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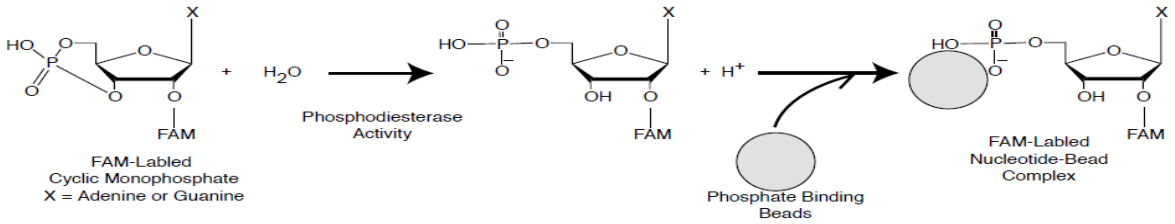
Data Sheet

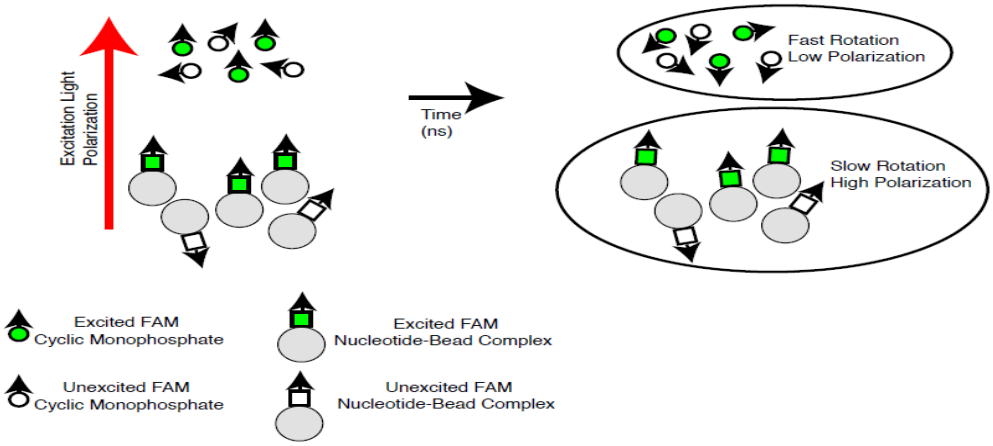
PDE4D3 Assay Kit

Catalog # 60346
Size: 96 reactions

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 Assay Kit* is designed for identification of inhibitors of PDE4D3 using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE4D3 to the binding agent.

Phosphodiesterases catalyze the hydrolysis of the phosphodiester bond in dye-labeled cyclic monophosphates. Beads selectively bind the phosphate group in the nucleotide product. This increases the size of the nucleotide relative to unreacted cyclic monophosphate. In the polarization assay, dye molecules with absorption transition vectors parallel to the linearly-polarized excitation light are selectively excited. Dyes attached to the rapidly-rotating cyclic monophosphates will obtain random orientations and emit light with low polarization. Dyes attached to the slowly-rotating nucleotide-bead complexes will not have time to reorient and therefore will emit highly polarized light.





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The *PDE4D3 Assay Kit* comes in a convenient 96-well format, with purified PDE4D3 enzyme, fluorescently labeled PDE4D substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE4D3 Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE4D3 reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE4D3 for 1 hour. Second, a binding agent is added to the reaction mix to produce a change in fluorescent polarization that can then be measured using a fluorescence reader equipped for the measurement of fluorescence polarization.

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	
60390	Binding Agent	100 µl	+4°C	
60391	Binding Agent Diluent (cAMP)	10 ml	+4°C	
	Black, low binding, microtiter plate	1	Room temp.	

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

REFERENCE: Chandrasekaran A, *et al., Cell Signal.* 2008; **20(1):** 139-53.

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

Step 1:

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

2) Add 25 µl of FAM-Cyclic-3´,5´-AMP (200 nM) to each well designated “Positive Control”, “Test Inhibitor”, and “Substrate Control”.

3) Add 20 µl of PDE assay buffer to each well designated “Substrate Control" and 45 µl of PDE assay buffer to each well designated “Blank”.

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4) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the wells labeled "Positive Control", "Substrate Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

5) 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 -70°C immediately. *Note: PDE4D3 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE4D3 in PDE buffer to 2.5 pg/µl (0.05 ng/reaction)*. Initiate reaction by adding 20 µl of diluted PDE4D3 to the wells designated "Positive Control" and "Test Inhibitor." Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: Optimal enzyme concentration may vary with the specific activity of the enzyme.*

7) Incubate at room temperature for 1 hour.

	Positive Control	**Test Inhibitor**	**Substrate Control**	**"Blank" Negative Control**
FAM-Cyclic-3',5'-AMP (200 nM)	25 µl	25 µl	25 µl	–
PDE assay buffer	–	–	20 µl	45 µl
Inhibitor (in PDE assay buffer)	–	5 µl	–	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl	5 µl
PDE4D3 (2.5 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

1) Mix **binding agent** thoroughly and dilute **binding agent** 1:100 with binding agent diluent.

2) Add 100 µl diluted binding agent to each microwell. Incubate at room temperature for 1 hour with slow shaking.

3) Read the fluorescent polarization of the sample in a microtiter-plate reader equipped for the measurement of fluorescence polarization, capable of excitation at wavelengths ranging from 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

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CALCULATING RESULTS:

Definition of Fluorescence Polarization

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular.

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$FP(measured) = \frac{([I_{\parallel}]-G*[I_{\perp}])}{([I_{\parallel}]+G*[I_{\perp}])} * 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

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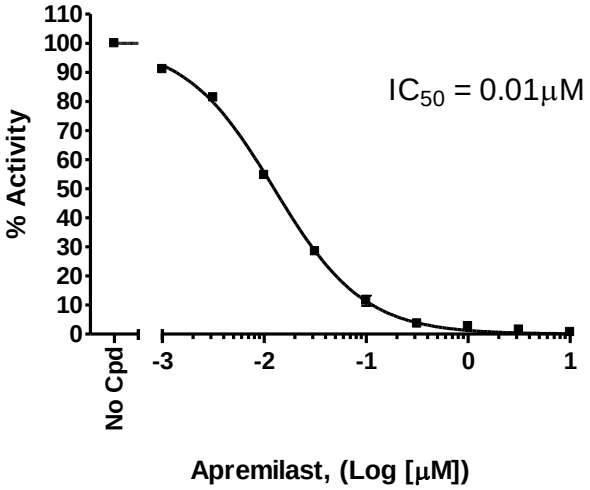
EXAMPLE OF ASSAY RESULTS:

PDE4D3 Activity

Substrate Conc. = 100nM (cAMP)



Inhibition of PDE4D3 by Apremilast, measured using the *PDE4D3 Assay Kit*, BPS Bioscience # 60346. Fluorescence polarization was measured at 528 nm using a Tecan M1000 fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS :

Product Name	Catalog #	Size
PDE4D2	60048	5 µg
PDE4D3	60046	5 µg
PDE4D7	60047	5 µg
PDE Assay Kit	60300	96 rxns.
PDE4D2 Assay Kit	60345	96 rxns.
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.
PDE4D2 TR-FRET Assay Kit	60707	96 rxns.
PDE4D3 TR-FRET Assay Kit	60701	96 rxns.
PDE4D7 TR-FRET Assay Kit	60708	96 rxns.

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