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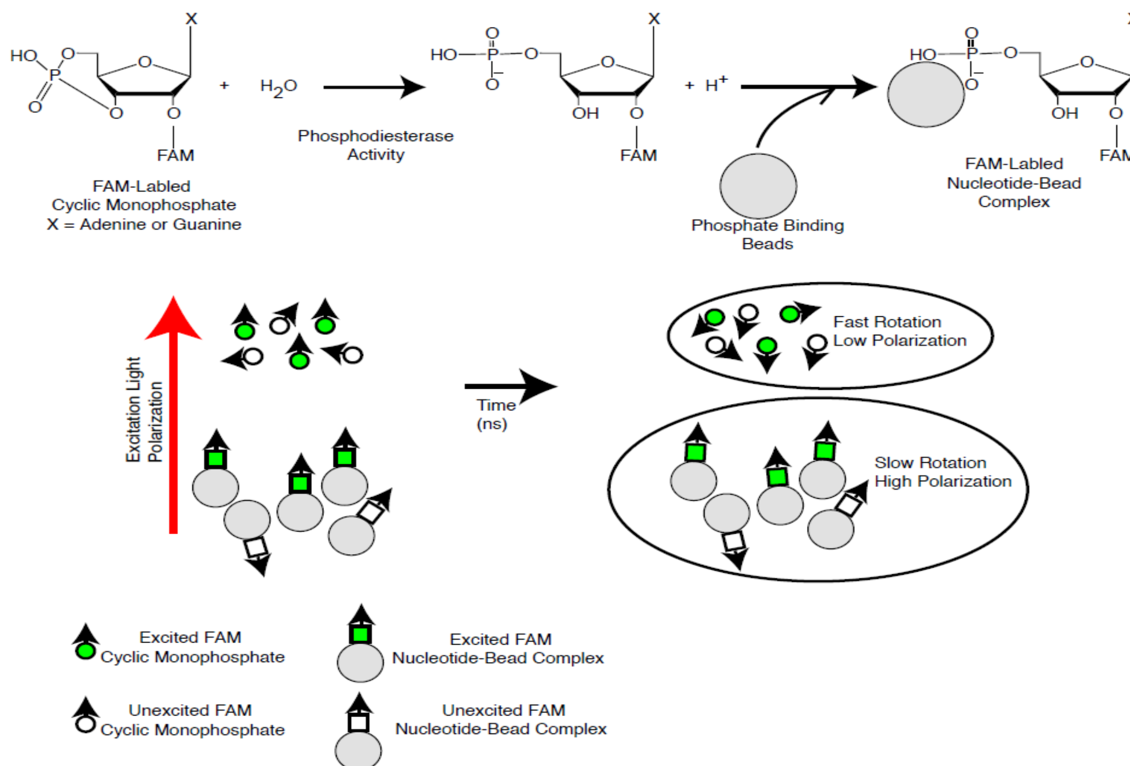
WEST BIOSCIENCE

Data Sheet
PDE Assay Kit
Catalog # w70311

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. The *PDE Assay Kit* is designed for identification of PDE inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDEs 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 PDE inhibitor screening assay kit comes in a convenient 96-well format, with fluorescently labeled substrate (cAMP and cGMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE reactions. First, the fluorescently labeled cAMP or cGMP is incubated with a sample containing PDE2A (cAMP-dependent) or PDE5A (cGMP-dependent) 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	
w70031	PDE2A recombinant enzyme	1 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
w70061	PDE5A	1 µg	-80℃	
	FAM-Cyclic-3′, 5′-AMP: 20 µM	50 µl	-80℃	
	FAM-Cyclic-3’, 5’-GMP: 20 µM	50 µl	-80℃	
	PDE assay buffer	25 ml	-20℃	
	Binding Agent	200 µl	+4℃	
	Binding Agent Diluent (cAMP)	10 ml	+4℃	
	Binding Agent Diluent (cGMP)	10 ml	+4℃	
	Black, low binding, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable to measure 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): Maurice DH. *Front. Biosci*. 2005; **10:**1221-8.

