

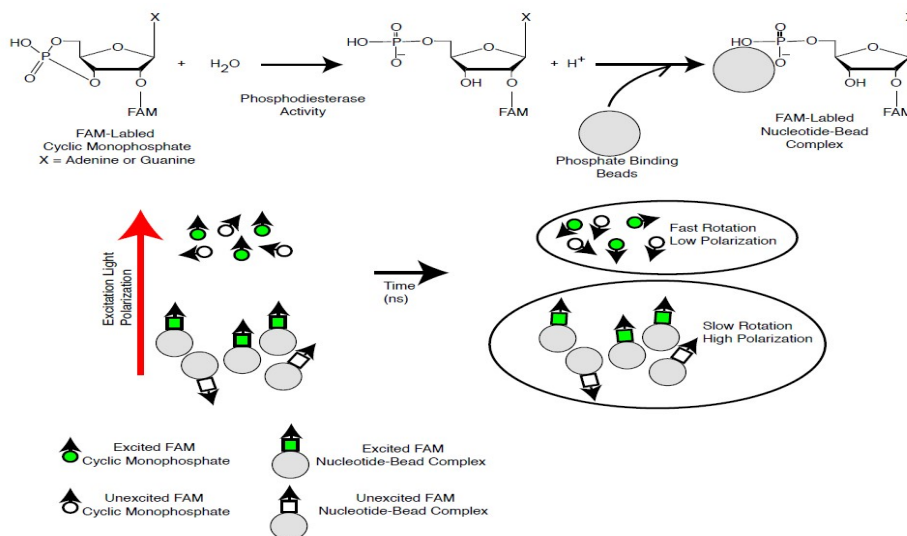
## Data Sheet

### ***Dog PDE3A Assay Kit***

**Catalog #79735**  
**Size: 96 reactions**

**DESCRIPTION:** Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cAMP signaling. Dog PDE3A, also known as cGMP-inhibited phosphodiesterase, has been implicated in cardiovascular function and fertility. The Dog PDE3A Assay Kit is designed for identification of inhibitors of canine PDE3A using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by Dog PDE3A 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 Dog PDE3A Assay Kit comes in a convenient 96-well format, with purified Dog PDE3A1 enzyme, fluorescently labeled substrate (cAMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the Dog PDE3A Assay Kit is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for Dog PDE3A reactions. First, the

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fluorescently labeled cAMP is incubated with a sample containing Dog PDE3A 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	
100263	Dog PDE3A recombinant enzyme	>1 µg	-80°C	<b>(Avoid freeze/thaw cycles!)</b>
60200	FAM-Cyclic-3', 5'-AMP (20 µM)	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
60390	Binding Agent	100 µl	+4°C	
60391	Binding Agent Diluent (cAMP)	10 ml	+4°C	
79685	Black, low binding, microtiter plate	1	Room temp.	

#### MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable to measure fluorescence polarization.

Adjustable micropipettor and sterile tips.

1,4-Dithiothreitol (DTT) 1 M in anhydrous DMSO.

#### 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:

- Hung, S.H., *et al. J. Biol. Chem.* **281 (39)**, 29236-29244 (2006)
- Hambleton, R., *et al. J. Biol. Chem.* **280 (47)**, 39168-39174 (2005)

#### ASSAY PROTOCOL:

***All samples and controls should be tested in duplicate.***

##### Step 1:

- Dilute 20 µM **FAM-Cyclic-3', 5'-AMP** stock 100-fold with **PDE assay 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.

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- Dilute 1M 1,4-Dithiothreitol (DTT) 1:500 into the diluted **FAM-Cyclic-3',5'-AMP**. For example, add 10  $\mu$ l DTT (1M) to 5 ml of diluted FAM-Cyclic-3', 5'-AMP (200 nM).
- Add 25  $\mu$ l of **FAM-Cyclic-3',5'-AMP** (200 nM) to each well designated "Positive Control," "Test Inhibitor," and "Substrate Control."
- Add 45  $\mu$ l of **PDE assay buffer** to each well designated "Blank" and add 20  $\mu$ l of **PDE assay buffer** to each well designated "Substrate Control."
- Add 5  $\mu$ l of inhibitor solution to each well designated "Test Inhibitor." For the wells labeled "Positive Control," "Substrate Control," and "Blank," add 5  $\mu$ l of the same solution without inhibitor (**PDE assay buffer**).
- Thaw **Dog PDE3A** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **Dog PDE3A** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at  $-80^{\circ}\text{C}$  immediately. *Note: Dog PDE3A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Positive Control	Test Inhibitor	Substrate Control	"Blank" Negative Control
FAM-Cyclic-3',5'-AMP (200 nM)	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l	-
PDE assay buffer	-	-	20 $\mu$ l	45 $\mu$ l
Inhibitor (in PDE assay buffer)	-	5 $\mu$ l	-	-
PDE assay buffer (no inhibitor)	5 $\mu$ l	-	5 $\mu$ l	5 $\mu$ l
Dog PDE3A (2.5 pg/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	-	-
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

- Dilute **Dog PDE3A** in **PDE assay buffer** to 2.5 pg/ $\mu$ l (0.05 ng/reaction)\*. Initiate reaction by adding 20  $\mu$ l of **Dog PDE3A** (2.5 pg/ $\mu$ l) 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.*
- Incubate the plate at room temperature for 1 hour.

## Step 2:

- Mix **Binding Agent** thoroughly and dilute **Binding Agent** 1:100 with **Binding Agent Diluent**.

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- 2) Add 100  $\mu$ l of diluted **Binding Agent** to each microwell. Incubate at room temperature for 30 minutes 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  $485 \pm 5$  nm and detection of emitted light ranging from  $528 \pm 10$  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".

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

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.

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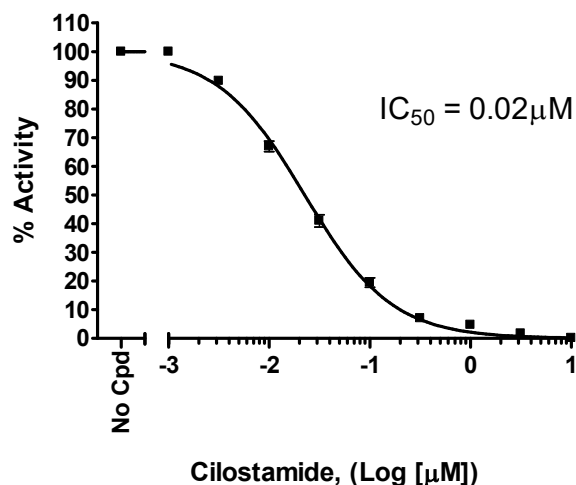
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**EXAMPLE OF ASSAY RESULTS:**

**Dog PDE3A Activity**

Substrate Conc. = 100nM (cAMP)



Inhibition of Dog PDE3A by Cilostamide measured using the *Dog PDE3A Assay Kit*, BPS Bioscience #79735. Fluorescence polarization was measured at 528 nm using a Tecan M1000 fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

**RELATED PRODUCTS :**

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
PDE3A (Mouse)	60036	5 µg
PDE3A (Dog)	100264	5 µg
PDE3A (Human)	60030	10 µg
PDE3A (669-end) (Human)	60032	5 µg
PDE3B (Human)	60031	10 µg
Mouse PDE3A Assay Kit	79606	96 rxns.
PDE1B Assay Kit	60311	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE3B Assay Kit	60331	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE4D Assay Kit	60345	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
PDE3A TR-FRET Assay Kit	60706	96 rxns.
FAM-cAMP Substrate	60200	100 nmol

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