

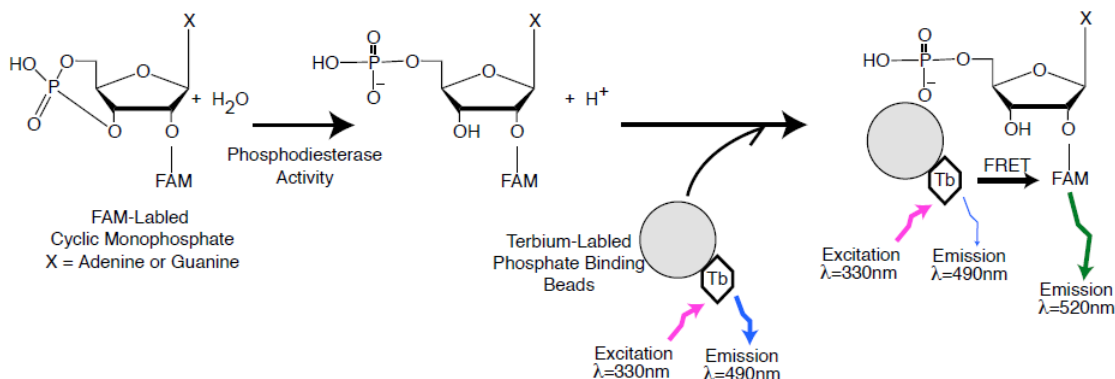
Data Sheet

PDE10A1 TR-FRET Assay Kit

Catalog # 60709

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE10A is a dual substrate PDE highly expressed in striatal medium spiny neurons. PDE10A inhibitors can improve the cognitive symptoms of schizophrenia, and exhibit potential therapeutic value for Huntington's disease. PDE10A1 is located in cytosol, whereas PDE10A2 is a membrane-associated protein.

The *PDE10A1 TR-FRET Assay Kit* is designed for identification of inhibitors of PDE10A1 using TR-FRET (Time Resolved Fluorescence Resonance Energy Transfer) technology. The assay is based on the generation of FAM-labeled nucleotide monophosphates by the phosphodiesterase. These phosphate groups bind to terbium-labeled nanoparticles, resulting in energy transfer from the terbium to the FAM, which emits a fluorescent signal at 520 nm. The change in fluorescent intensity can be easily measured using a fluorescence plate reader.



The *PDE10A1 TR-FRET Assay Kit* comes in a convenient 96-well format, with purified PDE10A1 enzyme, fluorescently labeled PDE substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. Using this kit, only two simple steps on a microtiter plate are required for the PDE10A1 activity assay. First, the fluorescent-labeled cAMP is incubated with a sample containing PDE10A1 for 1 hour. Second, a binding agent and a terbium donor are added to the reaction mix and incubated for 1 hour. Then, fluorescence intensity can be measured using a fluorescence reader.

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: 6 months from date of receipt when stored as directed.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: support@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

COMPONENTS:

Catalog #	Component	Amount	Storage	
60099	PDE10A1 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	
60394	Tb donor	50 µl	-80°C	
60390	Binding Agent	200 µl	+4°C	
78422	Binding Buffer A	20 ml	+4°C	
78423	Binding Buffer B	20 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 Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

REFERENCE(S):

- 1) Akinori Nishi and Gretchen L. Snyder. *J Pharmacol Sci.*, 2010, **114**: 6 – 16.
- 2) Kenji Omori and Jun Kotera. *Circulation Research*. 2007; **100**: 309-327.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Protocol for PDE10A1 assay

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.
- 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".
- 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) Thaw PDE10A1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. *Note: PDE10A1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: support@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

- 5) Dilute PDE10A1 in PDE buffer to 1.25 pg/ μ l (25 pg/reaction) in PDE buffer*. Add 20 μ l of PDE assay buffer to the wells designated as the "Blank" and "Substrate Control". Initiate reaction by adding 20 μ l of PDE10A1 (1.25 pg/ μ l) 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.*

	Blank	Substrate Control	Positive Control	Test Inhibitor
FAM-Cyclic-3',5'-AMP (200 nM)	–	25 μ l	25 μ l	25 μ l
PDE assay buffer	45 μ l	20 μ l	–	–
Test Inhibitor	–	–	–	5 μ l
Inhibitor Buffer (no inhibitor)	5 μ l	5 μ l	5 μ l	–
PDE10A1 (1.25 pg/ μ l)	–	–	20 μ l	20 μ l
Total	50 μl	50 μl	50 μl	50 μl

- 6) Incubate at room temperature for 1 hour.

