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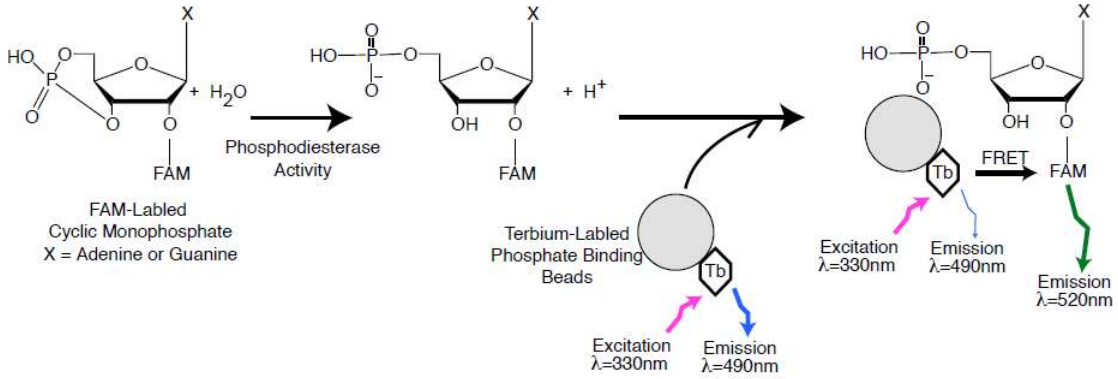
Data Sheet
PDE4D3 TR-FRET Assay Kit
Catalog # 60701

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE4D is a regulator of airway smooth-muscle contractility, and has been identified as a potential risk predictor for ischemic stroke. Additionally, PDE4D has been associated with asthma pathophysiology and bone formation. The PDE4D gene encodes at least 9 different isoforms.

The *PDE4D3 TR-FRET Assay Kit* is designed for identification of inhibitors of PDE4D3 using TR-FRET (Time Resolved Fluorescence Resonance Energy Transfer) technology. The assay is based on the generation of FAM-labeled nucleotide monophosphates by the phosphodiesterase. These phosphate groups bind to terbium-labeled nanoparticles, resulting in energy transfer from the terbium to the FAM, which emits a fluorescent signal at 520 nm. The change in fluorescent intensity can be easily measured using a fluorescence plate reader.



The *PDE4D3 TR-FRET Assay Kit* comes in a convenient 96-well format, with purified PDE4D3 enzyme, fluorescently labeled PDE substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. Using this kit, only two simple steps on a microtiter plate are required for the PDE4D3 activity assay. First, the fluorescent-labeled cAMP is incubated with a sample containing PDE4D3 for 1 hour. Second, a binding agent and a terbium donor are added to the reaction mix and incubated for 1 hour. Then, fluorescence intensity can be measured using a fluorescence reader.

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: 6 months from date of receipt when stored as directed.

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COMPONENTS:

Catalog #	Component	Amount	Storage	
60046	PDE4D3 recombinant enzyme	1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3′, 5′-AMP: 20 µM	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
	Tb donor	30 µl	-80°C	
60390	Binding Agent	200 µl	+4°C	
	Binding Buffer A	20 ml	+4°C	
	Binding Buffer B	20 ml	+4°C	
79685	Black, low binding NUNC microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

REFERENCE(S): Bender A., *et al., Pharmacol. Rev.* 2006; **58:** 488-520.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Protocol for PDE4D3 assay

Step 1:

1) Dilute 20 µM FAM-Cyclic-3',5'-AMP substrate stock solution 100-fold with PDE buffer to make a 200 nM solution. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

2) Add 25 µl of FAM-Cyclic-3',5'-AMP (200 nM) to each well designated "Substrate Control", "Positive Control", and "Test Inhibitor". Add 25 µl of PDE assay buffer to each well designated "Tb-only Control ".

3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". Add 5 µl of the same solution without inhibitor (inhibitor buffer) to the "Tb-only Control", "Substrate Control" and "Positive Control".