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

A. Protocol for PDE2A assay

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℃.

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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".

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 PDE2A on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE2A enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE2A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE2A in PDE buffer to 25 pg/µl (0.5 ng/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE2A 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	–
PDE2A (25 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. 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 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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B. Protocol for PDE5A assay

Step 1:

1) Dilute 20 µM FAM-Cyclic-3',5'-GMP substrate stock solution 100-fold with PDE buffer to make a 200 nM solution. Make only 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 for the "Substrate Control", "Positive Control", and "Test Inhibitor". Add 25 µl of PDE assay buffer to each well designated for the "Blank".

3) Add 5 µl of inhibitor solution of each well designated for "Test Inhibitor". Add 5 µl of the same solution without inhibitor (Inhibitor buffer), to the "Positive Control", "Substrate Control", and "Blank".

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

5) Thaw PDE5A on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of tube. Aliquot PDE5A enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE5A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE5A in PDE buffer to 6 pg/µl (120 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE5A 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
Inhibitor Buffer (no inhibitor)	5 µl	5 µl	5 µl	–
PDE5A (6 pg/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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Step 2:

1) Shake the tube containing the binding agent to ensure it is thoroughly mixed. Dilute binding agent 1:100 with the cGMP 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 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

C. 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".

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

OR

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

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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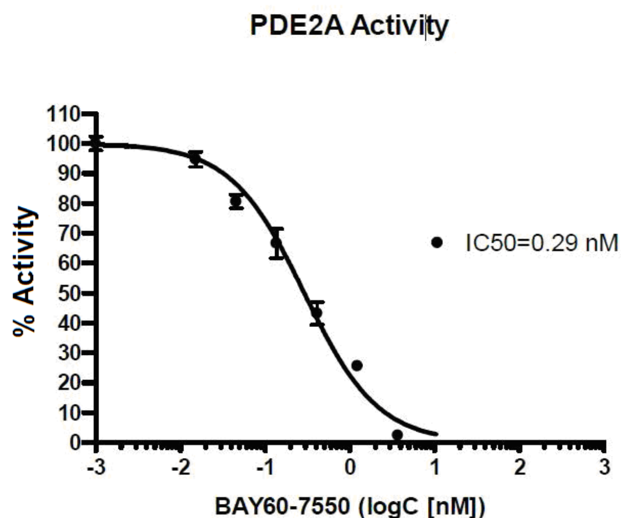
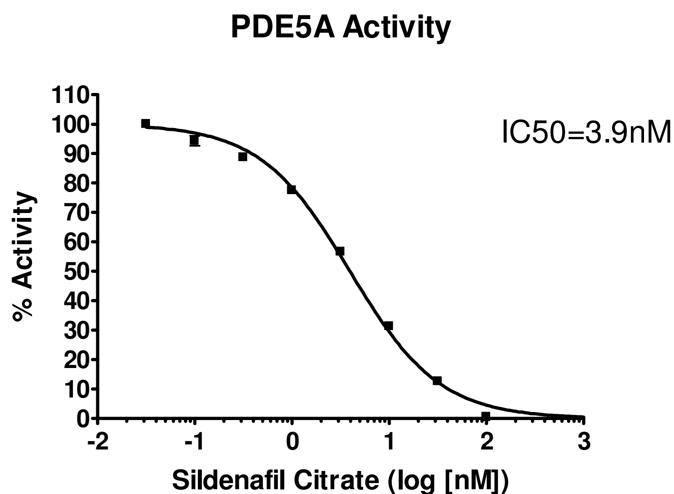
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE2A by BAY60-7550, measured using the *PDE Assay Kit*, West Bioscience # w70311. Fluorescence polarization was measured at 528 nm using a Bio-Tek fluorescent microplate reader.



Inhibition of PDE5A by Sildenafil Citrate, measured using the *PDE Assay Kit*, West Bioscience # w70311. 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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