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

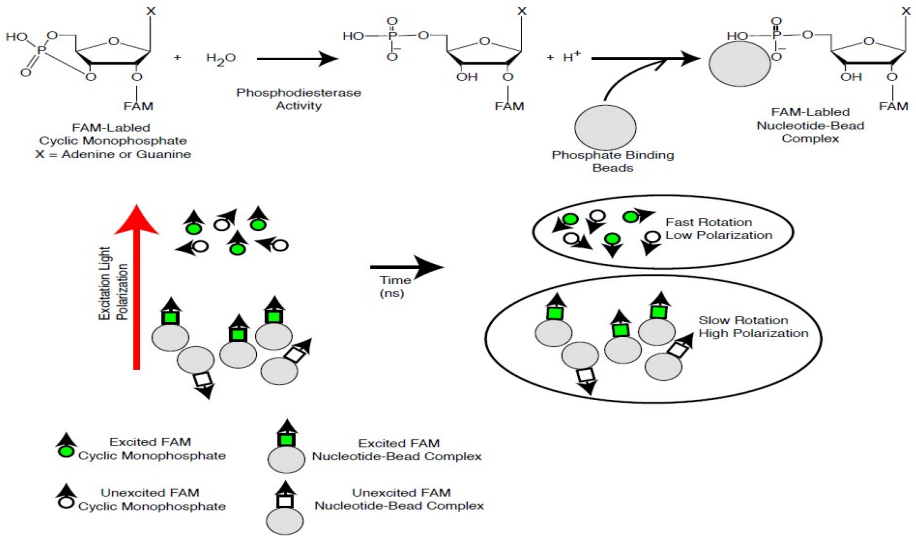
## *Rat PDE7A Assay Kit*

**Catalog # 79634**
**Size: 96 reactions**

**DESCRIPTION:** Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cAMP signaling. Rat PDE7A, also known as high affinity cAMP-specific phosphodiesterase 7A, has been implicated in cardiovascular function and fertility.

The Rat PDE7A Assay Kit is designed for identification of inhibitors of Rat PDE7A using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by Rat 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.



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

The Rat PDE7A *Assay Kit* comes in a convenient 96-well format, with purified Rat PDE7A enzyme, fluorescently labeled substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *Rat PDE7A Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for Rat PDE7A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing Rat PDE7A 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 | |
|---|---|---|---|---|
| 60036 | Rat 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 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. Morales-Garcia JA, , *et al.* (2011). Phosphodiesterase 7 Inhibition Preserves Dopaminergic Neurons in Cellular and Rodent Models of Parkinson Disease. Amédée T, ed. *PLoS ONE*. **6(2):**e17240.

2. Giembycz, M & J. Smith, S. (2006). Phosphodiesterase 7 (PDE7) as a therapeutic target. *Drugs of The Future - DRUG FUTURE*. **31(3):** 207.

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

**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). If inhibitor is dissolved in DMSO, make sure final DMSO concentration in the assay does not exceed 1%.

6) Thaw **Rat PDE7A** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **Rat PDE7A** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: Rat PDE7A 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 |
| Rat PDE7A (0.02 ng/µl) | 20 µl | 20 µl | – | – |
| **Total** | **50 µl** | **50 µl** | **50 µl** | **50 µl** |

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

7) Dilute **Rat PDE7A** in **PDE assay buffer** to 0.02 ng/µl (0.4 ng/reaction)*. Initiate reaction by adding 20 µl of diluted **Rat PDE7A** (0.02 ng/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.

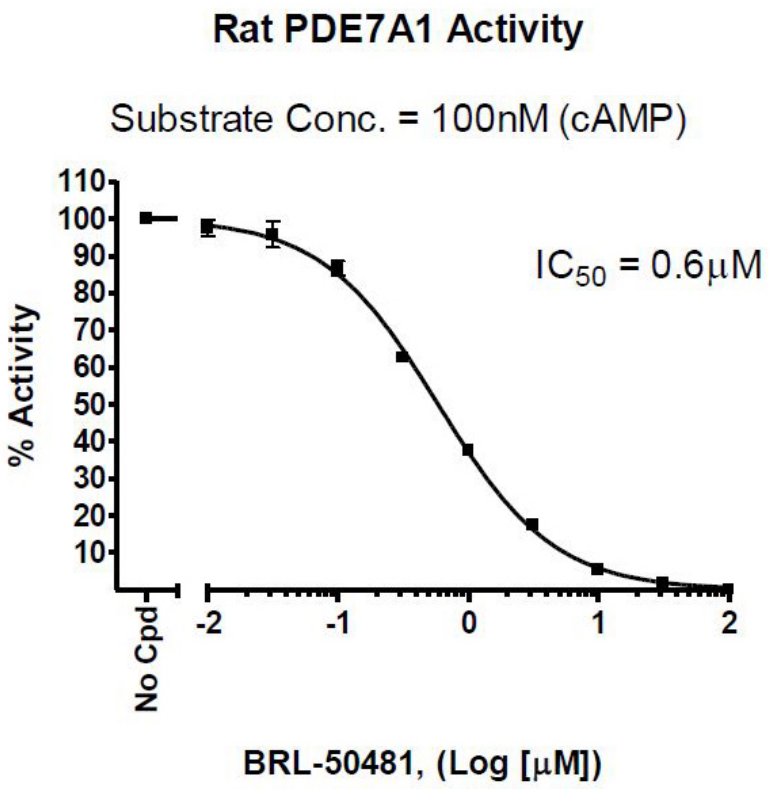
The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

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

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

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 Rat PDE7A by BRL-50481 measured using the *RAT PDE7A Assay Kit*, BPS Bioscience #79634. 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*

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

**RELATED PRODUCTS :**

| Product Name | Catalog # | Size |
|---|---|---|
| Rat PDE7A-HEK293 Recombinant Cell Line | 60408 | 2 vials |
| Rat PDE7A | 60074 | 10 µg |
| Rat PDE7B | 60075 | 10 µg |
| Rat PDE4B | 60049 | 5 µg |
| Rat PDE4D | 60054 | 5 µg |
| Rat PDE1B | 60009 | 10 µg |
| Rat PDE2A | 60022 | 5 µg |
| Rat PDE10A | 60102 | 5 µg |
| PDE4D2 | 60048 | 5 µg |
| PDE4D3 | 60046 | 5 µg |
| PDE4D7 | 60047 | 5 µg |
| PDE7A/CRE Reporter - HEK293 Cell Line | 60413 | 2 vials |
| PDE7A-HEK293 Recombinant Cell Line | 60407 | 2 vials |
| PDE7A-HEK293 Recombinant Cell Line | 60412 | 2 vials |
| PDE4A1A | 60040 | 10 µg |
| PDE4B1 | 60041 | 10 µg |
| PDE4B2 | 60042 | 5 µg |
| 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. |
| PDE4D2 Assay Kit | 60345 | 96 rxns. |
| PDE4D Cell-Based Activity Assay Kit | 60505 | 500 rxns. |