

Description

The PDE4D2 TR-FRET Assay Kit is designed to provide fast and easy identification of inhibitors of PDE4D2 (phosphodiesterase 4 D2) using Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET). The PDE4D2 TR-FRET Assay Kit comes in a convenient 96-well format, with enough recombinant purified PDE4D2 enzyme, fluorescently labeled PDE substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions.

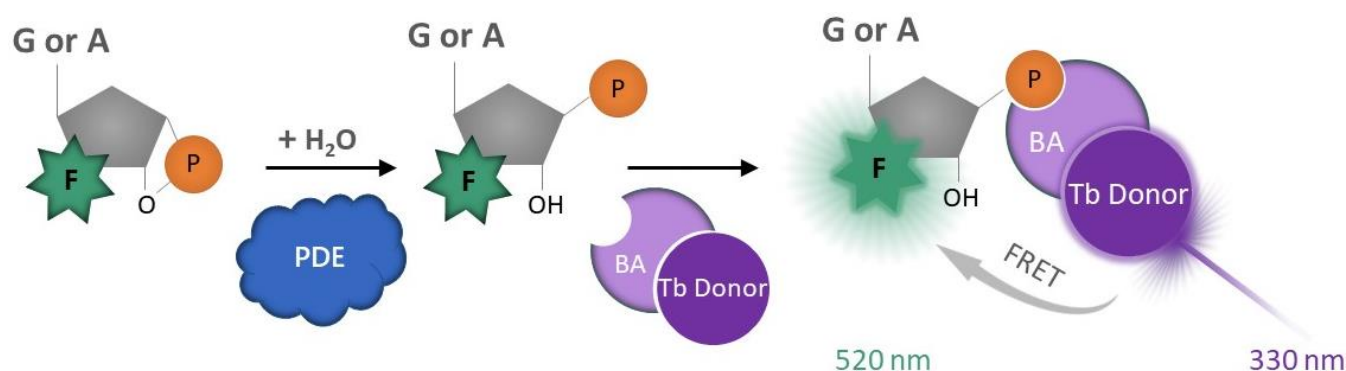


Figure 1: Illustration of the assay principle.

The reaction uses a fluorescein-conjugated (FAM) cyclic monophosphate nucleotide. Phosphodiesterase PDE4D2 catalyzes the hydrolysis of the phosphodiester bond in the cyclic monophosphate nucleotide, releasing the phosphate group for binding. The phosphate group binds to a “Binding Agent” (BA) that is recognized by terbium-labeled donor beads. This results in energy transfer from the terbium to FAM, which emits a fluorescent signal at 520 nm. If unbound to the phosphate group, the terbium-labeled beads emit at $\lambda=490$ nm. The fluorescent intensity is measured using a fluorescence plate reader capable of TR-FRET reading and an increase in 520 nm corresponds directly to the activity of PDE4D2. In this assay, the intensity of the signal at 520 nm is directly proportional to PDE4D2 enzymatic activity.

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 and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
60048	PDE4D2, GST-Tag*	>1 µg	-80°C
60200	FAM-Cyclic-3', 5'AMP**	1.2 nmoles**	-80°C
60393	PDE Assay Buffer (Incomplete)	25 ml	-20°C
60394	Tb Donor	50 µl	-80°C
82782	PDE Binding Agent (TR-FRET)	200 µl	+4°C
78422	Binding Buffer A	20 ml	+4°C
78423	Binding Buffer B	20 ml	+4°C
82735	0.5 M DTT	200 µl	-20°C
79685	Low binding, black 96-well plate	1	Room Temperature

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

** FAM-Cyclic-3', 5'-AMP is provided as a powder. 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 Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

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," "Positive Control," 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 Apremilast 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://www.bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com).

Step 1:

1. Prepare **Complete PDE Assay Buffer** by adding 20 µl of **0.5 M DTT** to 10 ml of **PDE Assay Buffer (Incomplete)**.
2. Thaw **PDE4D2** on ice. Briefly spin the tube containing the enzyme to recover its full content.
3. Dilute PDE4D2 with Complete PDE Assay Buffer to 10 pg/µl (40 µl/well), by performing a serial dilution. For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com).
4. Add 40 µl diluted PDE4D2 to the “Positive Control” and “Test Inhibitor” wells.
5. Add 40 µl of Complete PDE Assay Buffer to the “Blank” 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.
 - 6.1 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

6.2 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%.

