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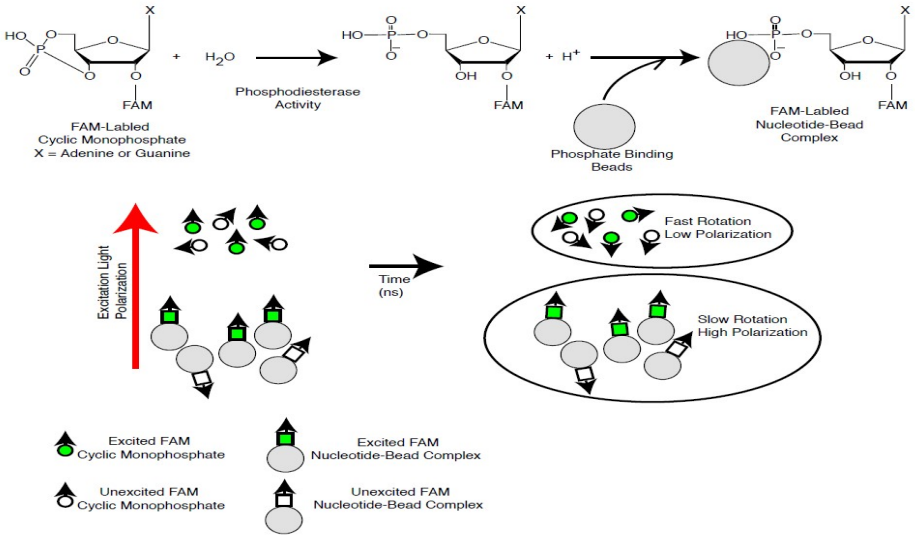
Data Sheet

Mouse PDE5A1 Assay Kit

Catalog # 79602
Size: 96 reactions

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cGMP signaling. Mouse PDE5A, also known as cGMP-inhibited phosphodiesterase, has been implicated in cardiovascular function and fertility. The Mouse PDE5A1 Assay Kit is designed for identification of inhibitors of Mouse PDE5A1 using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by Mouse PDE5A1 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 Mouse PDE5A1 *Assay Kit* comes in a convenient 96-well format, with purified Mouse PDE5A1 enzyme, fluorescently labeled substrate (cGMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *Mouse PDE5A1 Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for Mouse PDE5A1 reactions. First, the fluorescently labeled cGMP is incubated with a sample containing Mouse 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 equipped for the measurement of fluorescence polarization.

COMPONENTS:

Catalog #	Component	Amount	Storage	
60051	Mouse PDE5A1 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.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable to measure fluorescence polarization.
Adjustable micropipettor and sterile tips.
1,4-Dithiothreitol (DTT), 1M in anhydrous DMSO

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.

REFERENCES:
1. Tommpo, *et al.*, *Adv Neurobiol.* 2017;**17**:103-131.
2. Clapcote SJ, *et al*., *NeuroReport.* 2007; Nov; **18**(17):1841-1844.

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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 assay 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) Dilute 1M 1,4-Dithiothreitol (DTT) 1:500 into the diluted **FAM-Cyclic-3´,5´-GMP**. For example, add 10 µl DTT (1M) to 5 ml of diluted FAM-Cyclic-3´, 5´-GMP (200 nM).

3) Add 25 µl of **FAM-Cyclic-3´,5´-GMP** (200 nM) to each well designated "Positive Control," "Test Inhibitor," and "Substrate Control."

4) Add 45 µl of **PDE assay buffer** to each well designated "Blank" and add 20 µl of **PDE assay buffer** to each well designated "Substrate Control."

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

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

	Positive Control	Test Inhibitor	Substrate Control	"Blank" Negative Control
FAM-Cyclic-3',5'-GMP (200 nM)	25 µl	25 µl	25 µl	–
PDE assay buffer	–	–	20 µl	45 µl
Inhibitor (in PDE assay buffer)	–	5 µl	–	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl	5 µl
Mouse PDE5A1 (2.5 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

7) Dilute **Mouse PDE5A1** in **PDE assay buffer** to 2.5 pg/µl (0.05 ng/reaction)*. Initiate reaction by adding 20 µl of **Mouse PDE5A1** (2.5 pg/µl) 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.*

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8) Incubate the plate at room temperature for 1 hour.

Step 2:

1) Mix **Binding Agent** thoroughly and dilute **Binding Agent** 1:100 with **Binding Agent Diluent**.

2) Add 100 µl of 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 equipped for the measurement of fluorescence polarization, 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.

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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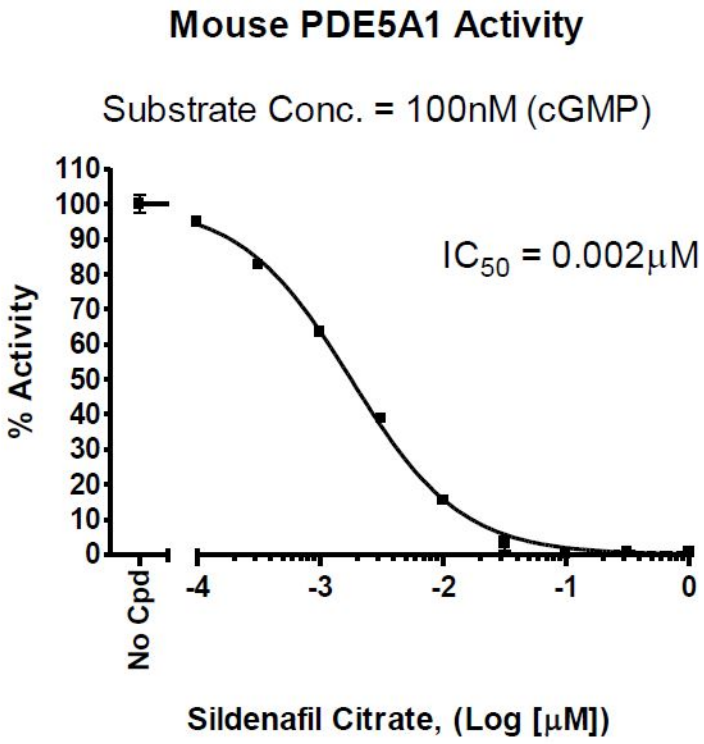
EXAMPLE OF ASSAY RESULTS:



Inhibition of Mouse PDE5A1 by Sildenafil Citrate measured using the *Mouse PDE5A1 Assay Kit*, BPS Bioscience #79602. 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 Name	Catalog #	Size
PDE5A1, human	60050	10 µg
PDE5A1, mouse	60051	10 µg
PDE5 Assay Kit	60350	96 rxns.
FAM-cGMP Substrate	60201	100 nmol
PDE Assay Kit	60300	96 rxns.
PDE1B Assay Kit	60311	96 rxns.
PDE3B Assay Kit	60331	96 rxns.
PDE4A Assay Kit	60340	96 rxns4
PDE4A1A	60040	10 µg
PDE4B1	60041	10 µg
PDE4B2	60042	5 µg
Dog PDE4B	60055	5 µg
Rat PDE4B	60049	5 µg
PDE4C1	60044	5 µg
PDE4D2	60048	5 µg
PDE4D3	60046	5 µg
PDE4D7	60047	5 µg
PDE4B2 Assay Kit	60343	96 rxns.
PDE4A1A Assay Kit	60340	96 rxns.
PDE4D2 Assay Kit	60345	96 rxns.
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.
Sildenafil Citrate	27105	25 mg
Rolipram	27005	10 mg

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