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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PDE3A TR-FRET Assay Kit is designed to provide fast and easy identification of inhibitors of PDE3A (phosphodiesterase 3A) using Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET). The PDE3A TR-FRET Assay Kit comes in a convenient 384-well format, with enough recombinant purified PDE3A (amino acids 669-end) enzyme, fluorescently labeled PDE substrate (cAMP), binding agent, and PDE assay buffer for 384 enzyme reactions.

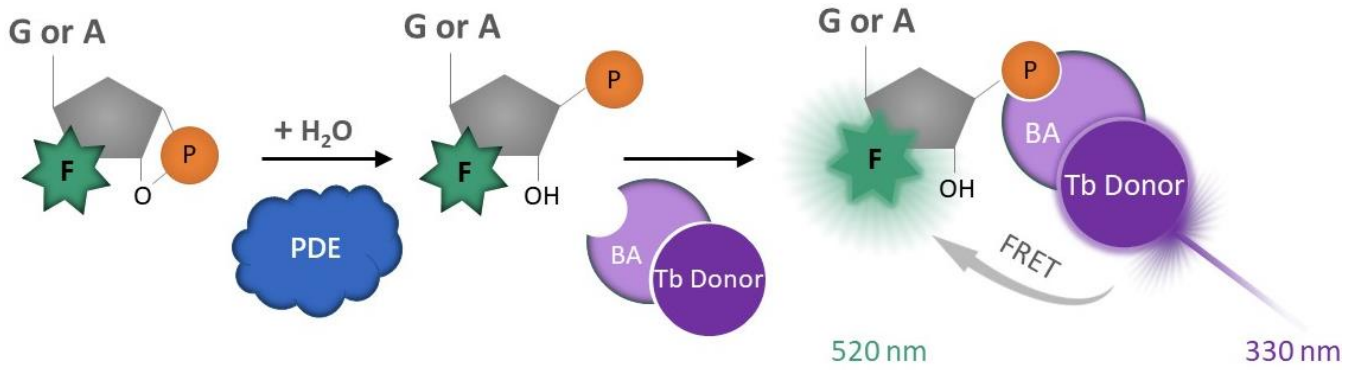

Figure 1: Illustration of the assay principle.

The reaction uses a fluorescein-conjugated (FAM) cyclic monophosphate nucleotide. Phosphodiesterase PDE3A 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 λ=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 PDE3A.

Background

Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cGMP signaling. PDE3A, also known as cGMP-inhibited phosphodiesterase, is activated by protein kinase A and B, and inhibited by cGMP. It has been implicated in cardiovascular functions by regulating the contractility of the heart muscle and vascular smooth muscle, and platelet aggregation. It has also been linked to fertility based on its expression in oocytes. Inhibitors for PDE3A and PDE3B showed some promise for cardiovascular disease treatment but can result in side effects such as arrhythmia. PDE3A has been found in lung and breast cancer, where it mediates cancer cell stemness by downregulating the cAMP/PKA pathway, and its level links to clinical prognosis. The use of PDE3A inhibitors results in reduction of breast cancer metastasis in animal models and may prove a powerful tool in cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
60032	PDE3A, (669-end), His-GST-Tags*	>1 µg	-80°C
60200	20 µM FAM-Cyclic-3', 5'-AMP	50 µl	-80°C
60393	PDE Assay Buffer	25 ml	-20°C
60394	Tb Donor	50 µl	-80°C
60390	Binding Agent	200 µl	+4°C
78422	Binding Buffer A	20 ml	+4°C
78423	Binding Buffer B	20 ml	+4°C
781209	Black 384-well plate	1	Room Temperature

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

Materials Required but Not Supplied

Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET).

Storage Conditions

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", "Positive Control", "Substrate Control" and "Test Inhibitor" conditions.

Step 1:

1. Dilute 20 µM FAM-Cyclic-3',5'-AMP 100-fold with PDE Assay Buffer to make a 200 nM solution.

2. Add 12.5 µl of diluted FAM-Cyclic-3',5'-AMP to each well designated "Substrate Control", "Positive Control" and "Test Inhibitor".

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

4. 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.

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

   For the positive and negative controls, use PDE Assay Buffer (Diluent Solution).

   4.2 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).

   The final concentration of DMSO should not exceed 1%.

5. Add 2.5 µl of inhibitor solution to each well designated “Test Inhibitor”.

6. Add 2.5 µl of Diluent Solution to the “Blank”, “Substrate Control” and “Positive Control” wells.

7. Thaw **PDE3A** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

   Note: PDE3A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

8. Dilute PDE3A in PDE Assay Buffer to 0.025 ng/µl (10 µl/well).

9. Add 10 µl of PDE Assay Buffer to the wells designated as "Substrate Control”.

10. Initiate the reaction by adding 10 µl of diluted PDE3A to the “Positive Control” and “Test Inhibitor”. Discard any remaining diluted enzyme after use.

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

Component	**Blank**	**Substrate Control**	**Positive Control**	**Test Inhibitor**
Diluted 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 PDE3A (0.025 ng/µl)	-	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Step 2:

1. Prepare **Binding Dilution Buffer** by mixing equal volumes of **Binding Buffer A** and **Binding Buffer B**.
2. Mix **Binding Agent** thoroughly.
3. Dilute Binding Agent 1:50 with Binding Dilution Buffer.
4. Dilute the Tb Donor 1:200 in the diluted Binding Agent solution.
5. Add 100 µl 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
Emission Wavelength	490±10
Lag Time	50 µs
Integration Time	50 µs
Excitation Wavelength	330±20
Emission Wavelength	520±10
Lag Time	50 µs
Integration Time	50 µs

CALCULATING RESULTS: Calculate the FRET value by using the following formula:

$$FRET = \frac{S_{520} - \left(\frac{Tb_{520}}{Tb_{490}} \times S_{490}\right)}{S_{490}} \times 1000$$

S_{520} = Sample value measured at 520 nm, S_{490} = Sample value measured at 490 nm, Tb_{520} = Tb only or Blank value measured at 520 nm, Tb490 = Tb only or Blank value measured at 490 nm.

The FRET value calculated for the “Substrate Control” should be subtracted from all other measurements and can be set as 0%. The FRET value from the “Positive Control” can be set as 100% activity.

The percent of activity (% Activity) can be measured as follows:

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

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{Sub}$ = FRET value for the Substrate Control, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

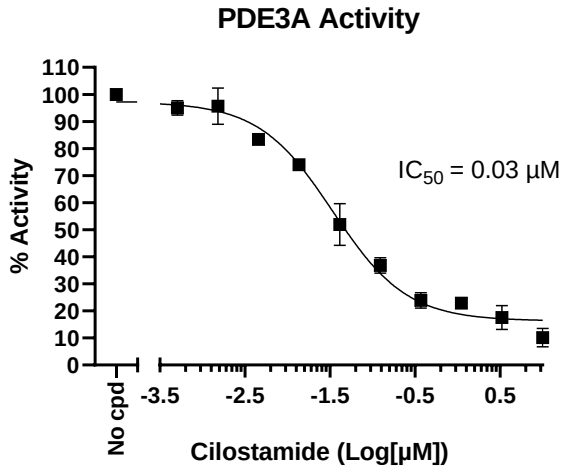

Figure 1: Inhibition of PDE3A activity by Cilostamide.
PDE3A activity was measured in the presence of increasing concentrations of Cilostamide (MedChemExpress #HY-101312). The "Substrate Control" value was subtracted from all other values. Results are expressed as the percentage of activity (in which activity in the absence of inhibitor is set at 100%).

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

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

Related Products

Products	*Catalog #*	*Size*
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