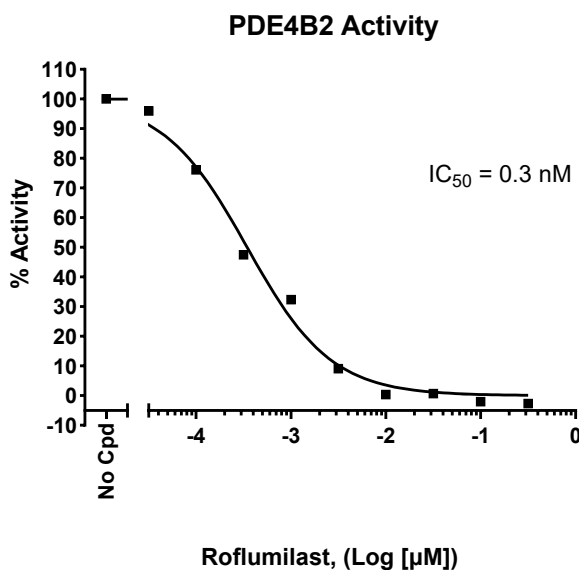


Figure 3: Inhibition of PDE4B2 by Roflumilast.

PDE4B2 was incubated with increasing concentrations of Roflumilast (#27645) in the presence of 200 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.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

References

Ahmed W., *et al.*, 2021 *Biomedicine & Pharmacotherapy* 134:111128.

Huang W., *et al.*, 2020 *Nat Commun* 11:3191.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PDE5A1 Assay Kit	60351	384 reactions
PDE5A1, GST-Tag Recombinant	60050	10 µg
Tadalafil	27657	50 mg/100 mg/500 mg
Vardenafil HCl Trihydrate	27776	10 mg/100 mg
PDE7A/CRE Reporter – HEK293 Recombinant Cell Line	60413	2 vials
PDE10A – HEK293 Recombinant Cell Line	60410	2 vials

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