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Data Sheet

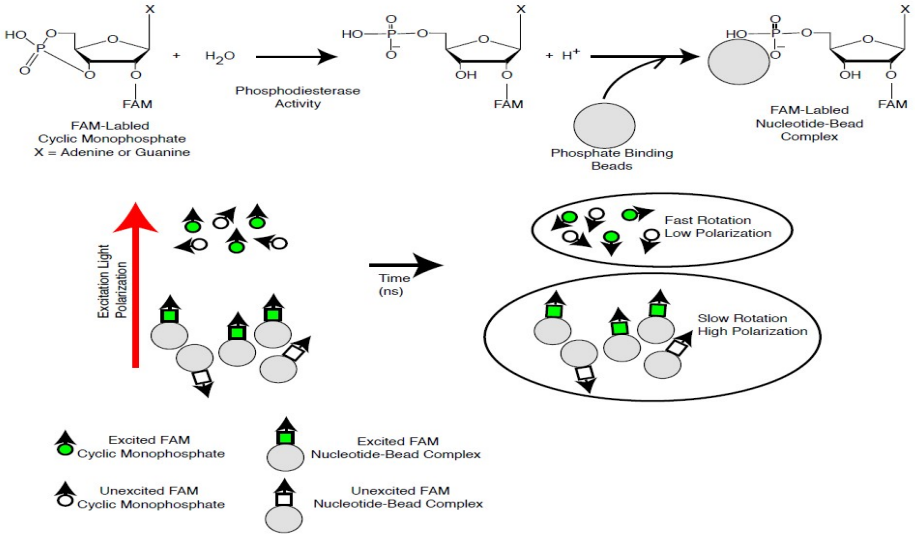
Rat PDE4B Assay Kit

Catalog #79571
Size: 96 reactions

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE4 selective inhibitors are currently in clinical trials for the treatment of diseases related to inflammatory disorders. Altered activity of PDE4B has been associated with schizophrenia and bipolar disorder. It has also recently been found to modulate cognition, as reduction in PDE4B activity improves memory and long-term plasticity in mouse models.

The Rat PDE4B Assay Kit is designed for identification of inhibitors of rat PDE4B (rPDE4B) using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by rPDE4B 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 *Rat PDE4B Assay Kit* comes in a convenient 96-well format, with purified rPDE4B enzyme, fluorescently labeled rPDE4B substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *Rat PDE4B Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for rPDE4B reactions. First, the fluorescently labeled cAMP is incubated with a sample containing rPDE4B 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	
60049	Rat PDE4B 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	
79685	Black, low binding, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable to measure fluorescence polarization.
Adjustable micropipettor and sterile tips.
1,4-Dithiothreitol (DTT).

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

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

REFERENCES:
1. Clapcote SJ, *et al.*, *Adv Neurobiol.* 2017;**17**:103-131.
2. Braun, N.N., *et al.*, *NeuroReport.* 2007; Nov; **18**(17):1841-1844.

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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 assay 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) Dilute 1 M 1,4-Dithiothreitol (DTT) 1:500 into the diluted **FAM-Cyclic-3´,5´-AMP**. For example, add 10 µl DTT (1 M) to 5 ml of diluted FAM-Cyclic-3´, 5´-AMP (200 nM).

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

4) Add 45 µl of **PDE assay buffer** to each well designated "Blank" and add 20 µl of **PDE assay buffer** to each well designated "Substrate Control."

5) 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).

	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
rPDE4B (1 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

6) Thaw **rPDE4B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **rPDE4B** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: rPDE4B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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7) Dilute **rPDE4B** in **PDE assay buffer** to 1 pg/µl (0.02 ng/reaction)*. Initiate reaction by adding 20 µl of **rPDE4B** (1 pg/µl) to the wells designated "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.*

8) Incubate at room temperature for 1 hour.

Step 2:

1) Mix **Binding Agent** thoroughly and dilute **Binding Agent** 1:100 with **Binding Agent Diluent**.

2) Add 100 µl of diluted **Binding Agent** to each microwell. Incubate at room temperature for 30 minutes 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 485 ± 5 nm and detection of emitted light ranging from 528 ± 10 nm. Blank value is subtracted from all other values.

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".

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

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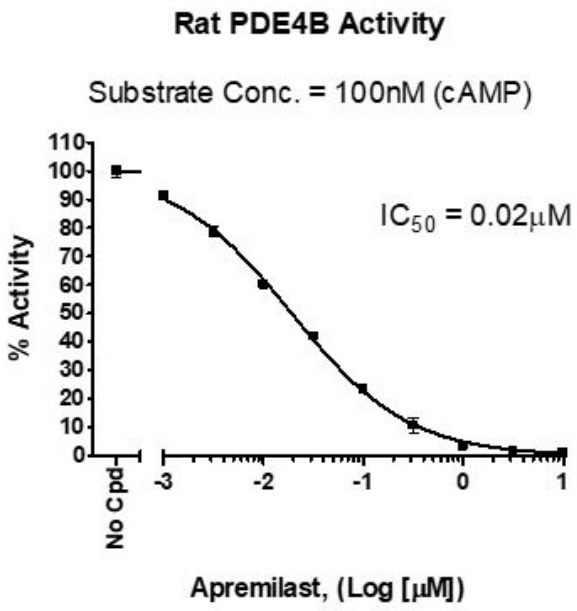
The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

EXAMPLE OF ASSAY RESULTS:



Inhibition of rPDE4B by Apremilast measured using the r*PDE4B Assay Kit*, BPS Bioscience #79571. 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*

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RELATED PRODUCTS :

Product Name	Catalog #	Size
PDE4A1A	60040	10 µg
PDE4B1	60041	10 µg
PDE4B2	60042	5 µg
Dog PDE4B	60055	5 µg
Rat PDE4B	60049	5 µg
PDE4C1	60044	5 µg
PDE4D2	60048	5 µg
PDE4D3	60046	5 µg
PDE4D7	60047	5 µg
PDE Assay Kit	60300	96 rxns.
PDE4B2 Assay Kit	60343	96 rxns.
PDE4A1A Assay Kit	60340	96 rxns.
PDE4D2 Assay Kit	60345	96 rxns.
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.

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