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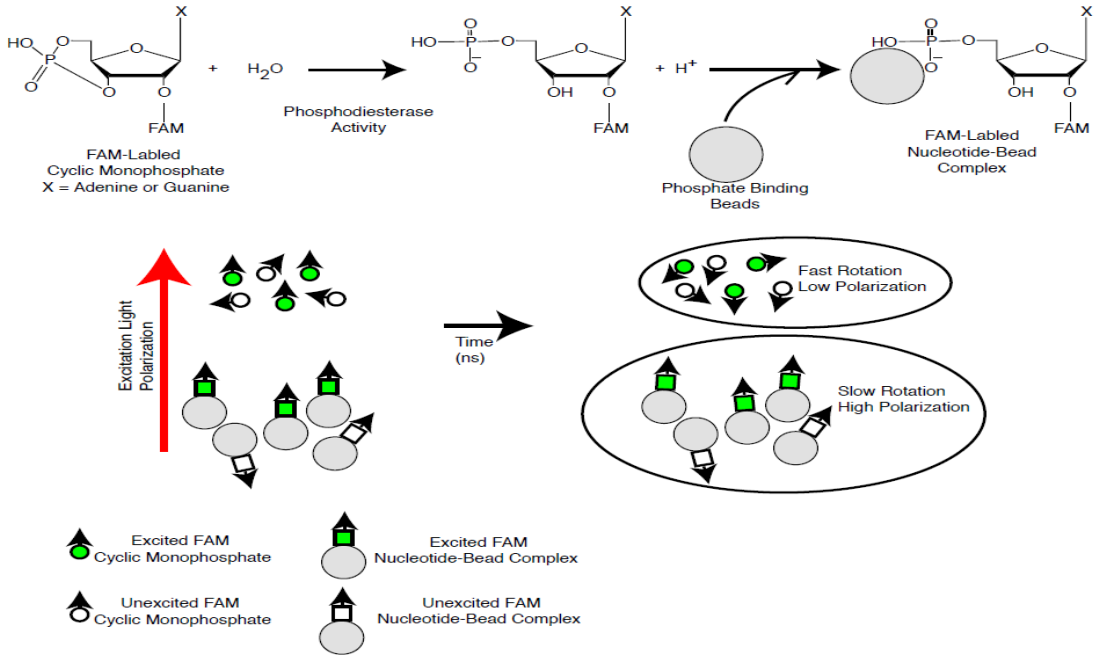
Data Sheet

PDE1B Assay Kit

Catalog # 60311

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE1B is a calcium-dependent cyclic nucleotide phosphodiesterase that is highly expressed in the striatum. It plays a physiological role in the central nervous system, and PDE1B activity has been linked to impaired cognition and spatial learning. The PDE1B Assay Kit is designed for identification of PDE1B inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by 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 PDE1B inhibitor screening assay kit comes in a convenient 96-well format, including purified PDE1B enzyme, fluorescently labeled PDE1B substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the PDE1B Assay Kit is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE1B reactions. First, the fluorescently labeled cAMP is incubated with a sample containing 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	
60011	PDE1B recombinant enzyme	1 µg	-70°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3′, 5′-AMP: 20 µM	50 µl	-70°C	
	PDE assay buffer	25 ml	-20°C	
	Binding Agent	100 µl	+4°C	
	Binding Agent Diluent	10 ml	+4°C	
VWR, 62408-936	Black, low binding NUNC microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring fluorescence polarization

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

REFERENCE: Siuciak JA, et al. (2007) *Neuropharmacology* **53(1):**113-24.

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

Step 1:

1) Dilute cAMP 20 µM 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 "Substrate Control", "Positive Control", and "Test Inhibitor". Add 25 µl of PDE assay buffer to each well designated "Blank".

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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 "Blank", "Substrate Control" and "Positive Control".

4) Add 20 µl of PDE assay buffer to the wells designated as the "Blank" and "Substrate Control".

5) Thaw PDE1B on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE1B enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: PDE1B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE1B in PDE buffer to 0.15 ng/µl (3 ng/reaction)*. Keep diluted enzyme on ice. Initiate reaction by adding 20 µl of diluted PDE1B 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.*

7) In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at room temperature for 1 hour.

	Blank	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	–
PDE1B (0.15 ng/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

1) Shake the tube containing the binding agent to ensure it is thoroughly mixed. 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.

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3) Read the fluorescent polarization of the sample in a microtiter-plate reader 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.

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. G= 0.87 for the Bio-Tek “Synergy 2” microplate reader used in our facilities.

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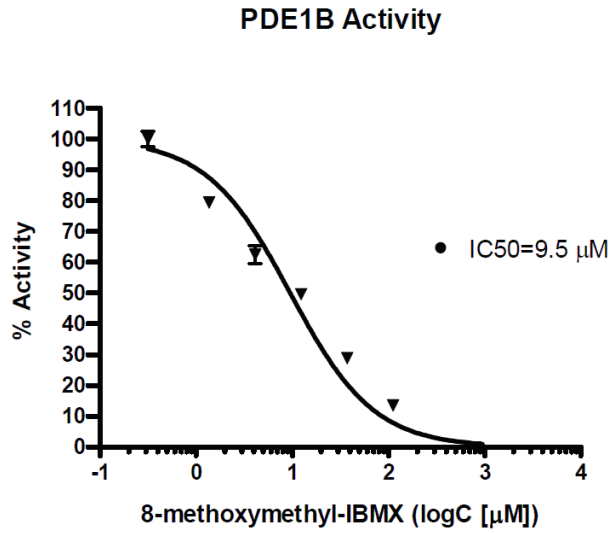
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE1B, Cat. #60011, by 8-methoxymethyl-IBMX, measured using the PDE1B Assay Kit, Cat. # 60311. Fluorescence polarization was measured at 528 nm using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS :

Product	Cat. #	Size
PDE1A	60010	10 µg
PDE1B	60011	10 µg
PDE1C	60012	10 µg
PDE Assay Kit	60300	100 rxns.
PDE2A Assay Kit	60320	100 rxns.
PDE3A Assay Kit	60330	100 rxns.
PDE3B Assay Kit	60331	100 rxns.
PDE4A Assay Kit	60340	100 rxns.
PDE4D Assay Kit	60345	100 rxns.
PDE10A Assay Kit	60400	100 rxns.