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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet
PDE6C Assay Kit
Catalog # 79501

DESCRIPTION: Phosphodiesterases (PDEs) play an important role in dynamic regulation of cAMP and cGMP signaling. In particular, PDE6C, also known as cGMP-inhibited phosphodiesterase, is involved in light detection and cone phototransduction. PDE6C encodes the alpha-prime subunit of cone-specific phosphodiesterase, an enzyme found exclusively in the light-detecting (photoreceptor) cells called cones. Cone cells are found in a specialized tissue at the back of the eye known as the retina, and mutations in PDE6c are associated with eye diseases including Cone Dystrophy 4 and Achromatopsia.

The *PDE6C Assay Kit* is designed for identification of PDE6C inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE6C 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 PDE6C inhibitor screening assay kit comes in a convenient 96-well format, with purified PDE6C enzyme, fluorescently labeled PDE6 substrate (cGMP), binding agent, and PDE assay buffer for 100 enzyme reactions. The key to the *PDE6C Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE6C reactions. First, the fluorescently labeled cGMP is incubated with PDE6C for 1 hour. Second, the 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.

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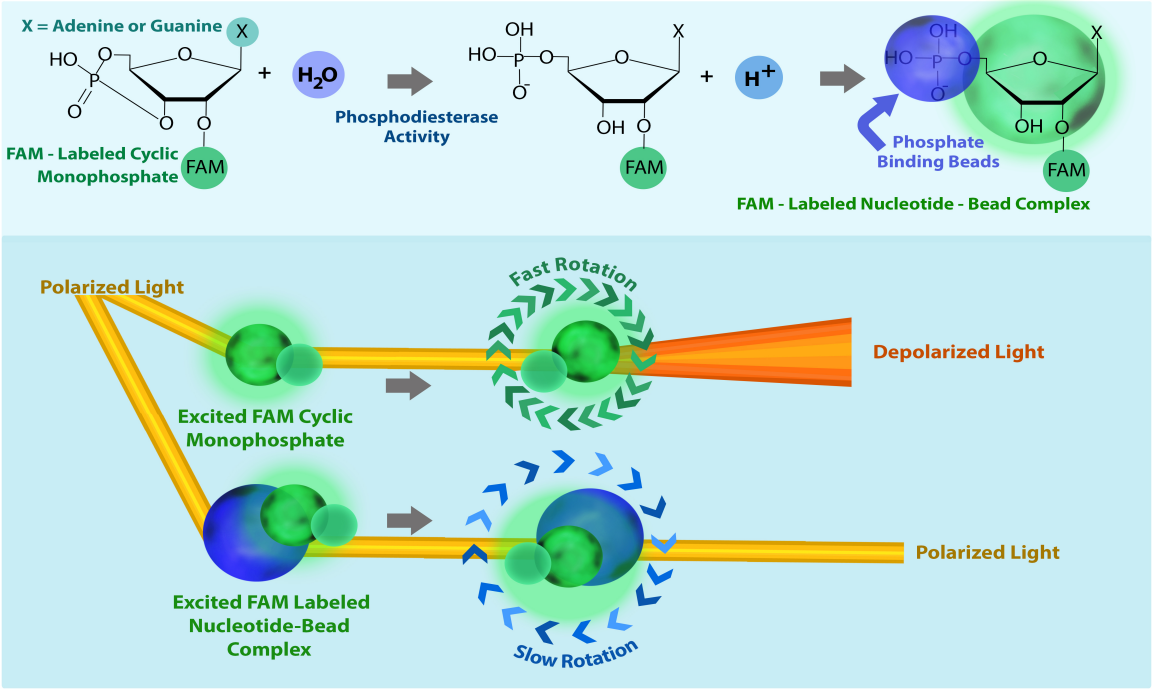

COMPONENTS:

Catalog #	Component	Amount	Storage	
60062	PDE6C	1 µg	-80°C	***(Avoid freeze/thaw cycles!)***
60201	FAM-Cyclic-3′, 5′-GMP: 20 µM	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
60390	Binding Agent	100 µl	+4°C	
60392	Binding Agent Diluent (cGMP)	10 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 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: Martinez S.E. *et al., J. Biol. Chem*. 2008 Sep 19; **283(38):**25913-9.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Dilute 20 µM FAM-Cyclic-3′,5′-GMP 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′-GMP (200 nM) to each well designated for the "Substrate Control", "Positive Control", and "Test Inhibitor". Add 25 µl of PDE assay buffer to each well designated for the "Blank".

3) Add 5 µl of inhibitor solution of 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 for the "Blank" and "Substrate Control".

5) Thaw PDE6C on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot PDE6C enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: PDE6C is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute PDE6C in PDE buffer to 25 pg/µl (500 pg/reaction) in PDE buffer*. Initiate reaction by adding 20 µl of diluted PDE6C 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 the plate at room temperature for 1 hour.

	Blank	Substrate Control	Positive Control	Test Inhibitor
FAM-Cyclic-3',5'-GMP (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	–
PDE6C (25 pg/µl)	–	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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Step 2:

1) Shake the tube containing the binding agent to ensure it is thoroughly mixed. 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 30 minutes with slow shaking.

3) Read the fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 485 ± 5 nm and detection of emitted light ranging from 528 ± 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".

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

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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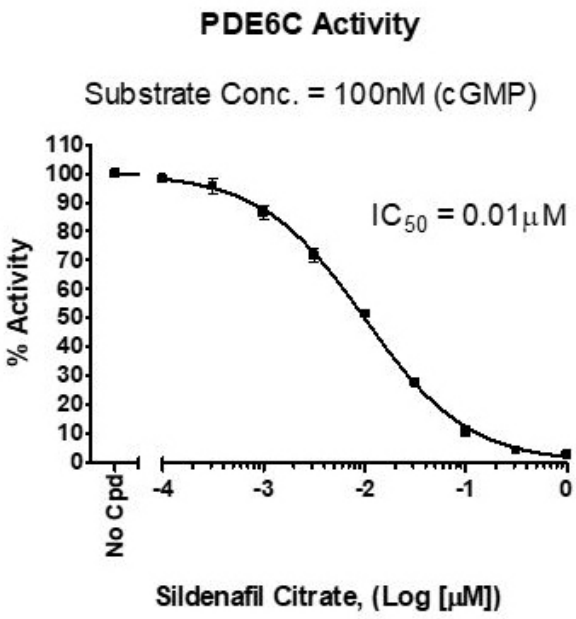
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE6C by Sildenafil Citrate and Tadalafil, measured using the PDE6C Assay Kit, BPS Bioscience # 79501. 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

RELATED PRODUCTS :

Product	Catalog #	Size
PDE6C, human	60062	5 µg
PDE6C, mouse	60065	5 µg
PDE5A1, human	60050	10 µg
PDE5A1, mouse	60051	10 µg
PDE 5A1 Assay Kit	60350	96 rxns.
PDE1B Assay Kit	60311	96 rxns.
PDE3B Assay Kit	60331	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cGMP Substrate	60201	100 nmol
Sildenafil Citrate	27105	25 mg
Tadalafil	27657	50 mg