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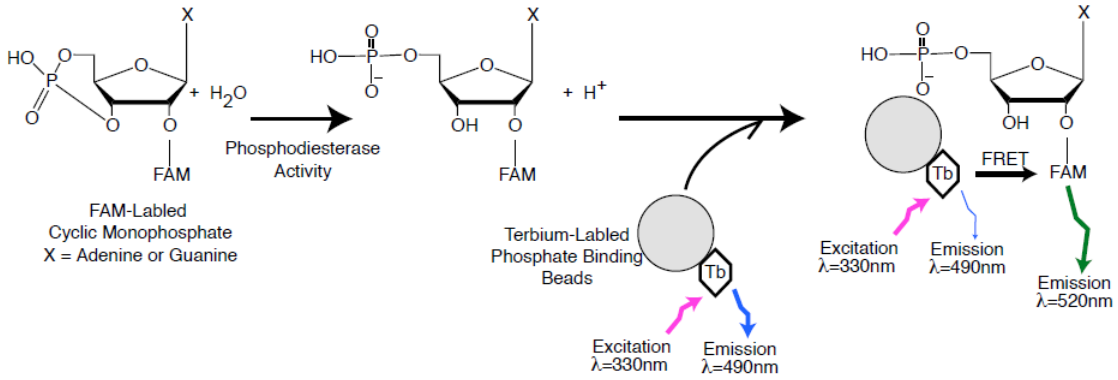
Data Sheet
PDE1C TR-FRET Assay Kit
Catalog # 60705

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE1C is a calmodulin-dependent PDE that is expressed principally in human myocardium. The *PDE1C TR-FRET Assay Kit* is designed for identification of inhibitors of PDE1C using TR-FRET (Time Resolved Fluorescence Resonance Energy Transfer) technology. The assay is based on the generation of FAM-labeled nucleotide monophosphates by the PDE1C. 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 *PDE1C TR-FRET Assay Kit* comes in a convenient 96-well format, with purified PDE1C enzyme, fluorescently labeled PDE1C 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 PDE1C activity assay. First, the fluorescent-labeled cAMP is incubated with a sample containing PDE1C 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	
60013	PDE1C recombinant enzyme	1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3′, 5′-AMP: 20 µM	20 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)

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):
Vandeput. F., *et al. J. Biol. Chem.* 2007; **282(45)**: 32749-32757.

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

Protocol for PDE1C 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.

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”.

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4) Thaw PDE1C on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. *Note: PDE1C is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute PDE1C in PDE buffer to 2.5 pg/µl (50 pg/reaction) in PDE buffer*. Add 20 µl of PDE assay buffer to the wells designated as the "Blank" and "Substrate Control". Initiate reaction by adding 20 µl of PDE1C (2.5 pg/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*.

	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	–
PDE1C (2.5 pg/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) 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 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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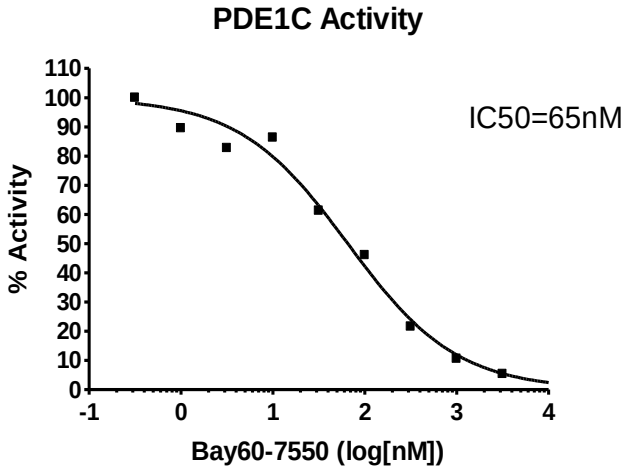
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE1C by Bay60-7550, measured using the *PDE1C TR-FRET Assay Kit*, BPS Bioscience # 60705. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS :

Product	Catalog #	Size
PDE1A1	60010	10 µg
PDE1B	60011	10 µg
PDE1C	60013	10 µg
PDE1C (mouse)	60012	10 µg
PDE4D3	60046	5 µg
PDE1B TR-FRET Assay Kit	60704	96 rxns.
PDE4D3 TR-FRET Assay Kit	60701	96 rxns.
PDE1A Assay Kit	60310	96 rxns.
PDE1C Assay Kit	60311	96 rxns.
PDE1C Assay Kit	60312	96 rxns.
PDE2A Assay Kit	60320	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE5A Assay Kit	60350	96 rxns.
PDE7A Assay Kit	60370	96 rxns.
PDE7B Assay Kit	60371	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
PDE11A Assay Kit	60411	96 rxns.