4) Thaw PDE4D3 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE4D3 enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80 °C immediately. *Note: PDE4D3 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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5) Dilute PDE4D3 in PDE buffer to 3 pg/µl (60 pg/reaction) in PDE buffer*. Add 20 µl of PDE assay buffer to the wells designated as the "Tb-only Control" and "Substrate Control". Initiate reaction by adding 20 µl of PDE4D3 (3 pg/µl) to the wells designated for the "Positive Control" and "Test Inhibitor". Discard any remaining diluted enzyme after use. **Note: optimal enzyme concentration may vary with the specific activity of the enzyme*.

	Tb only Control	Substrate Control	Positive Control	Test Inhibitor
FAM-Cyclic-3',5'-AMP (200 nM)	–	25 µl	25 µl	25 µl
PDE assay buffer	45 µl	20 µl	–	–
Test Inhibitor	–	–	–	5 µl
Inhibitor Buffer (no inhibitor)	5 µl	5 µl	5 µl	–
PDE4D3 (3 pg/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

6) Incubate at room temperature for 1 hour.

Step 2:

1) Make binding dilution buffer by mixing equal volumes of Binding buffer A and Binding buffer B. For example, mix 1 ml Binding buffer A with 1 ml Binding buffer B.
2) Mix **binding agent** thoroughly and dilute **binding agent** 1:50 with binding dilution buffer made in Step 1.
3) Add Tb donor (1:1,000 dilution) to the mixture in Step 2.
4) Add 100 µl to each well. Incubate at room temperature for 1 hour with slow shaking.
5) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

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

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CALCULATING RESULTS:

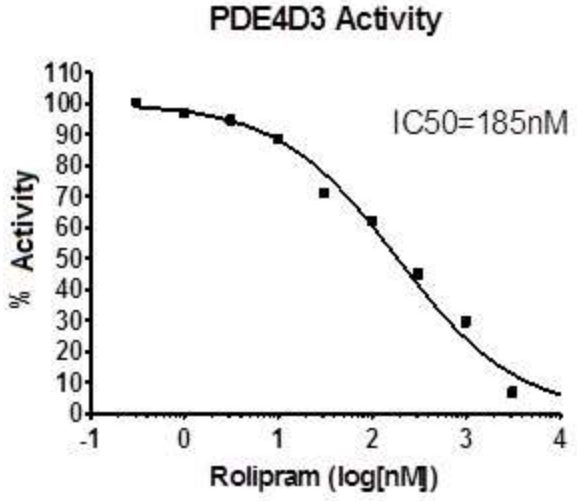
$$FRET = \frac{S_{520} - \left(\frac{Tb_{520}}{Tb_{490}} \times S_{490}\right)}{S_{490}} \times 1000$$

Where S_{520} = Sample 520 nm reading, S_{490} = Sample 490 nm reading, Tb_{520} = Tb only 520 nm reading, Tb_{490} = Tb only 490 nm reading. When percentage activity is calculated, the FRET value from substrate only control can be set as zero percent activity and the FRET value from positive control can be set as one hundred percent activity.

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

Where $FRET_s$ = Sample FRET, $FRET_{Sub}$ = Substrate only control FRET, and $FRET_P$ = Positive control FRET.

EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE4D3 by Rolipram, measured using the *PDE4D3 TR-FRET Assay Kit*, BPS Bioscience # 60701. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS :

Product	Cat. #	Size
PDE4D2	60048	5 µg
PDE4D3	60046	5 µg
PDE4D7	60047	5 µg
PDE4A1A	60040	10 µg
PDE4A10	60038	10 µg
PDE4A4B	60039	10 µg
PDE4B1	60041	10 µg
PDE4B2	60042	10 µg
PDE4C1	60044	5 µg
PDE FP Assay Kit	60300	96 rxns.
PDE4A FP Assay Kit	60340	96 rxns.
PDE4B FP Assay Kit	60343	96 rxns.
PDE4D3 FP Assay Kit	60345	96 rxns.

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