6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

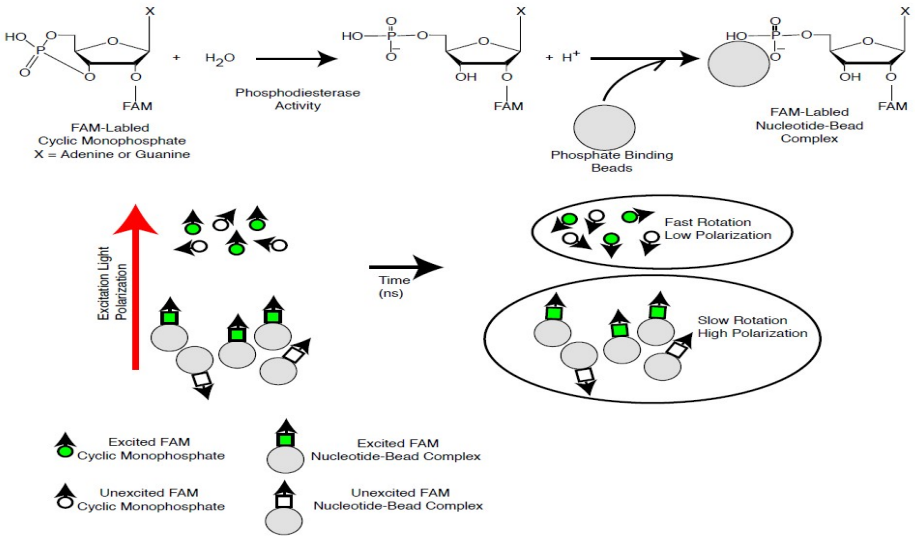
Rat PDE1B Assay Kit

Catalog #79662
Size: 96 reactions

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cAMP signaling. Rat PDE1B, also known as calmodulin-dependent phosphodiesterase, is a dual specificity phosphodiesterase has been implicated in allergy, asthma, hypertension, and schizophrenia. PDE1B plays an important role in T cell activation and survival, and that PDE1B-targeted therapy might prove beneficial in leukemias and inflammatory disorders.

The Rat PDE1B Assay Kit is designed for identification of inhibitors of Rat PDE1B using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by Rat PDE1B 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 PDE1B *Assay Kit* comes in a convenient 96-well format, with purified Rat PDE1B enzyme, fluorescently labeled substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *Rat PDE1B Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for Rat PDE1B reactions. First, the fluorescently labeled cAMP is incubated with a sample containing Rat PDE1B 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	
60009	Rat PDE1B 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.	

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

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) Wenogle, L.P., *et al*. Phosphodiesterase 1: A Unique Drug Target for Degenerative Diseases and Cognitive Dysfunction. *Adv Neurobiol.* 2017;**17:**349-384. **53(1):** 113-24.

2) Reed T., Repaske D., *et al.* Phosphodiesterase 1B Knock-Out Mice Exhibit Exaggerated Locomotor Hyperactivity and DARPP-32 Phosphorylation in Response to Dopamine Agonists and Display Impaired Spatial Learning, *J. Neuroscience* 15 June 2002, **22 (12):**5188-5197.

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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 1M 1,4-Dithiothreitol (DTT) 1:500 into the diluted **FAM-Cyclic-3´,5´-AMP**. For example, add 10 µl DTT (1M) to 5 ml of diluted FAM-Cyclic-3´, 5´-GMP (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
Rat PDE1B (50 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

6) Thaw **Rat PDE1B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **Rat PDE1B** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: Rat PDE1B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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7) Dilute **Rat PDE1B** in **PDE assay buffer** to 50 pg/µl (1 ng/reaction)*. Initiate reaction by adding 20 µl of **Rat PDE1B** (50 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 the plate 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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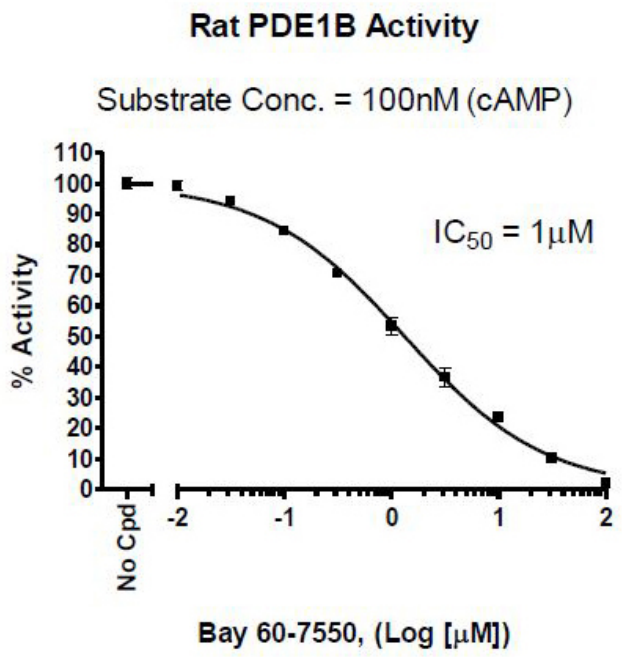
The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

EXAMPLE OF ASSAY RESULTS:



Inhibition of Rat PDE1B by Bay 60-7550, measured using the *Rat PDE1B Assay Kit*, BPS Bioscience #79662. 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
RAT PDE1B	60009	10 µg
Rat PDE2A	60022	5 µg
Rat PDE4B	60049	5 µg
Rat PDE4D	60054	5 µg
Rat PDE7A	60074	10 µg
Rat PDE7B	60075	10 µg
Rat PDE10A	60102	5 µg
Mouse PDE1C	60012	10 µg
Mouse PDE3a	60036	5 µg
Mouse PDE2a V1	60017	5 µg
Rat PDE4B Assay Kit	79571	96 rxns.
Rat PDE7A Assay Kit	79634	96 rxns.
rPDE7A-HEK293 Recombinant Cell Line	60408	2 vials
Mouse PDE3A1 Assay Kit	79606	96 rxns.
Mouse PDE5A1 Assay Kit	79602	96 rxns.