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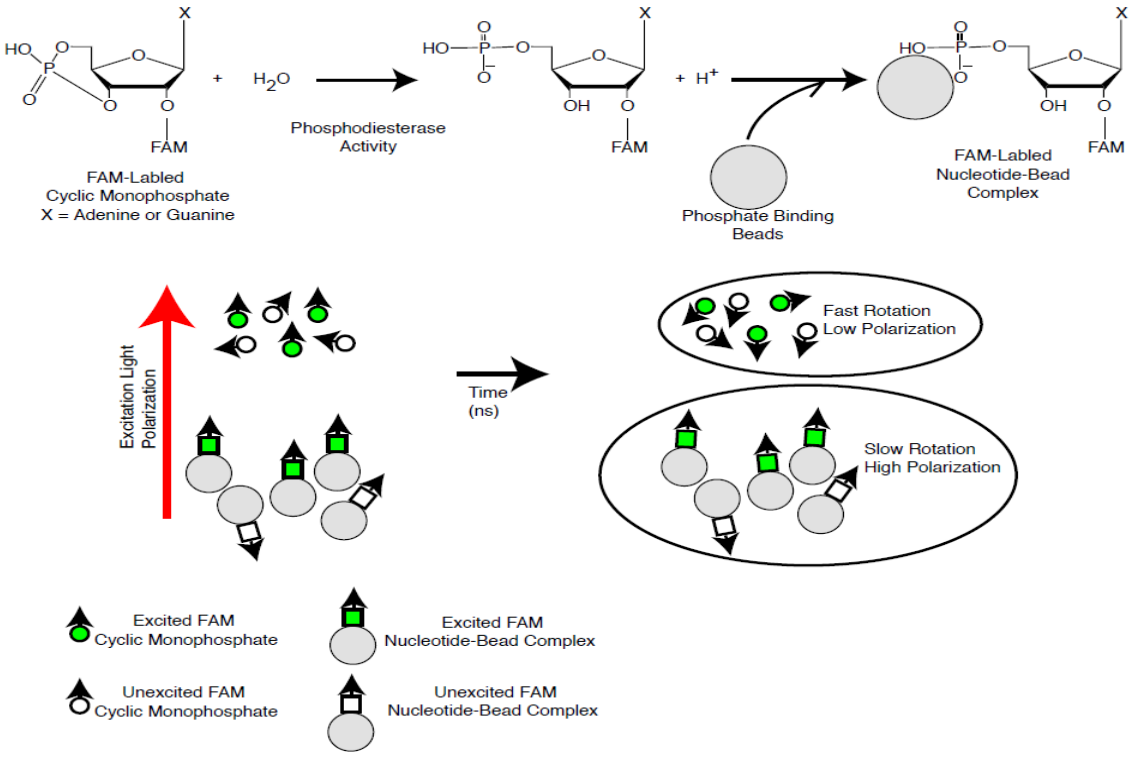
Data Sheet
PDE5A Assay Kit
Catalog # 60350

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE5A, also known as cGMP-inhibited phosphodiesterase, has been implicated in cardiovascular function and fertility. The *PDE5A Assay Kit* is designed for identification of PDE5A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE5A 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 PDE5A inhibitor screening assay kit comes in a convenient 96-well format, with purified PDE5A enzyme, fluorescently labeled PDE5 substrate (cGMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE5A Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE5A reactions. First, the fluorescently labeled cGMP is incubated with PDE5A for 1 hour. Second, the 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	
60050	PDE5A	>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.	

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: 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 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°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.”

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3) Add 5 µl of inhibitor solution of 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 for the "Blank" and "Substrate Control."

5) Thaw PDE5A on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot PDE5A enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -70°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 10 pg/µl (200 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE5A 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 the plate 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	–
PDE5A (10 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 binding agent diluent.

2) Add 100 µl diluted binding agent to each microwell. 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:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

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. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

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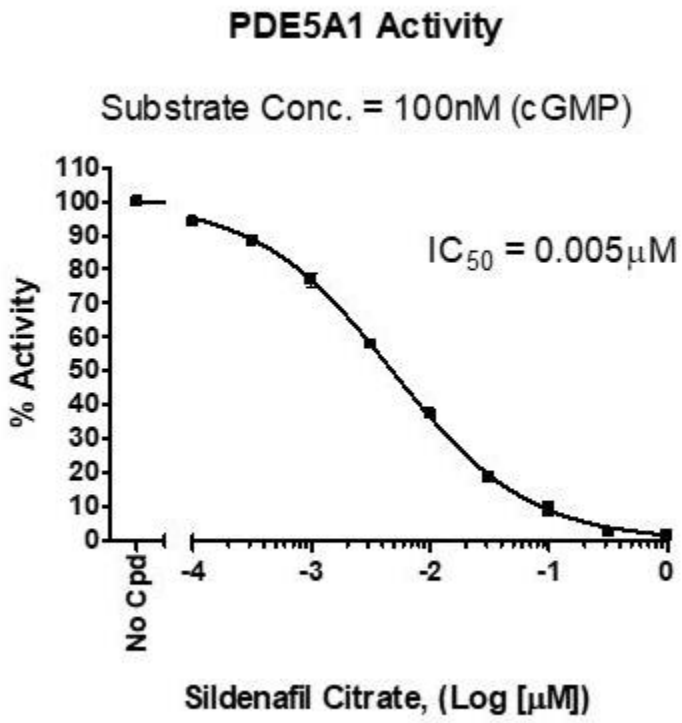
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE5A by Sildenafil Citrate, measured using the PDE5A Assay Kit, BPS Bioscience # 60350. 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

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RELATED PRODUCTS :

Product	Catalog #	Size
PDE5A, human	60050	10 µg
PDE5A, mouse	60051	10 µg
PDE Assay Kit	60300	96 rxns.
PDE1B Assay Kit	60311	96 rxns.
PDE3B Assay Kit	60331	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE4D Assay Kit	60345	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cGMP Substrate	60201	100 nmol
Sildenafil Citrate	27105	25 mg
Rolipram	27005	10 mg

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