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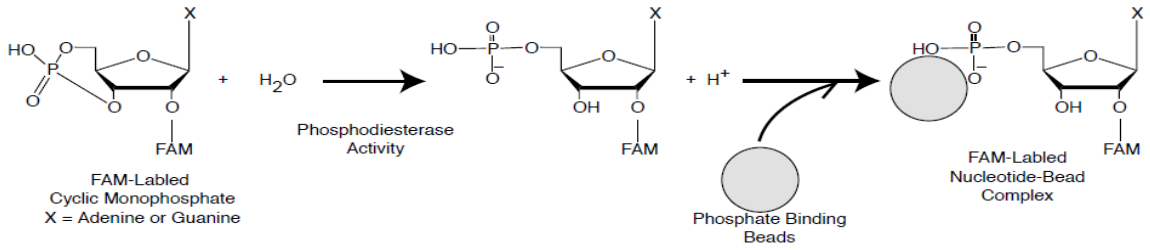
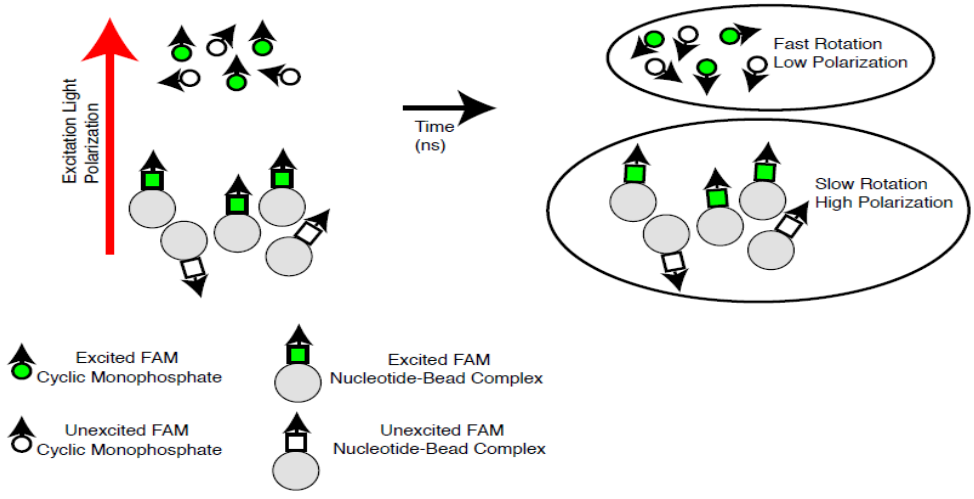
Data Sheet
PDE3B Assay Kit
Catalog # 60383

BACKGROUND: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE3B, also known as cGMP-inhibited phosphodiesterase, is involved in mediating the antilipolytic and anti glycogenlytic effects of insulin in adipose and liver tissues. PDE3B can hydrolyze both cAMP and cGMP.

DESCRIPTION: The PDE3B Assay Kit is designed for identification of PDE3B inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE3B 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 PDE3B inhibitor screening assay kit comes in a convenient 384-well format, including purified PDE3B enzyme, fluorescently labeled PDE3B substrate (cAMP), binding agent, and PDE assay buffer for 400 enzyme reactions. The key to the *PDE3B Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE3B reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE3B 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.

COMPONENTS:

Catalog #	Component	Amount	Storage	
60031	PDE3B 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	250 µl	+4°C	
60391	Binding Agent Diluent (cAMP)	25 ml	+4°C	
	Black, low binding, 384 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.

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

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** substrate stock solution 100-fold with **PDE Assay 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 12.5 µl of **FAM-Cyclic-3',5'-AMP** (200 nM) to each well designated "Substrate Control," "Positive Control," and "Test Inhibitor." Add 12.5 µl of **PDE Assay Buffer** to each well designated "Blank."

3) Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor." Add 2.5 µl of 10% DMSO in water (inhibitor buffer) to the wells labeled "Blank," "Substrate Control," and "Positive Control."

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4) Add 10 µl of **PDE Assay Buffer** to the wells designated as the "Blank" and "Substrate Control."

5) Thaw **PDE3B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **PDE3B** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PDE3B** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **PDE3B** in **PDE Assay Buffer** to 4 pg/µl (40 pg/reaction)*. Initiate reaction by adding 10 µl of diluted **PDE3B** 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) Incubate at room temperature for 1 hour.

	Blank	Substrate Control	Positive Control	Test Inhibitor
FAM-Cyclic-3',5'-AMP (200 nM)	–	12.5 µl	12.5 µl	12.5 µl
PDE assay buffer	22.5 µl	10 µl	–	–
Test Inhibitor	–	–	–	2.5 µl
10% DMSO in water (Inhibitor Buffer)	2.5 µl	2.5 µl	2.5 µl	–
PDE3B (4 pg/µl)	–	–	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Step 2:

1) Shake the tube containing the binding agent to ensure it is thoroughly mixed. Mix **binding agent** thoroughly and dilute **binding agent** 1:100 with the cAMP **Binding Agent Diluent .**

2) Add 50 µl diluted **Binding Agent** to each well. Incubate at room temperature for 20 minutes with slow shaking.

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

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

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

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

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".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

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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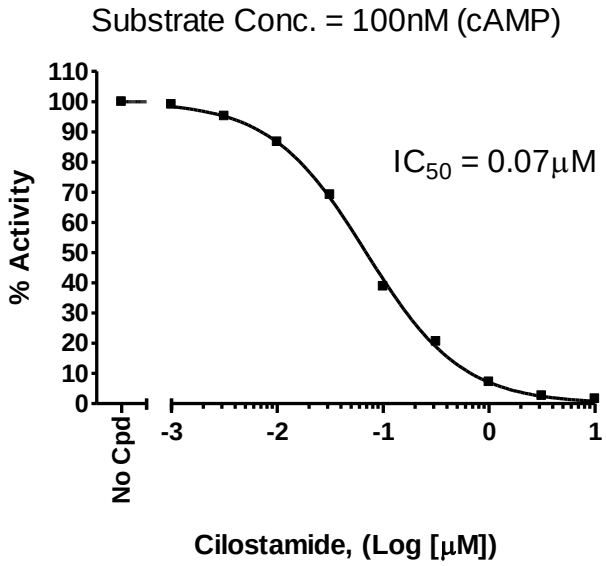
EXAMPLE OF ASSAY RESULTS:

PDE3B Activity



Inhibition of PDE3B by Cilostamide, measured using the *PDE3B Assay Kit*, BPS Bioscience #60383. Fluorescence polarization was measured at 528 nm using a Tecan M100 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	Catalog #	Size
PDE3B	60031	10 µg
PDE1A1	60010	10 µg
PDE1B	60011	10 µg
PDE2A1	60020	5 µg
PDE3A (484-end)	60030	10 µg
PDE3A (669-end)	60032	10 µg
PDE3A TR-FRET Assay Kit	60706	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE1A Assay Kit	60310	96 rxns.
PDE1B Assay Kit	60311	96 rxns.
PDE2A Assay Kit	60320	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cAMP Substrate	60200	100 nmole.

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