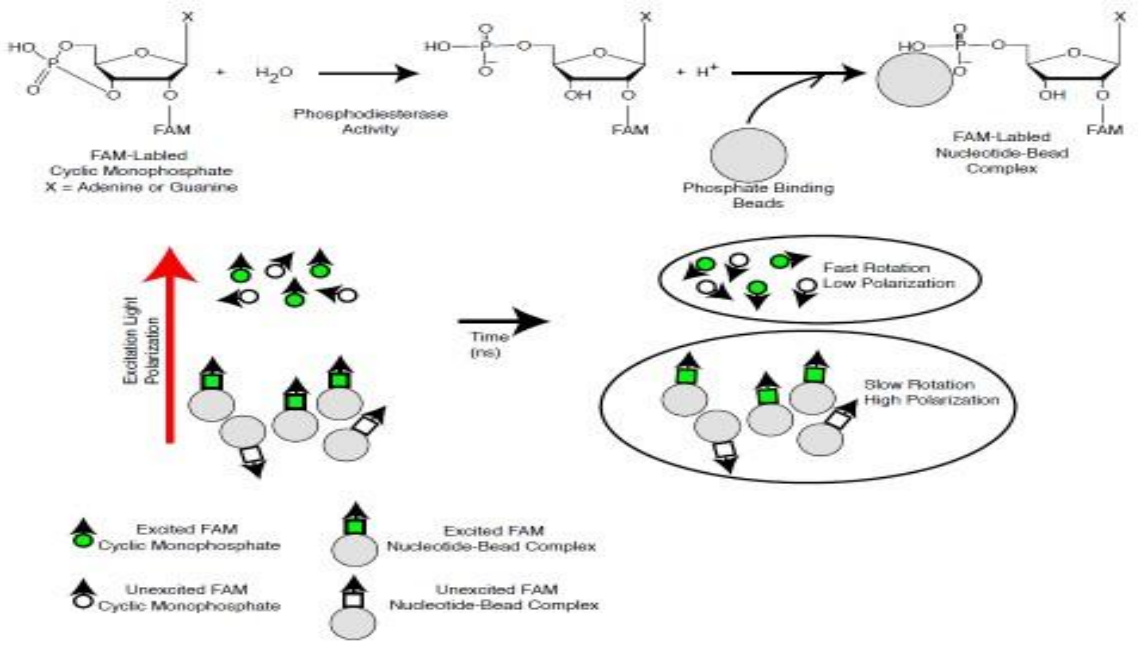
PDE4B2 Assay Kit

GenWay ID : GWB-A8C9F0

Legacy ID: 40-054-165281

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.



The PDE4B2 inhibitor screening 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.

COMPONENTS:

Avoid freeze/thaw cycles!	*Amount*	*Storage*
PDE4B2 recombinant enzyme (BPS #60042)	*5 µg*	*-80°C*
FAM-Cyclic-3', 5'-AMP: 20 µM	*50 µl*	*-80°C*
PDE assay buffer	*12 ml*	*-20°C*

	Amount	*Storage*
Binding Agent	*100 µl*	*+4°C*
Binding Agent Diluent (cAMP)	*10 ml*	*+4°C*
Black, low binding NUNC microtiter plate	*1*	*Room temp.*

APPLICATIONS: *Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.*

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) *Dilute cAMP 20 µM 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) *Dilute PDE4B2 in PDE buffer to 7.5 pg/µl (0.15 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -70°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. *Note: optimal enzyme concentration may vary with the specific activity of the enzyme.*

3) *In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at room temperature for 1 hour.*

	cAMP Positive Control	cAMP Sample	cAMP Negative Control	"Blank" Negative Control
PDE4B2 (7.5 pg/µl)	*20 µl*	*20 µl*	–	–
FAM-Cyclic-3',5'-AMP (200 nM)	*25 µl*	*25 µl*	*25 µl*	–
Inhibitor (in PDE assay buffer)	–	*5 µl*	–	–
PDE assay buffer	*5 µl*	–	*25 µl*	*50 µl*
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

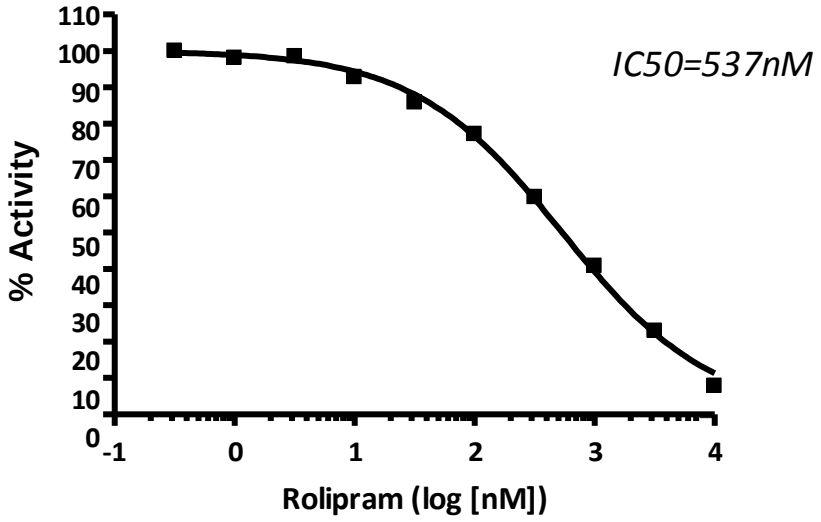
1) Dilute binding agent 1:200 with binding agent diluent.

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 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.

PDE4B2 Activity

Substrate Conc.=100nM cAMP



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. G= 0.87 for the Bio-Tek "Synergy 2" microplate reader used in our facilities.

REFERENCES:

1. *Cullen MD, et al. Bioorg Med Chem Lett. 2008; 18(4):1530-3.*
2. *Méhats C, et al. BMC Pregnancy Childbirth. 2007; 7 Suppl 1:S12.*