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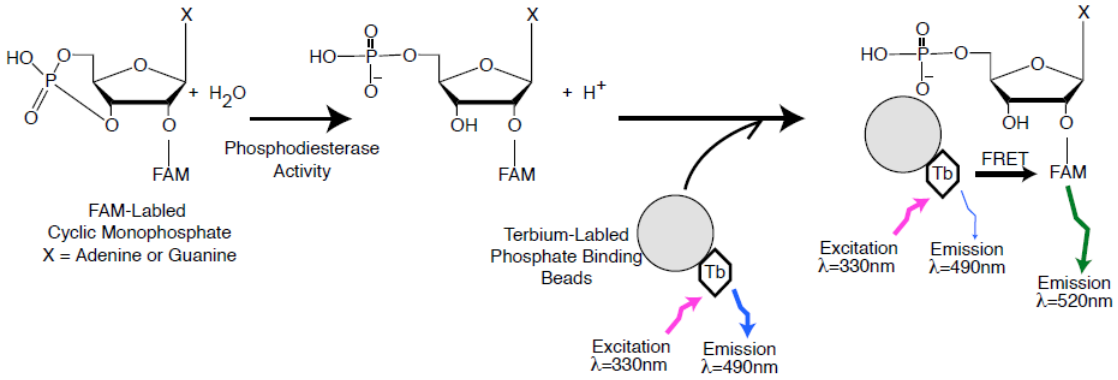
Data Sheet
PDE1B TR-FRET Assay Kit
Catalog # w70715

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE1B is a calcium/calmodulin-dependent cyclic nucleotide phosphodiesterase that is highly expressed in the striatum. It plays a physiological role in the central nervous system, and PDE1B activity has been linked to impaired cognition and spatial learning.

The *PDE1B TR-FRET Assay Kit* is designed for identification of inhibitors of PDE1B using TR-FRET (Time Resolved Fluorescence Resonance Energy Transfer) technology. The assay is based on the generation of FAM-labeled nucleotide monophosphates by the PDE1B. 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 *PDE1B TR-FRET Assay Kit* comes in a convenient 96-well format, with purified PDE1B enzyme, fluorescently labeled PDE1B 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 PDE1B activity assay. First, the fluorescent-labeled cAMP is incubated with a sample containing PDE1B for 1 hour. Second, a binding agent and a terbium donor are added to the reaction mix and incubated for 30 minutes. Then, fluorescence intensity can be measured using a fluorescence reader.

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COMPONENTS:

Catalog #	Component	Amount	Storage	
w70022	PDE1B recombinant enzyme	1 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
w70211	FAM-Cyclic-3′, 5′-AMP: 20 µM	20 µl	-80℃	
w70404	PDE assay buffer	25 ml	-20℃	
	Tb donor	30 µl	-80℃	
w70401	Binding Agent	200 µl	+4℃	
	Binding Buffer A	20 ml	+4℃	
	Binding Buffer B	20 ml	+4℃	
VWR w72419-936	Black, low binding NUNC 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)

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

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

REFERENCE(S): Siuciak JA, *et al.* (2007) *Neuropharmacology* **53(1):**113-24.

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

Protocol for PDE1B assay

Step 1:

1) Dilute 20 µM FAM-Cyclic-3′,5′-AMP substrate stock solution 100-fold with PDE assay buffer to make a 200 nM solution. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20℃.

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 “Tb-only Control ".

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 “Tb-only Control”, “Substrate Control” and “Positive Control”.

4) Thaw PDE1B on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE1B enzyme into single-use

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aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: PDE1B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute PDE1B in PDE buffer to 0.1 ng/µl (2 ng/reaction) in PDE buffer*. Add 20 µl of PDE assay buffer to the wells designated as the "Tb-only Control " and "Substrate Control". Initiate reaction by adding 20 µl of PDE1B (0.1 ng/µl) 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*.

	Tb Only Control	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	–
PDE1B (0.1 ng/µl)	–	–	20 µl	20 µl
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 volume Binding Buffer A and Binding Buffer B. For example, mix 1 ml Binding Buffer A with 1 ml Binding Buffer B.

2) Dilute Binding Agent 1:50 with binding dilution buffer made in Step 1.

3) Add Tb donor (1:1,000 dilution) to the mixture in Step 2.

4) Add 100 µl 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.

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Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	330±20
Emission Wavelength	490±10
Lag Time	50 µs
Integration Time	50 µs
Excitation Wavelength	330±20
Emission Wavelength	520±10
Lag Time	50 µs
Integration Time	50 µs

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.

$$\%\ Activity = \frac{FRET_s - FRET_{Sub}}{FRET_P - FRET_{Sub}} \times 100\%$$

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

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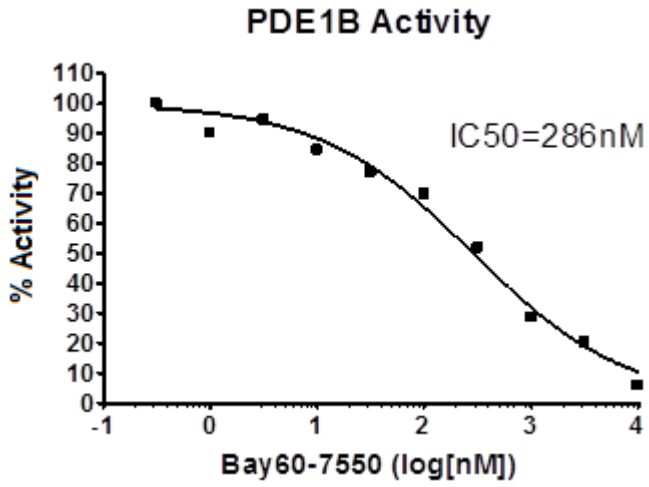
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE1B by Bay60-7550, measured using the *PDE1B TR-FRET Assay Kit*, West Bioscience # w70715. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*