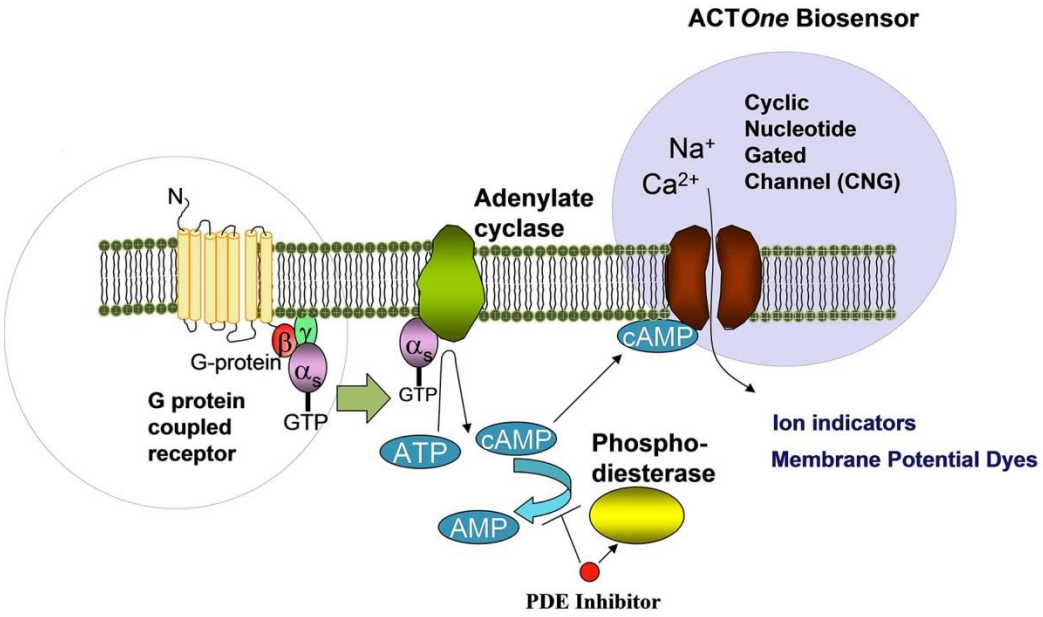
ACTOne™ Human Phosphodiesterase II A (PDE2A) Cell Line

Catalog Number: CL-03-PDE2A

Description

cAMP is a key second messenger involved extensively in cellular signal transduction pathways associated with the majority of G-protein coupled receptors (GPCRs). The activation of these GPCRs by neurotransmitters, lipids, nucleotides, peptides and hormones results in the activation or the inhibition of plasma membrane-bound adenylate cyclase through heterotrimeric G-proteins.

Human PDE2A ACTOne™ is a HEK293-CNG-Gs cell line that expresses human PDE2A. HEK293-CNG-Gs cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG-Gs cells (originally developed by BD Biosciences by introducing Gs-GPCR in HEK-293 CNG cells) (Cat# CL-03-PC10)

Gene/Enzyme

PDE2A (Genbank Locus ID 5138)

Applications

- cAMP dependent human PDE2A cell based assay
- cell based high-throughput screening of human PDE2A inhibitors

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Functional Test

- this cell line has been tested positive for PDE2A specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin and 5 µg/ml blasticidin.
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Assay materials not included

Membrane Potential Dye (10x) Kit (Fluorescent)	eENZYME CA-M165
Biocoat Poly-D-Lysine coated 384-well black/clear plate	BD 354663
Phosphodiesterase (PDE) inhibitor Ro 20-1724 (200 mM stock in DMSO, store at -20°C)	Sigma B8279
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma D8537
Bay 60-7550 (20 mM stock in DMSO, store at -20°C)	Cayman Chemical 10011135

Cell culture materials not included

DMEM, high glucose, with glutamine	Biosource International P104G-000
Fetal bovine serum	Invitrogen 26140-079
Trypsin-EDTA solution (10x)	Sigma T4174
G418 sulfate	Cellgro 61-234-RG
Puromycin	Clontech 8052-2
Blasticidin S HCl	Invitrogen R210-01

Cell Culture Protocol (provided with the cell line)

cAMP Assay Protocol (provided with the cell line)

- Kinetic assay with on-line compound addition
- Endpoint assay or kinetic assay with off-line compound addition

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Data Example

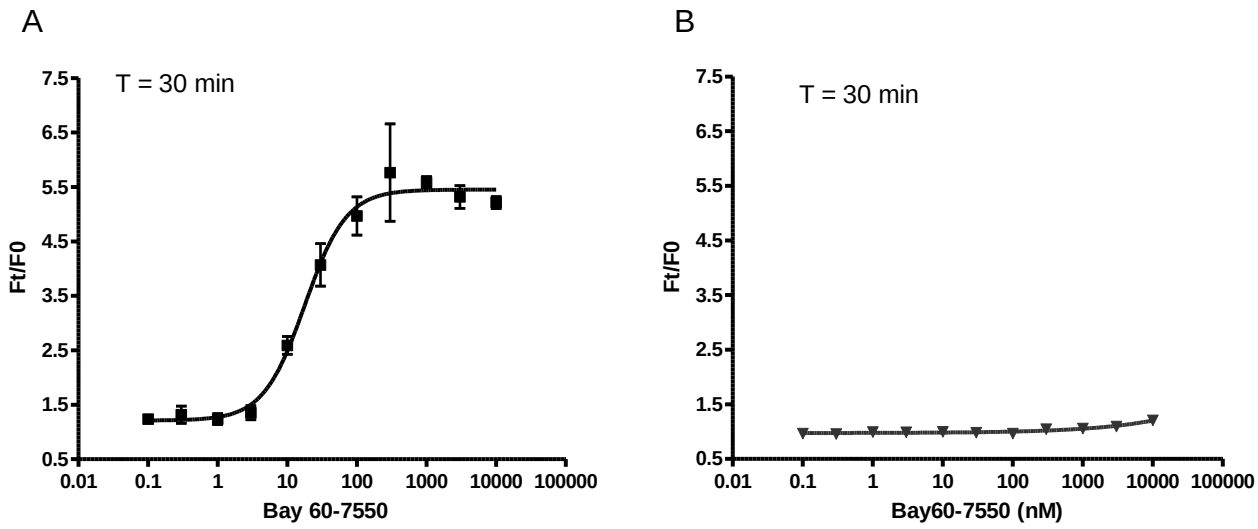

Figure 1. Response of ACT*One* PDE2A cell line & parental cell line to Bay 60-7550.

ACTOne PDE2A cells and parental cells (CL-03-PDE2A) were plated overnight in 20 μl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μl/well of membrane potential dye. After 2 hours of incubation at room temperature, baseline was recorded using a FlexStation (Molecular Devices) (F0). 10 μl of PDE inhibitors at various concentrations were added to the cell plate, and the data was recorded 30 minutes (Ft) after drug addition. Dose response curves were generated by Prism.

A. Dose response curve of Bay 60-7550 in ACT*One* PDE2A cell line. EC50 = 17.8 nM in the presence of 10 μM of Ro20-1724

B. Parental cells do not respond to Bay 60-7550 in the presence of 10 μM of Ro20-1724

Notice to Purchaser

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