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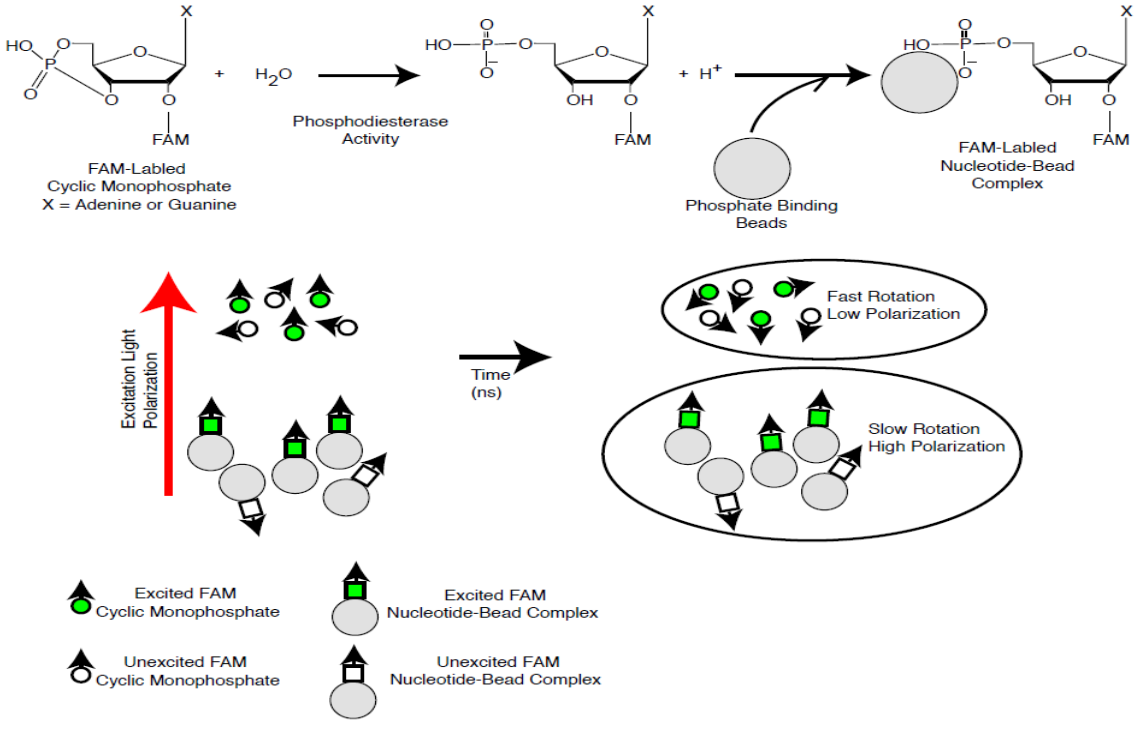
Data Sheet
PDE9A2 Assay Kit
Catalog # 60381

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE9A2 is primarily expressed in spleen, small intestine, and brain. PDE9 inhibitors have been studied as therapeutics for treatment of cardiovascular diseases, diabetes, and neurodegenerative disorders. The *PDE9A2 Assay Kit* is designed for identification of PDE9A2 inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE9A2 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 PDE9A2 inhibitor screening assay kit comes in a convenient 96-well format, including purified PDE9A2 enzyme, fluorescently labeled PDE9 substrate (cGMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE9A2 Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE9A2 reactions. First, the fluorescently labeled cGMP is incubated with a sample containing PDE9A2 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	
60090	PDE9A2 recombinant enzyme*	>1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60201	FAM-Cyclic-3′, 5′-GMP(20 µM)	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
60390	Binding Agent	100 µl	+4°C	
60392	Binding Agent Diluent (cGMP)	10 ml	+4°C	
79685	Black, low binding, microtiter plate	1	Room temp.	

*We have provided additional material in this vial for ease of retrieval.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring fluorescence polarization
Adjustable micropipettor and sterile tips
Inhibitor Buffer (inhibitor solution without inhibitor)

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. (2010) *J. Med Chem.* **53 (4)**: 1726-31.

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

Step 1:

1) Dilute **FAM-Cyclic-3',5'-GMP** (20 µM) substrate stock solution 100-fold with **PDE assay buffer** to make a **FAM-Cyclic-3′, 5′-GMP** (**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'-GMP (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 10% DMSO in water (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 **PDE9A2 recombinant enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **PDE9A2 recombinant enzyme** into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE9A2 recombinant enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **PDE9A2 recombinant enzyme** in **PDE assay buffer*** to 0.08 pg/µl (1.6 pg/reaction).. Initiate reaction by adding 20 µl of diluted **PDE9A2 recombinant enzyme** 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’-GMP (200 nM)	–	25 µl	25 µl	25 µl
PDE assay buffer	45 µl	20 µl	–	–
Test Inhibitor	–	–	–	5 µl
10% DMSO in water (Inhibitor buffer)	5 µl	5 µl	5 µl	–
PDE9A2 (0.08 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 **Binding Agent Diluent (cGMP) .**

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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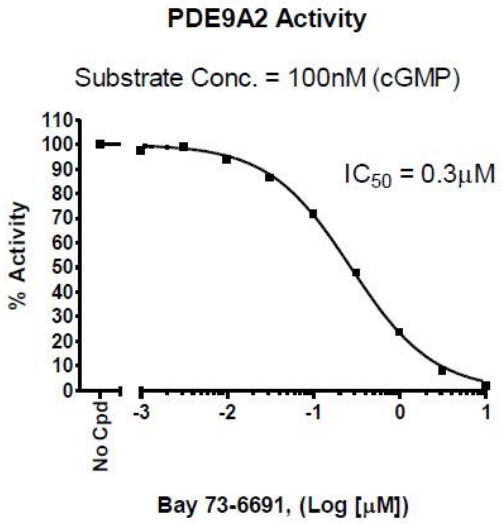
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE9A2 by Bay 73-6691, measured using the *PDE9A2 Assay Kit*, BPS Bioscience # 60381. 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*

RELATED PRODUCTS :

Product	**Catalog #**	**Size**
PDE9A2	60090	10 µg
PDE5A1	60050	10 µg
PDE6C	60060	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.
PDE7A Assay Kit	60370	96 rxns.
PDE7B Assay Kit	60371	96 rxns.
PDE8A Assay Kit	60380	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cGMP Substrate	60201	100 nmole.

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