7. Add 5 μ l of the diluted Test Inhibitor to the “Test Inhibitor” wells.
8. Add 5 μ l of the Diluent Solution to the “Blank” and “Positive Control” wells.
9. 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.
10. Initiate the reaction by adding 5 μ l of FAM-Cyclic-3',5'-AMP (2 μ M) to all wells.
11. Protect from light and incubate at Room Temperature (RT) for 1 hour.

Component	Blank	Positive Control	Test Inhibitor
FAM-Cyclic-3',5'-AMP	5 μ l	5 μ l	5 μ l
Complete PDE Assay Buffer	40 μ l	-	-
Test Inhibitor	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	-
Diluted PDE4D2 (10 pg/ μ l)	-	40 μ l	40 μ l
Total	50 μl	50 μl	50 μl

Step 2:

1. Prepare **Binding Dilution Buffer** by mixing equal volumes of **Binding Buffer A** and **Binding Buffer B**.
2. Gently mix the tube containing the **PDE Binding Agent**.
3. Prepare **Binding Agent Solution** by diluting Binding Agent 50-fold with **Binding Dilution Buffer**.
4. Dilute the Tb Donor 200-fold with diluted **Binding Agent Solution** (100 μ l/ well).
5. Add 100 μ l of the Tb Donor/Binding Agent mix to each well.
6. Incubate at RT for 1 hour with gentle agitation.
7. Read the fluorescence intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Two sequential measurements should be conducted. Tb-Donor emission should be measured at 490 nm followed by Acceptor emission at 520 nm. Data analysis is performed using the TR-FRET ratio (520 nm emission/490 nm emission).

Reading Mode	Time Resolved
Excitation Wavelength	330 (20 bandwidth)
Emission Wavelength	490 (10 bandwidth)
Lag Time	50 μ s
Integration Time	50 μ s
Excitation Wavelength	330 (20 bandwidth)
Emission Wavelength	520 (10 bandwidth)
Lag Time	50 μ s
Integration Time	50 μ s

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (520 nm emission/490 nm emission).

$$FRET = \frac{S_{520}}{S_{490}}$$

When percentage activity is calculated, the FRET value from the Blank can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ = FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_P$ = FRET value for the Positive Control (no inhibitor).

Example Results

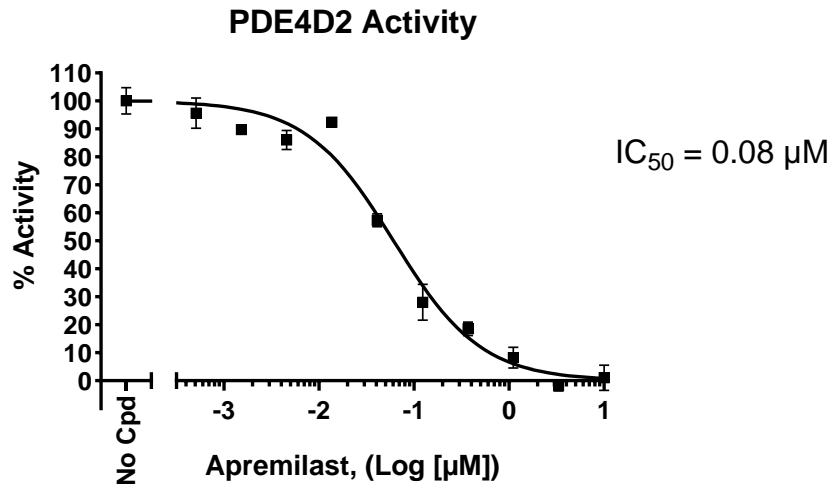


Figure 2: Inhibition of PDE4D2 activity by Apremilast.

PDE4D2 activity was measured in the presence of increasing concentrations of Apremilast (SelleckChem #S8034) concentrations. The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 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

Hao N., *et al.*, 2020, *Mol Cancer Ther* 19(3): 868-881.

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PDE3A Assay Kit	79736	96 reactions
PDE3A (Mouse) Assay Kit	79606	96 reactions
PDE3B Assay Kit	60331	96 reactions
PDE3B, GST-Tag Recombinant	60031	10 µg

Version 072225