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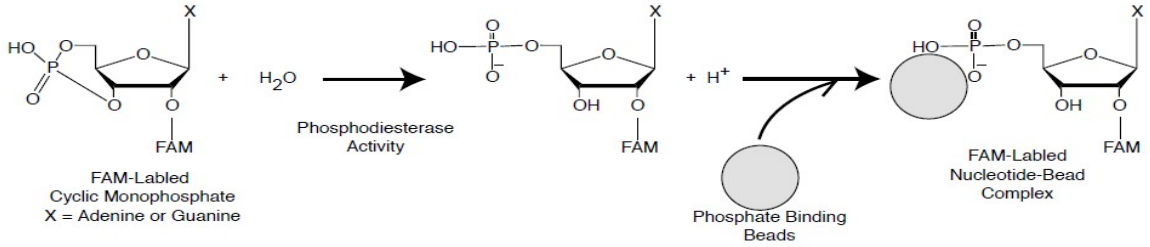
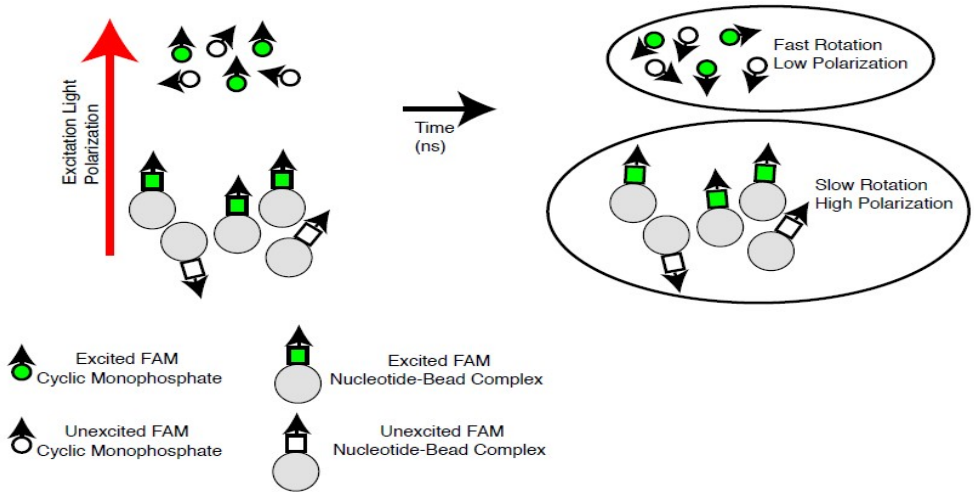
Data Sheet
PDE5A1 Assay Kit
Catalog #60351

BACKGROUND: Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cGMP signaling. PDE5A, also known as cGMP-inhibited phosphodiesterase, has been implicated in cardiovascular function and fertility.

DESCRIPTION: The PDE5A1 Assay Kit is designed for identification of PDE5A1 inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE5A to the binding agent. PDE5A catalyzes the hydrolysis of the phosphodiester bond in dye-labeled cyclic guanosine monophosphate. Nanoparticle beads selectively bind the phosphate group in the nucleotide product. This increases the size of the nucleotide relative to unreacted cyclic guanosine 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 PDE5A1 inhibitor screening assay kit comes in a convenient 384-well format, including purified PDE5A1 enzyme, fluorescently labeled PDE5A1 substrate (cGMP), binding agent, and PDE assay buffer for 384 enzyme reactions. The key to the *PDE5A1 Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE5A1 reactions. First, the fluorescently labeled cGMP is incubated with a sample containing PDE5A1 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*	
60050	PDE5A1	>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	250 µl	+4°C	
60392	Binding Agent Diluent (cGMP)	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(S):
Maurice DH. Front. Biosci. 2005; 10:1221-8.

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

Step 1:

1) Dilute 20 µM **FAM-Cyclic-3',5'-GMP** 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 diluted **FAM-Cyclic-3',5'-GMP** (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".

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3) Add 2.5 µl of inhibitor solution to each well designated “Test Inhibitor”. Add 2.5 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Blank”, “Substrate Control” and “Positive Control”.

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

5) Thaw **PDE5A1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **PDE5A1** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PDE5A1** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **PDE5A1** in **PDE Assay Buffer** to 10 pg/µl (100 pg/reaction) in PDE buffer*. Initiate reaction by adding 10 µl of diluted **PDE5A1** 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)	–	12.5 µl	12.5 µl	12.5 µl
PDE assay buffer	22.5 µl	10 µl	–	–
Test Inhibitor	–	–	–	2.5 µl
Inhibitor Buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
PDE5A1 (10 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 **cGMP 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".

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

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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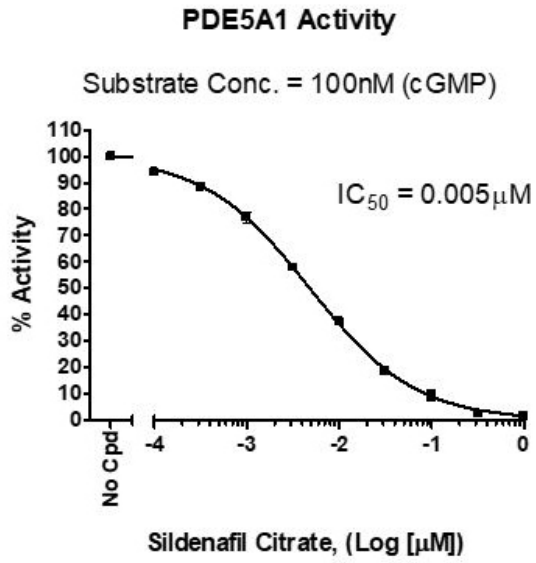
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE5A1 by Sildenafil Citrate, measured using the *PDE5A1 Assay Kit*, BPS Bioscience # 60351. 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.*

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RELATED PRODUCTS :

Product	Catalog #	Size
PDE5A	60050	10 µg
Mouse PDE5A	60051	5 µg
PDE1A1	60010	10 µg
PDE1B	60011	10 µg
PDE2A1	60020	5 µg
PDE3A	60030	10 µg
PDE5A Assay Kit	60350	96 rxns.
PDE Assay Kit	60300	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-cGMP Substrate	60201	100 nmole.

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