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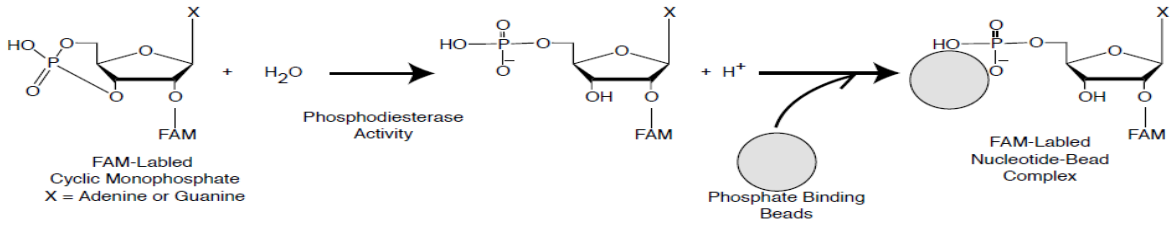
Data Sheet

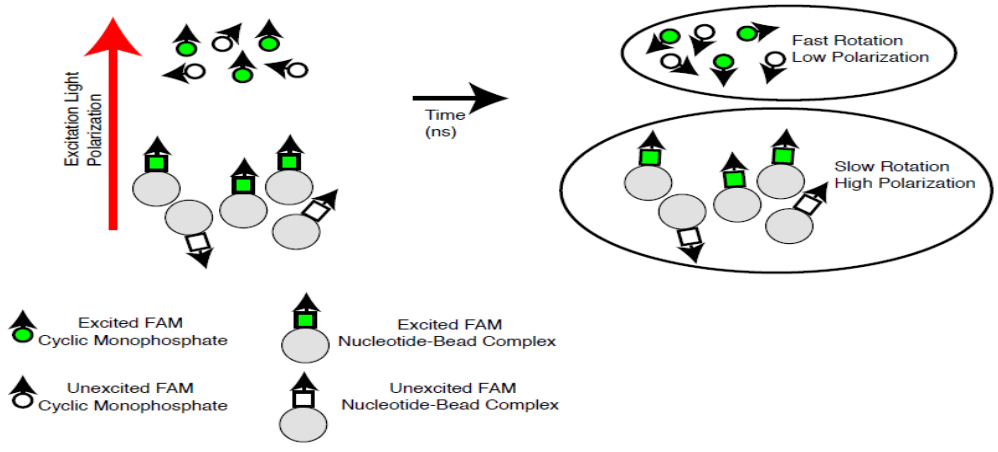
PDE3B Assay Kit

Catalog # w70342
Size: 96 reactions

DESCRIPTION: 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 antiglycogenlytic effects of insulin in adipose and liver tissues. 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 Assay Kit* comes in a convenient 96-well format, with purified PDE3B enzyme, fluorescently labeled PDE3B substrate (cAMP), binding agent, and PDE assay buffer for 100 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 equipped for the measurement of fluorescence polarization.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w70042	PDE3B recombinant enzyme	1 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
w70211	FAM-Cyclic-3´, 5´-AMP (20 µM)	50 µl	-80℃	
	PDE assay buffer	25 ml	-20℃	
	Binding Agent	100 µl	+4℃	
	Binding Agent Diluent	10 ml	+4℃	
	Black, low binding, 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 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℃.

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

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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”.

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 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 -70°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 buffer to 50 pg/µl (1 ng/reaction)*. Initiate reaction by adding 20 µl of diluted PDE3B 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.*

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
PDE3B (50 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

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

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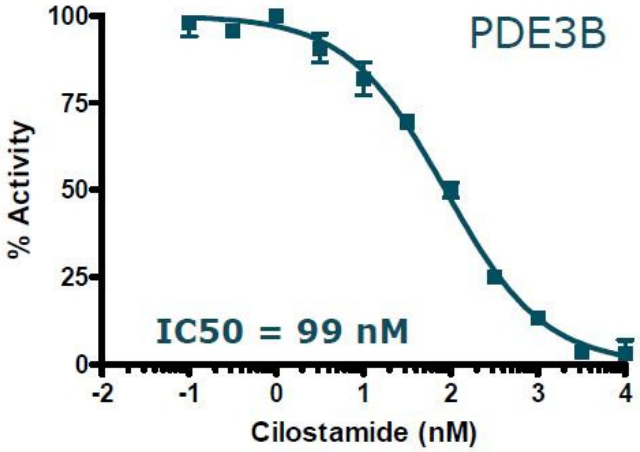
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE3B activity by cilostamide, measured using the PDE3B Assay Kit, West Bioscience # w70342. 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 West Bioscience, Inc. at sale@westbioscience.com*

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