

Phosphodiesterase 4B (PDE4B) ACTOne™ Stable Cell Line

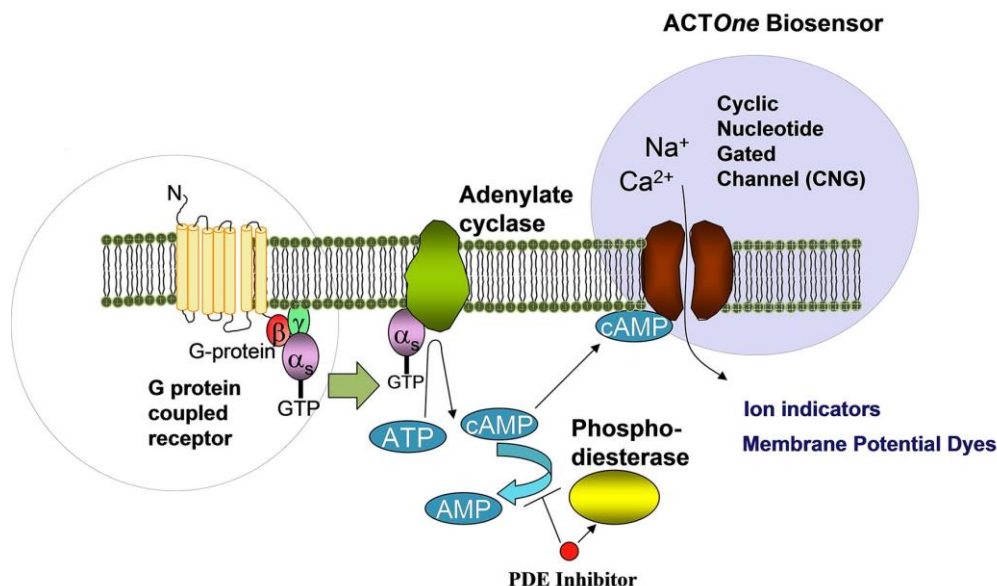
CATALOG NUMBER: CL-02-PDE4B

Introduction

Phosphodiesterase 4B belongs to the cyclic nucleotide phosphodiesterase (PDE) family. This PDE4B hydrolyzes specifically the second messenger cAMP, which is a regulator and mediator of several 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 PDE4B ACTOne™ is a CHO-K1-CNG cell line that expresses human PDE4B. 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

PDE4B (Genbank No. NP_002591.2)

Applications

- cAMP dependent human PDE4B cell based assay
- cell based high-throughput screening of endogenous human PDE4B inhibitors

Functional Test

- this cell line has been tested positive for PDE4B 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 10⁶ cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Cell Culture Medium

- DMEM/Nutrient F-12 Ham-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

Growth Properties

- Adherent

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.

Data Analysis

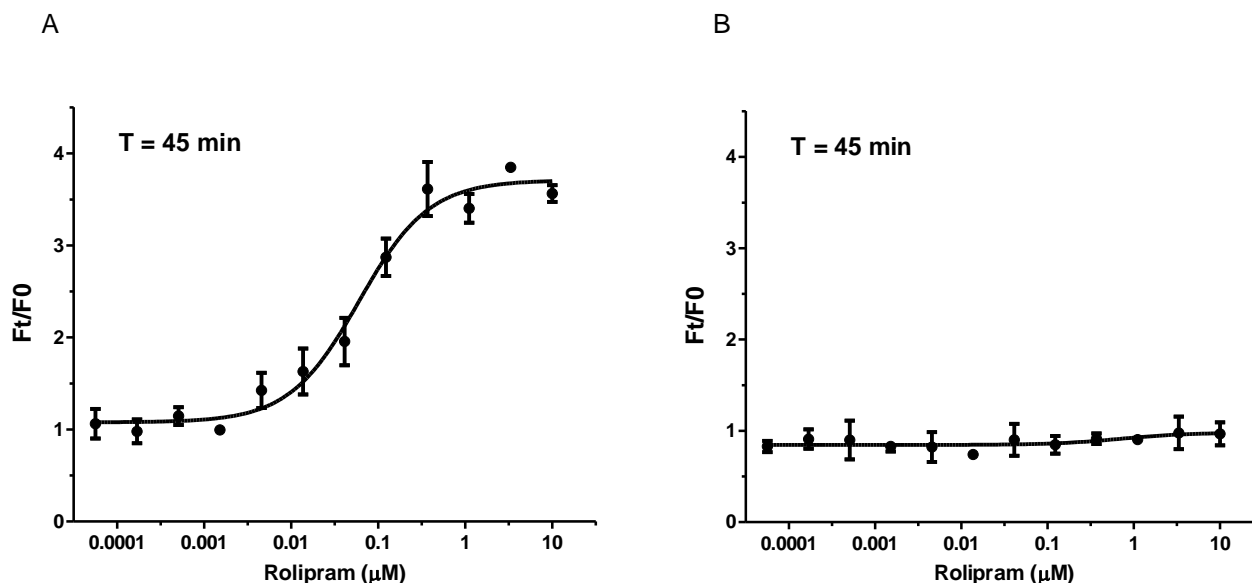


Figure 1. Response of ACTOne™ cAMP-PDE4B cell line & parental cell line to Rolipram

ACTOne™ cAMP-PDE4B 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 (Ft) after drug addition. Dose response curves were generated by Prism.

- A. Dose response curve of Rolipram in ACTOne™ cAMP-PDE4B cell line. IC50 =60.3 nM in the presence of 3 µM of Forskolin.**
- B. Parental cells do not respond to Rolipram in the presence of 3 µM of Forskolin.**

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