CREATIVE BIOMART®
Assay Kit

PDE3A Assay Kit

Product Information

Common Name

PDE3A

Cat.No. Kit-1834

Product Overview

The PDE3A Assay Kit is designed for identification of PDE3A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE3A to the binding agent. The key to the PDE3A Assay Kit is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE3A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE3B for 1 hour. Second, 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.

Applications

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

Storage

At least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

Size

96 reactions

Kit Components

PDE3A recombinant enzyme: >1 µg; -80°C
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

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Black, low binding, microtiter plate: 1; Room temp.

Materials Required but Not Supplied

Fluorescent microplate reader capable of measuring fluorescence polarization
Adjustable micropipettor and sterile tips.
1,4-Dithiothreitol (DTT) 1 M in anhydrous DMSO.

Assay Protocol

Step 1:
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.
2) Dilute 1M 1,4-Dithiothreitol (DTT) 1:500 into the diluted FAM-Cyclic-3´,5´-AMP. For example, add 10 µl DTT (1M) to 5 ml of diluted FAM-Cyclic-3´, 5´-AMP (200 nM).
3) Add 25 µl of FAM-Cyclic-3´,5´-AMP (200 nM) to each well designated "Positive Control", "Test Inhibitor", and "Substrate Control".
4) 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".
5) 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 (PDE assay buffer).
6) Thaw PDE3A on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot PDE3A enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. Note: PDE3A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
7) Dilute PDE3A in PDE assay buffer to 2.5 pg/µl (0.05 ng/reaction)*. Initiate reaction by adding 20 µl of diluted PDE3A 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.
8) Incubate at room temperature for 1 hour.
Positive Test Substrate "Blank"

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	Control Inhibitor	Control 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	–	–
PDE assay buffer (no inhibitor)	5 µl	–	5 µl	5 µl
PDE3A (2.5 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 485 ± 5 nm and detection of emitted light ranging from 528 ± 10 nm. Blank value is subtracted from all other values.

Analysis

Definition of Fluorescence Polarization:

P =(Ia - Ib) /(Ia -+ Ib)

where Ia = Intensity with polarizers parallel and Ib= 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) = ([Ia]-G*[Ib]) /([Ia]+G*[Ib])* 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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