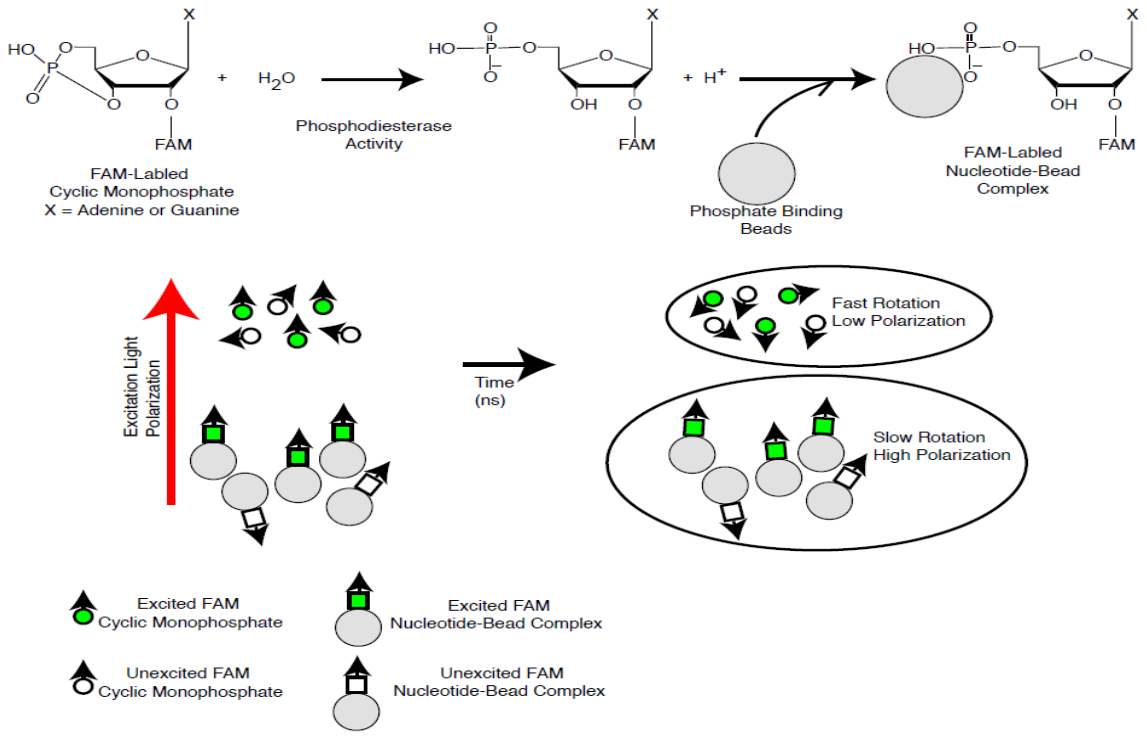
9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet
PDE10A Assay Kit
Catalog # w70411

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. The PDE10A Assay Kit is designed for identification of PDE10A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE10A 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.



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The PDE10A inhibitor screening assay kit comes in a convenient 96-well format, including purified PDE10A enzyme, fluorescently labeled PDE10A substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the PDE10A Assay Kit is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE10A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE10A 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	
w70111	PDE10A2 recombinant enzyme	100 ng	-80℃	***(Avoid freeze/ thaw cycles!)***
w70211	FAM-Cyclic-3', 5'-AMP: 20 µM	50 µl	-80℃	
	PDE assay buffer	25 ml	-20℃	
	Binding Agent	100 µl	+4℃	
	Binding Agent Diluent	10 ml	+4℃	
	Black, low binding, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring 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

REFERENCES:
1. Threlfell S, et al. (2009) *Pharmacol Exp Ther* **328(3):**785-95.
2. Menniti FS, et al. (2007) *Curr Opin Investig* Drugs **8(1):**54-9.

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℃.

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".

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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) Add 20 µl of PDE assay buffer to the wells designated as the "Blank" and "Substrate Control”.

5) Thaw PDE10A on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE10A enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: PDE10A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE10A to 10-20 pg/µl (200-400 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE10A 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*.

7) Incubate at room temperature for 1 hour.

	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	–
PDE10A2 (10-20 pg/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

1) Shake the tube containing the binding agent to ensure it is thoroughly mixed. 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 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.

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}\mu$= 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.

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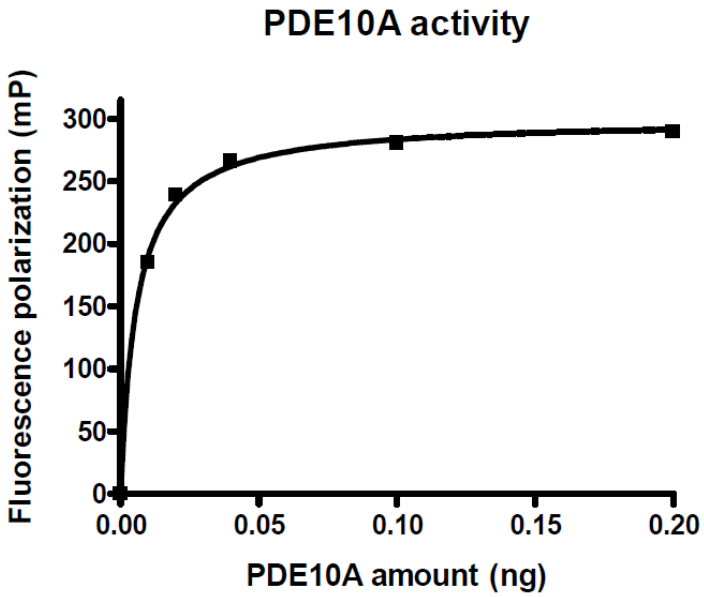
EXAMPLE OF ASSAY RESULTS:



Activity of PDE10A2, Cat. #60100, measured using the PDE10A Assay Kit, Cat. #w70411. Fluorescence polarization was measured at 528 nm using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*

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