

Phosphodiesterase 4A (PDE4A) ACTOne™ Stable Cell Line

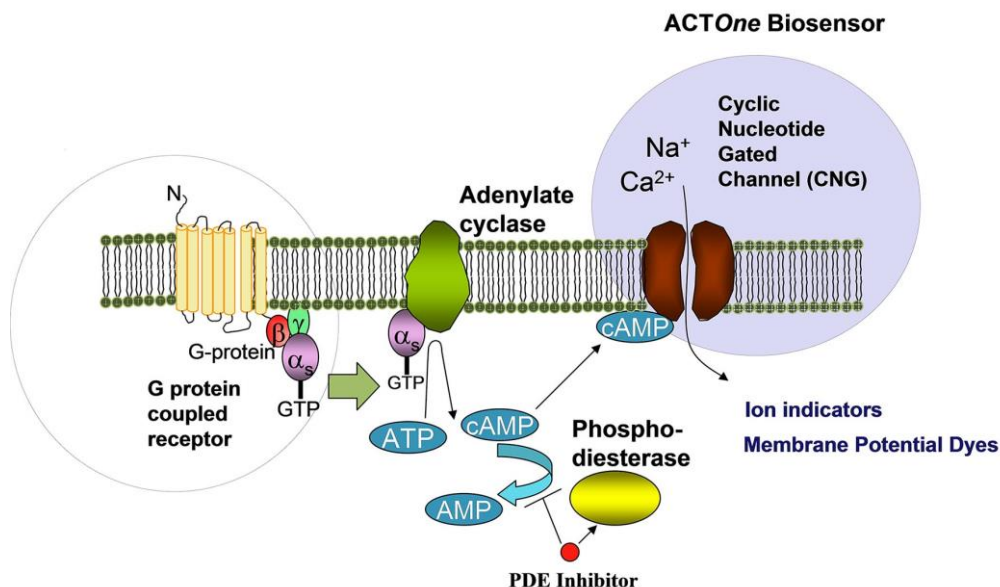
CATALOG NUMBER: CL-02-PDE4B

Introduction

Phosphodiesterase 4A belongs to the cyclic nucleotide phosphodiesterase (PDE) family. This PDE4B hydrolyzes specifically the second messenger cAMP, which is a regulator and mediator of a number of cellular responses to extracellular signals. Thus, by regulating the cellular concentration of cAMP, this protein plays a key role in many important physiological processes.

Description

Human PDE4A ACTOne™ is a CHO-K1-CNG cell line that expresses human PDE4A. CHO-K1--CNG 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

CHO-K1-CNG cells (originally developed by BD Biosciences) (Cat# CL-02-PC30)

Gene/Enzyme Introduced

PDE4A (Genbank No. NP_001104777)

Applications

- cAMP dependent human PDE4A cell based assay
- cell based high-throughput screening of endogenous human PDE4A inhibitors

Functional Test

- this cell line has been tested positive for PDE4A 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 (CL-02-PDE4A): 1 mL (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

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.

Cell Culture Medium

- DMEM/Nutrient F-12 Ham-10% FBS supplemented with 250 $\mu\text{g/ml}$ G418, 1 $\mu\text{g/ml}$ Puromycin and 5 $\mu\text{g/ml}$ blasticidin.
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Growth Properties

- Adherent

Data Analysis

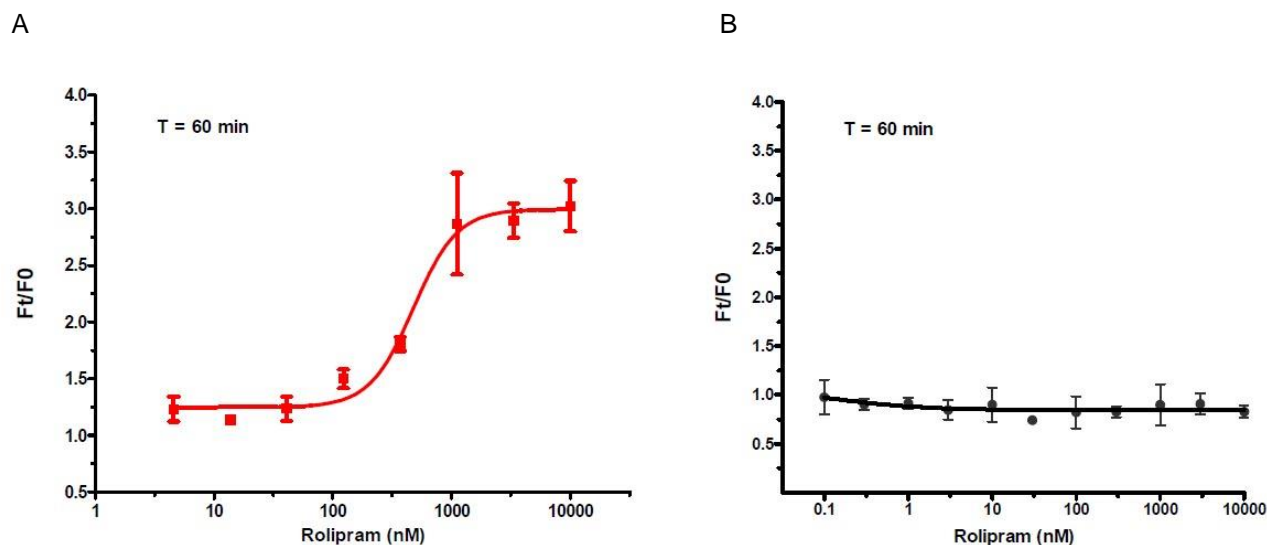


Figure 1. Response of ACTOne™ cAMP-PDE4A cell line & parental cell line to Rolipram

ACTOne™ cAMP-PDE4A cells and parental cells (Cat# CL-02-PC30) were plated overnight in 20 μl culture medium on a 384 well microplate. The next day, cells were dye-loaded with 20 μl /well of membrane potential dye (Cat# CA-M165). 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 45 minutes (F_t) after drug addition. Dose response curves were generated by Prism.

- Dose response curve of Rolipram in ACTOne™ cAMP-PDE4A cell line. IC₅₀ = 470 nM in the presence of 3 μM of Forskolin
- Parental cells do not respond to Rolipram in the presence of 3 μM of Forskolin

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