6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

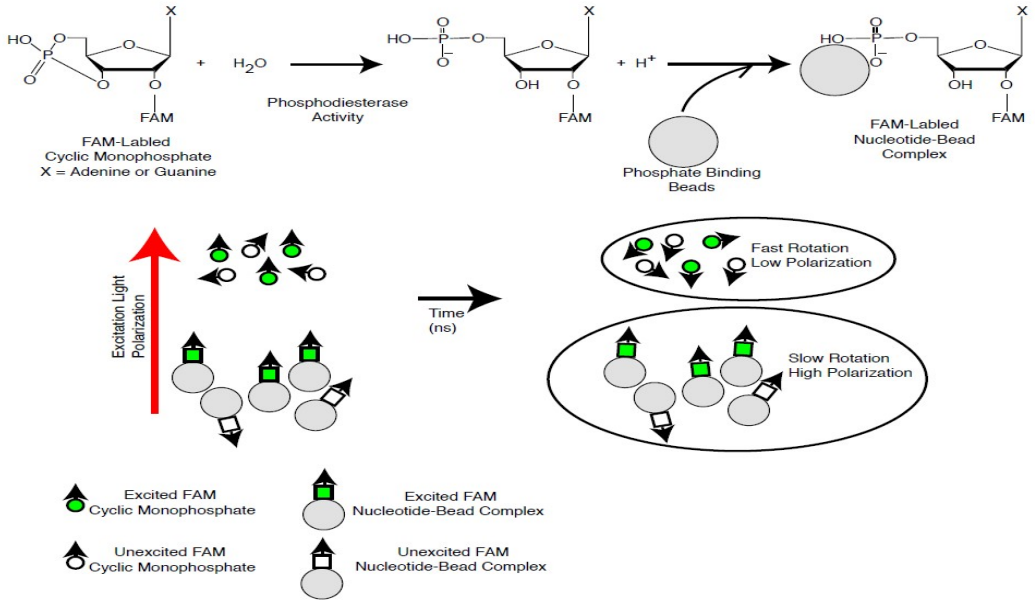
Mouse PDE1C Assay Kit

Catalog # 61312
Size: 96 reactions

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cAMP signaling. Mouse PDE1C, also known as cAMP-inhibited phosphodiesterase, has been implicated in cardiovascular function and fertility.

The Mouse PDE1C Assay Kit is designed for identification of inhibitors of Mouse PDE1C using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by Mouse PDE1C 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 PDE1C Assay Kit* comes in a convenient 96-well format, with purified Mouse PDE1C enzyme, fluorescently labeled substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *Mouse PDE1C Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for Mouse PDE1C reactions. First, the fluorescently labeled cAMP is incubated with a sample containing Mouse PDE1C 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	
60012	Mouse PDE1C recombinant enzyme	>1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3´, 5´-AMP (20 µM)	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
60390	Binding Agent	100 µl	+4°C	
60391	Binding Agent Diluent (cAMP)	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) 1 M 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**.** Knight WE, Chen S, Zhang Y, *et al.* PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction. (2016). *Proc. Natl. Acad. Sci.USA* **113(45):**E7116-E7125.
2. Vandeput, F., Wolda, S. L., Krall, J., Hambleton, R., Uher, L., McCaw, K. N., Radwanski, P. B., Florio, V., Movsesian, M. A. (2007).Cyclic nucleotide phosphodiesterase PDE1C1 in human cardiac myocytes**.** *J. Biol. Chem*. **282:** 32749-32757.

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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´-AMP** 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´-AMP**. 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´-AMP** (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 PDE1C** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **Mouse PDE1C** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: Mouse PDE1C 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'-AMP (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 PDE1C (0.5 pg/µl)	20 µl	20 µl	–	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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7) Dilute **Mouse PDE1C** in **PDE assay buffer** to 0.5 pg/µl (0.01 ng/reaction)*. Initiate reaction by adding 20 µl of diluted **Mouse PDE1C** (0.5 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.*

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".

$$FP(measured) = \frac{([I_{\parallel}]-G*[I_{\perp}])}{([I_{\parallel}]+G*[I_{\perp}])} * 1000$$

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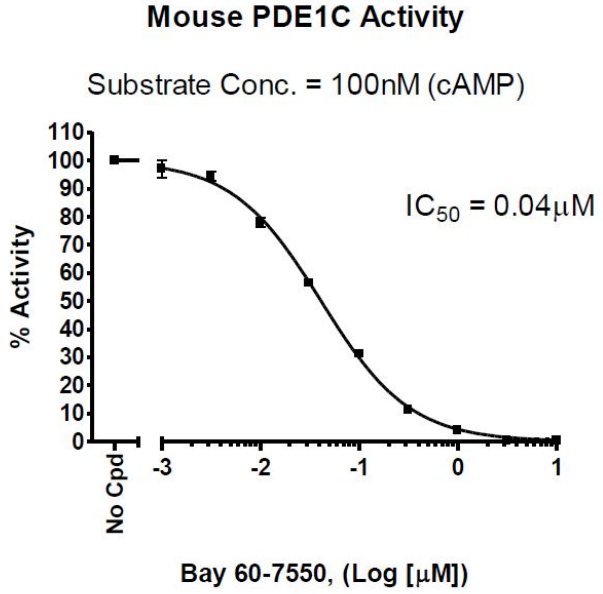
The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

EXAMPLE OF ASSAY RESULTS:



Inhibition of murine PDE1C by Bay 60-7550 measured using the *Mouse PDE1C Assay Kit*, BPS Bioscience #61312. 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
Mouse PDE1C	60012	10 µg
Mouse PDE3a	60036	5 µg
Mouse PDE2a V1	60017	5 µg
Mouse PDE2a V2	60018	5 µg
Mouse PDE2a V4	60019	5 µg
Mouse PDE6C	60065	5 µg
Mouse PDE5A	60051	10 µg
Mouse PDE7A	60072	10 µg
Mouse PDE7B	60073	10 µg
Mouse PDE10C	60101	10 µg
Mouse PDE11A	60064	5 µg
Rat PDE4B	60049	5 µg
rRat PDE4D	60054	5 µg
Rat PDE1B	60009	10 µg
Rat PDE2A	60022	5 µg
Rat PDE7A	60074	10 µg
Rat PDE7B	60075	10 µg
Rat PDE10A	60102	5 µg
PDE4B2	60042	5 µg
Mouse PDE3A1 Assay Kit	79606	96 rxns.
Mouse PDE5A1 Assay Kit	79602	96 rxns.
Mouse PDE5A1 Assay Kit	79602	96 rxns.
Dog PDE4B	60055	5 µg
Rat PDE4B Assay Kit	79571	96 rxns.
PDE Assay Kit	60300	96 rxns.
PDE4B2 Assay Kit	60343	96 rxns.
PDE4A1A Assay Kit	60340	96 rxns.
PDE7A2 Assay Kit	60345	96 rxns.
PDE7A Cell-Based Activity Assay Kit	60505	500 rxns.