

**Description**

The PDE1C TR-FRET Assay Kit is designed to provide fast and easy identification of inhibitors of PDE1C (phosphodiesterase 1C) using Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET). The PDE1C TR-FRET Assay Kit comes in a convenient 96-well format, with enough recombinant purified PDE1C enzyme, fluorescently labeled PDE substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions.

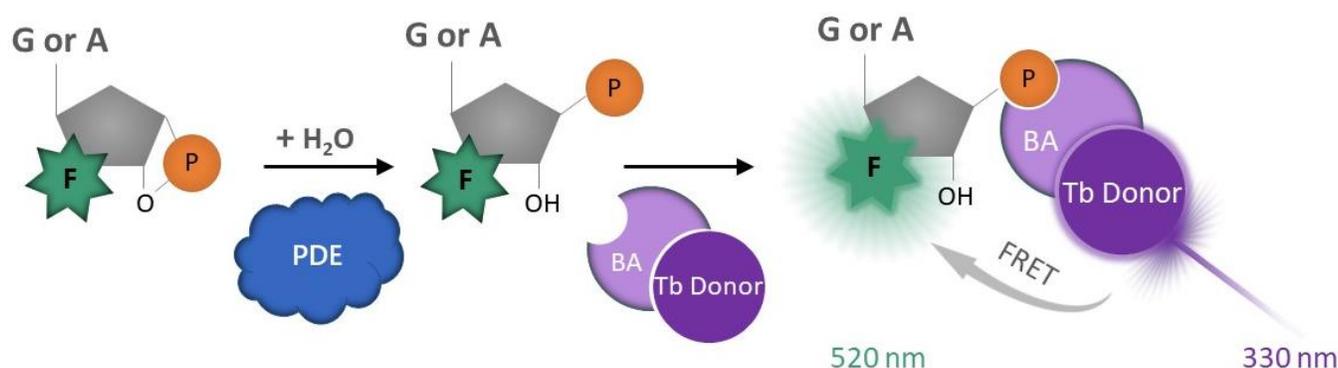


Figure 1: Illustration of the assay principle.

The reaction uses a fluorescein-conjugated (FAM) cyclic monophosphate nucleotide. Phosphodiesterase PDE1C 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 PDE1C. In this assay, the intensity of the signal at 520 nm is directly proportional to PDE1C 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. Among the 11 different families of PDEs, the phosphodiesterase 1 (PDE1) sub-family of enzymes hydrolyze both cAMP and cGMP in a mutually competitive manner. The catalytic activity of PDE1 is stimulated by their binding to  $\text{Ca}^{2+}$ /calmodulin, resulting in the integration of  $\text{Ca}^{2+}$  and cyclic nucleotide-mediated signaling in various diseases. The PDE1 family includes three subtypes, PDE1A, PDE1B and PDE1C, which differ for their relative affinities for cAMP and cGMP. These isoforms are differentially expressed throughout the body, including the cardiovascular, central nervous system and other organs. PDE1 increases vascular contraction of mesenteric arteries upon angiotensin II infusion in rats and also regulates cGMP hydrolysis in cardiomyocyte and vascular smooth muscle cells, which in turn controls smooth muscle tone. Inhibition of PDE1 attenuates adverse cardiac remodeling and may serve as a novel therapeutic approach to limit nitrate tolerance.

**Applications**

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
60013	PDE1C, 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. 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 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 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<sub>50</sub> 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/ProteinFAQs).
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/SerialDilutionProtocol).

**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 **PDE1C** on ice. Briefly spin the tube containing the enzyme to recover its full content.
3. Dilute **PDE1C** with Complete PDE Assay Buffer to 5 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 PDE1C** 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 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  $\mu$ l of the **Test Inhibitor** serial dilution to the “Test Inhibitor” wells.
8. Add 5  $\mu$ l of the **Diluent Solution** to the “Blank,” and “Positive Control” wells.
9. Resuspend one vial of **FAM-Cyclic-3',5'-AMP** in 600  $\mu$ l of Complete PDE Assay Buffer to make a 2  $\mu$ M solution.
10. Initiate the reaction by adding 5  $\mu$ l of **FAM-Cyclic-3',5'-AMP** (2  $\mu$ M) to all wells.
11. Protect from light and incubate at Room Temperature (RT) for 1 hour.

<b>Component</b>	<b>Blank</b>	<b>Positive Control</b>	<b>Test Inhibitor</b>
FAM-Cyclic-3',5'-AMP	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
Complete PDE Assay Buffer	40 $\mu$ l	-	-
Test Inhibitor	-	-	5 $\mu$ l
Diluent Solution	5 $\mu$ l	5 $\mu$ l	-
Diluted PDE1C (5 pg/ $\mu$ l)	-	40 $\mu$ l	40 $\mu$ l
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

**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  $\mu$ l mix/well).
5. Add 100  $\mu$ 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 $\mu$ s
Integration Time	50 $\mu$ s
Excitation Wavelength	330 (20 bandwidth)
Emission Wavelength	520 (10 bandwidth)
Lag Time	50 $\mu$ s
Integration Time	50 $\mu$ 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

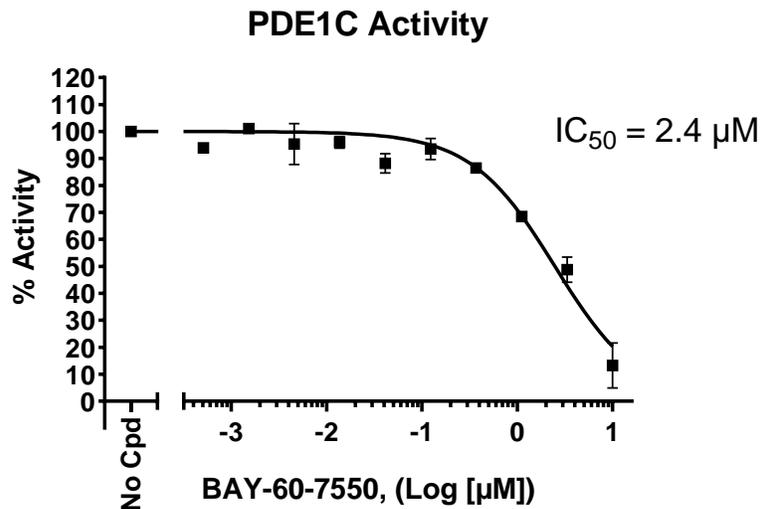


Figure 2: Inhibition of PDE1C activity by BAY-60-7550.

PDE1C activity was measured in the presence of increasing concentrations of BAY-60-7550 (MedChemExpress #HY-14992) 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](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For further questions, please visit [https://bpsbioscience.com/contactsupport@bpsbioscience.com](mailto:https://bpsbioscience.com/contactsupport@bpsbioscience.com)

## References

Ahmed W., et al., 2021 *Biomedicine & Pharmacotherapy* 134:111128.  
Huang W., et al., 2020 *Nat Commun* 11:3191.

## Related Products

Products	Catalog #	Size
PDE1B Assay Kit	60311	96 reactions
Mouse PDE1C Assay Kit	61312	96 reactions
PDE1B TR-FRET Assay Kit	60704	96 reactions
PDE1C Assay Kit	60312	96 reactions
PDE1B, GST-tag Recombinant	60011	10 µg
PDE1B-HEK293 Recombinant Cell Line	60402	2 vials

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