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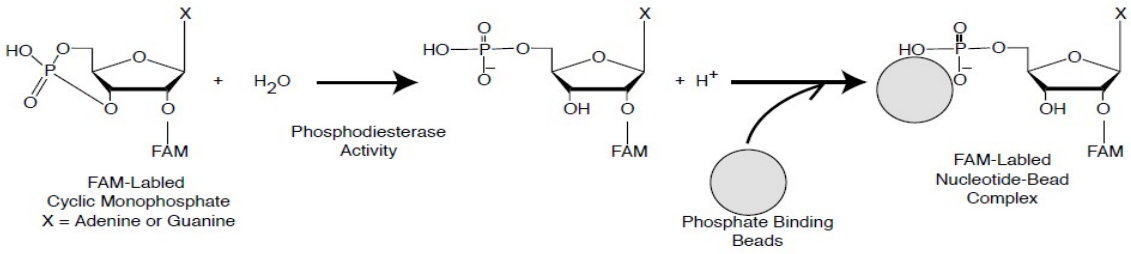
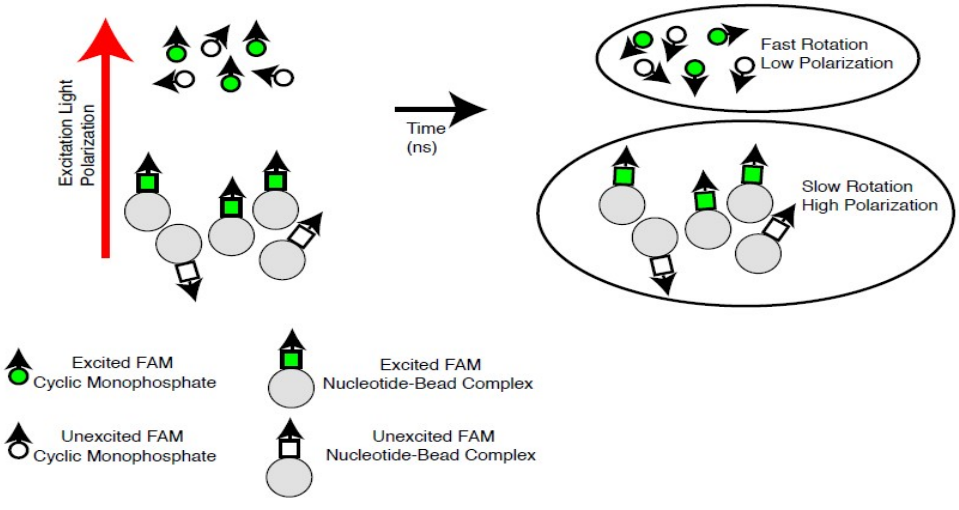
Data Sheet
PDE8A Assay Kit
Catalog # 60380

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE8 is involved in many biological processes, including T-cell activation, testosterone production, adrenocortical hyperplasia, and thyroid function. The *PDE8A Assay Kit* is designed for identification of PDE8A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE8A 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 PDE8A inhibitor screening assay kit comes in a convenient 96-well format, including purified PDE8A1 enzyme, fluorescently labeled PDE8A substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE8A Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE8A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE8A 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	
60080	PDE8A1 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 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(S): Wang, H., et al. *Biochemistry* 2008; **48:** 12760-8.

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 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 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 PDE8A1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE8A1 enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE8A1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE8A1 in PDE buffer to 2 pg/µl (40 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE8A1 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)	–	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	–
PDE8A1 (2 pg/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. Mix **binding agent** thoroughly and dilute **binding agent** 1:100 with the cAMP binding agent diluent**.**

2) Add 100 µl diluted binding agent to each well. Incubate at room temperature for 30 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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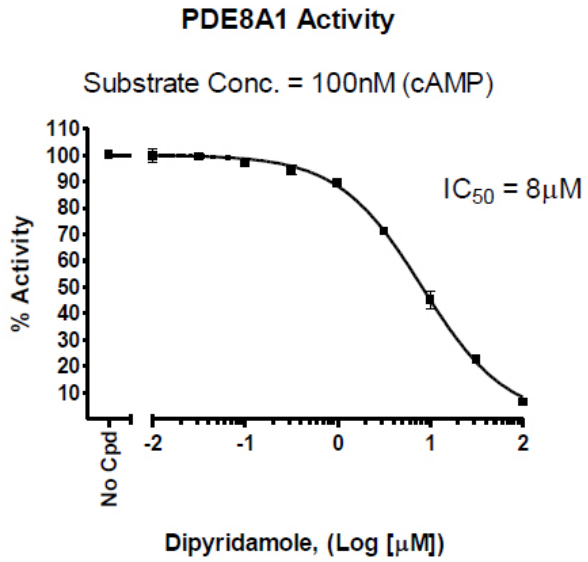
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE8A1 by Dipyridamole, measured using the *PDE8A Assay Kit*, BPS Bioscience # 60380. Fluorescence polarization was measured at 528 nm using a Tecan M-1000 fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS :

Product	Catalog #	Size
PDE8A1	60080	10 µg
PDE4A1A	60040	10 µg
PDE4B1	60041	10 µg
PDE7A	60070	10 µg
PDE7B	60071	10 µg
PDE1B Assay Kit	60311	96 rxns.
PDE2A Assay Kit	60320	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE5A Assay Kit	60350	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cAMP Substrate	60200	100 nmole.

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