Data Sheet

PDE4B2 Assay Kit

Catalog # AMS.60343
Size: 96 reactions

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. PDE4 selective inhibitors are currently in clinical trials for the treatment of diseases related to inflammatory disorders. Increased expression of PDE4B2 was observed in the near-term myometrium. PDE4B2 can be induced by its own substrate, under the control of one of the major utero-contractile agonist, PGE2. The PDE4B2 Assay Kit is designed for identification of inhibitors of PDE4B2 using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE4B2 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.

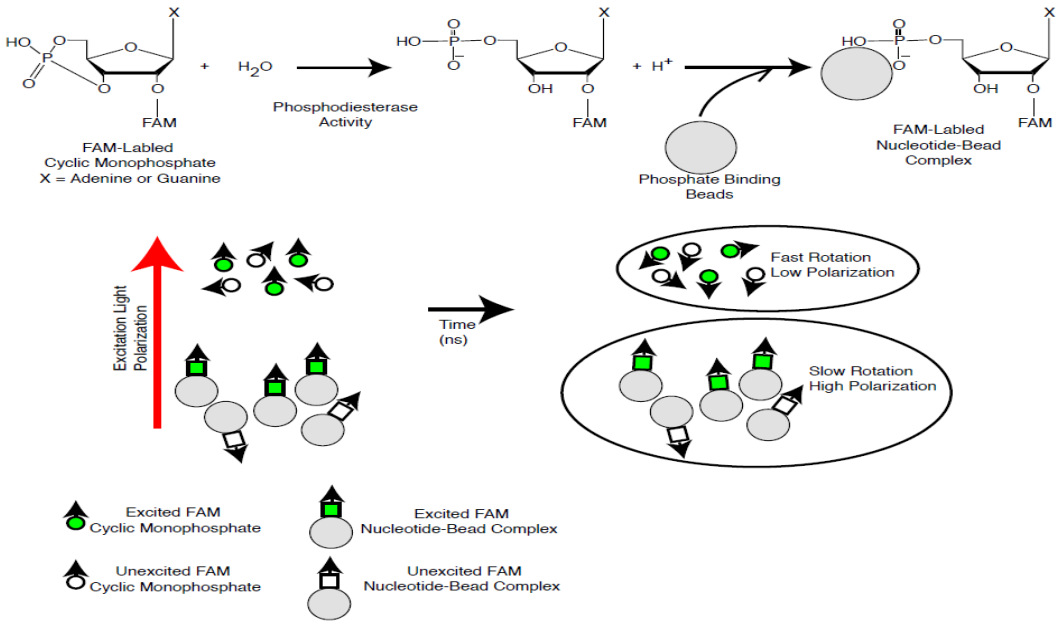
AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85



The *PDE4B2 Assay Kit* comes in a convenient 96-well format, with purified PDE4B2 enzyme, fluorescently labeled PDE4B2 substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE4B2 Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE4B2 reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE4B2 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	
60042	PDE4B2 recombinant enzyme	5 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3´, 5´-AMP (20 µM)	50 µl	-80°C	
	PDE assay buffer	25 ml	-20°C	
	Binding Agent	100 µl	+4°C	
	Binding Agent Diluent	10 ml	+4°C	
	Black, low binding, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable to measure 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: Chandrasekaran A, *et al., Cell Signal.* 2008; **20(1):** 139-53.

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 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) Add 25 µl of FAM-Cyclic-3´,5´-AMP (200 nM) to each well designated “Positive Control”, “Test Inhibitor”, and “Substrate Control”.

3) Add 20 µl of PDE assay buffer to each well designated “Substrate Control" and 45 µl of PDE assay buffer to each well designated “Blank”.

4) 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).

5) Thaw PDE4B2 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE4B2 enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: PDE4B2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE4B2 in PDE buffer to 7.5 pg/µl (0.15 ng/reaction)*. Initiate reaction by adding 20 µl of PDE4B2 (7.5 pg/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.*

7) Incubate at room temperature for 1 hour.

	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
PDE4B2 (7.5 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 microwell. Incubate at room temperature for 1 hour 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 475-495 nm and detection of emitted light ranging from 518-538 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".

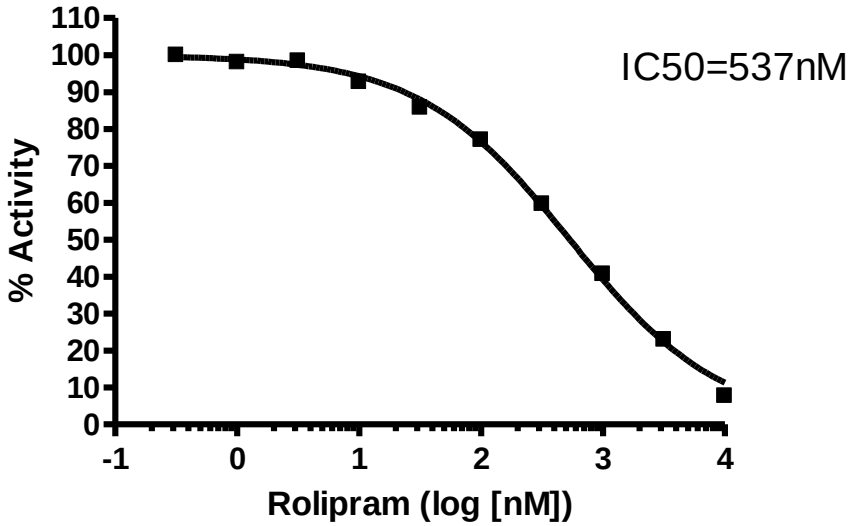
$$FP(measured) = \frac{([I_{\parallel}]-G*[I_{\perp}])}{([I_{\parallel}]+G*[I_{\perp}])} * 1000$$

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:

PDE4B2 Activity

Substrate Conc.=100nM cAMP



Inhibition of PDE4B2 by Rolipram, measured using the *PDE4B2 Assay Kit*, BPS Bioscience # 60343. Fluorescence polarization was measured at 528 nm using a Tecan M1000 fluorescent microplate reader. *Data shown is lot-specific.*

RELATED PRODUCTS :

Product Name	Catalog #	Size
PDE4A1A	60040	10 µg
PDE4B1	60041	10 µg
PDE4B2	60042	5 µg
PDE4C1	60044	5 µg
PDE4D2	60048	5 µg
PDE4D3	60046	5 µg
PDE4D7	60047	5 µg
PDE Assay Kit	60300	96 rxns.
PDE4A1A Assay Kit	60340	96 rxns.
PDE4D2 Assay Kit	60345	96 rxns.
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85