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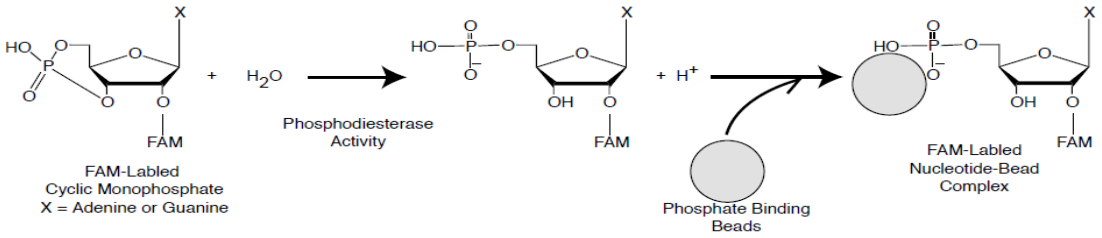
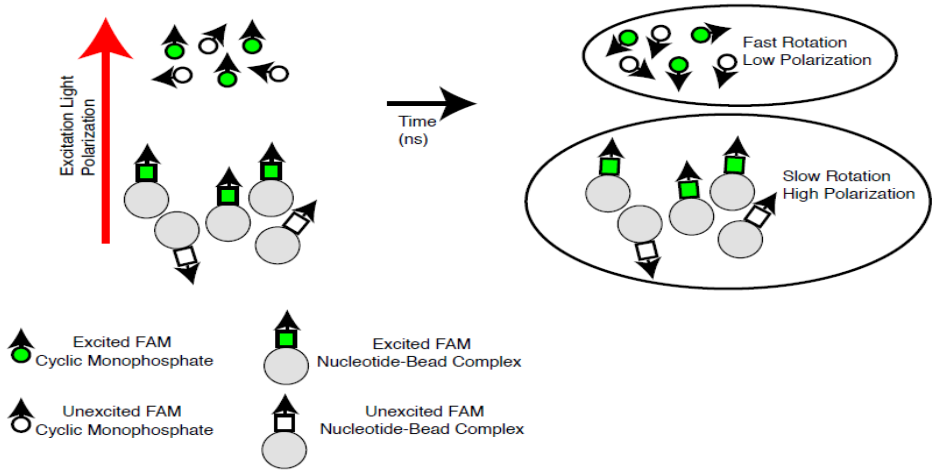
Data Sheet
PDE7A Assay Kit
Catalog # 60370

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE7A is widely expressed in various tissues including skeletal muscle, T lymphocytes, brain and pancreas and plays and important role in the regulation of osteoblastic differentiation. The *PDE7A Assay Kit* is designed for identification of PDE7A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE7A 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 PDE7A inhibitor screening assay kit comes in a convenient 96-well format, including purified PDE7A enzyme, fluorescently labeled PDE7A substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE7A Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE7A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE7A for 1 hour. Second, a binding agent is added to the reaction mix to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization.*

COMPONENTS:

Catalog #	Component	Amount	Storage	
60070	PDE7A 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 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):

1. Malik, R. et al. (2008) *Appl. Microbiol. Biotechnol.* ***77 (5)****: 1167-1173*.
2. Pekkinen, M. et al. (2008) *Bone.* ***43 (1)***: 84-91.

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

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°C.

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".

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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 PDE7A on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE7A enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE7A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE7A in PDE buffer to 20 pg/µl (400 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE7A 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	–
PDE7A (20 pg/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

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

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

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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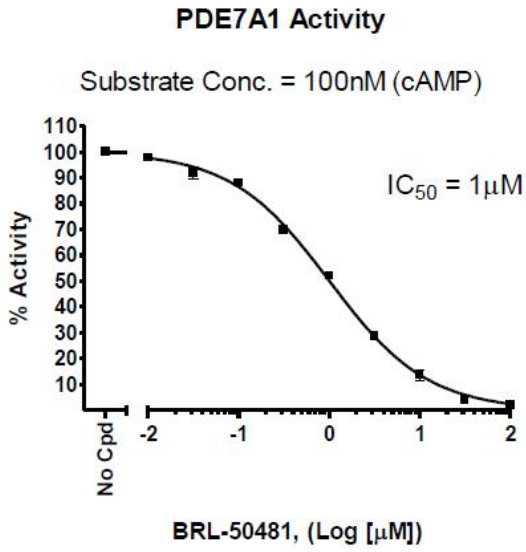
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE7A by BRL-50481, measured using the *PDE7A Assay Kit*, BPS Bioscience # 60370. 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*

RELATED PRODUCTS :

Product	**Catalog #**	**Size**
PDE7A	60070	10 µg
PDE8A1	60080	10 µg
PDE4A1A	60040	10 µg
PDE4B1	60041	10 µg
PDE7B	60071	10 µg
PDE1B Assay Kit	60311	96 rxns.
PDE2A Assay Kit	60320	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE5A Assay Kit	60350	96 rxns.
PDE8A Assay Kit	60380	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cAMP Substrate	60200	100 nmole.