Step 2:

- 1) Make binding dilution buffer by mixing equal volumes of Binding buffer A and Binding buffer B. For example, mix 1 ml Binding buffer A with 1 ml Binding buffer B.
- 2) Mix **binding agent** thoroughly and dilute **binding agent** 1:50 with binding dilution buffer made in Step 1.
- 3) Add Tb donor (1:200 dilution) to the mixture in Step 2.
- 4) Add 100 μ l to each well. Incubate at room temperature for 1 hour with slow shaking.
- 5) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	330 \pm 20
Emission Wavelength	490 \pm 10
Lag Time	50 μ s
Integration Time	50 μ s
Excitation Wavelength	330 \pm 20
Emission Wavelength	520 \pm 10
Lag Time	50 μ s
Integration Time	50 μ s

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: support@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

CALCULATING RESULTS:

$$FRET = \frac{S_{520} - \left(\frac{Tb_{520}}{Tb_{490}} \times S_{490} \right)}{S_{490}} \times 1000$$

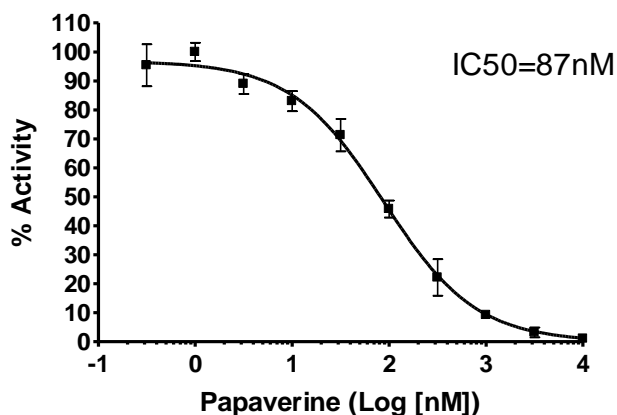
Where S_{520} = Sample 520 nm reading, S_{490} = Sample 490 nm reading, Tb_{520} = Tb only 520 nm reading, Tb_{490} = Tb only 490 nm reading. When percentage activity is calculated, the FRET value from substrate only control can be set as zero percent activity and the FRET value from positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{sub}}{FRET_p - FRET_{sub}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{sub}$ = Substrate only control FRET, and $FRET_p$ = Positive control FRET.

EXAMPLE OF ASSAY RESULTS:

PDE10A1 Activity



Inhibition of PDE10A1 by Papaverine, measured using the *PDE10A1 TR-FRET Assay Kit*, BPS Bioscience # 60709. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: support@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

RELATED PRODUCTS :

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
PDE10A1	60099	10 µg
PDE10A2	60100	10 µg
PDE10A (mouse)	60101	10 µg
PDE10A (rat)	60102	5 µg
PDE11A	60110	10 µg
PDE1B	60011	10 µg
PDE1C	60012	10 µg
PDE4D3	60046	5 µg
PDE10A FP Assay Kit	60400	96 rxns.
PDE11A FP Assay Kit	60411	96 rxns.
PDE1B FP Assay Kit	60311	96 rxns.
PDE1C FP Assay Kit	60312	96 rxns.
PDE4D3 FP Assay Kit	60345	96 rxns.
PDE1B TR-FRET Assay Kit	60704	96 rxns.
PDE1C TR-FRET Assay Kit	60705	96 rxns.
PDE4D3 TR-FRET Assay Kit	60701	96 rxns.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: support@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com