Phosphodiesterase 4C (PDE4C) ACTOne™ Stable Cell Line

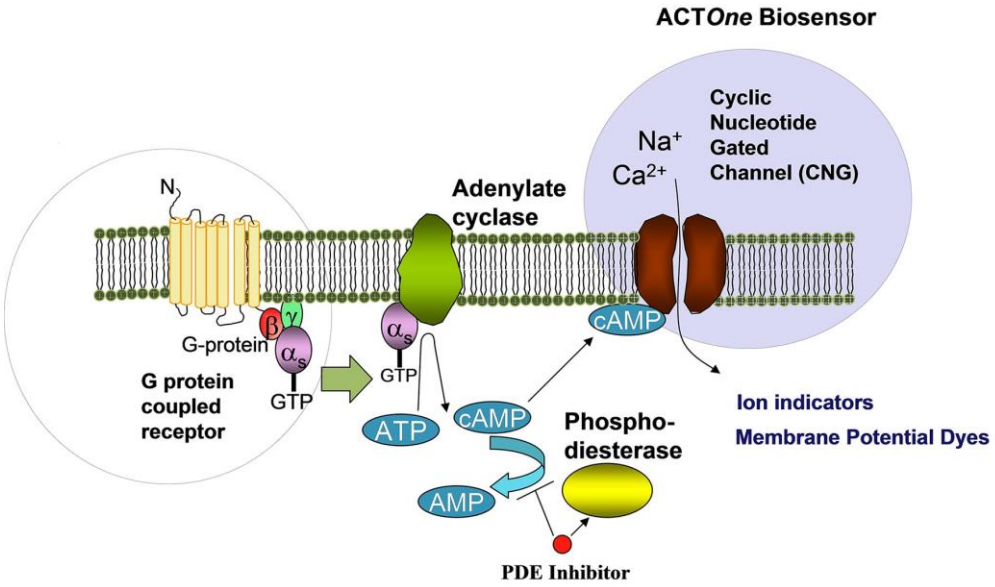
CATALOG NUMBER: CL-02-PDE4C

Introduction

The PDE4C receptor, also known as the Phosphodiesterase 4C receptor, is a member of the cyclic nucleotide phosphodiesterase (PDE) family, and PDE4 subfamily. This PDE hydrolyzes 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 PDE4C ACTOne™ is a CHO-K1-CNG cell line that expresses human PDE4C. 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

UniProtKB/Swiss-Prot for PDE4C Gene: PDE4C_HUMAN,Q08493

Applications

- cAMP dependent human PDE4B cell based assay
- cell based high-throughput screening of human PDE4C agonists/antagonists

Functional Tests

- 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 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Cell Culture Medium

- DMEM-F12 plus 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.

Assay materials not included：

Elite™ Membrane Potential Dye Kit	EENZYME Cat# CA-M165
Biocoat Poly-D-Lysine coated 384-well black/clear plate	BD 354663
Phosphodiesterase (PDE) inhibitor Rolipram (50mM stock in DMSO, store at -20°C)	
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma D8537
Isoproterenol (10mM stock in H2O)	Sigma I6504
Forskolin	

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
Blasticidi	

Data Analysis

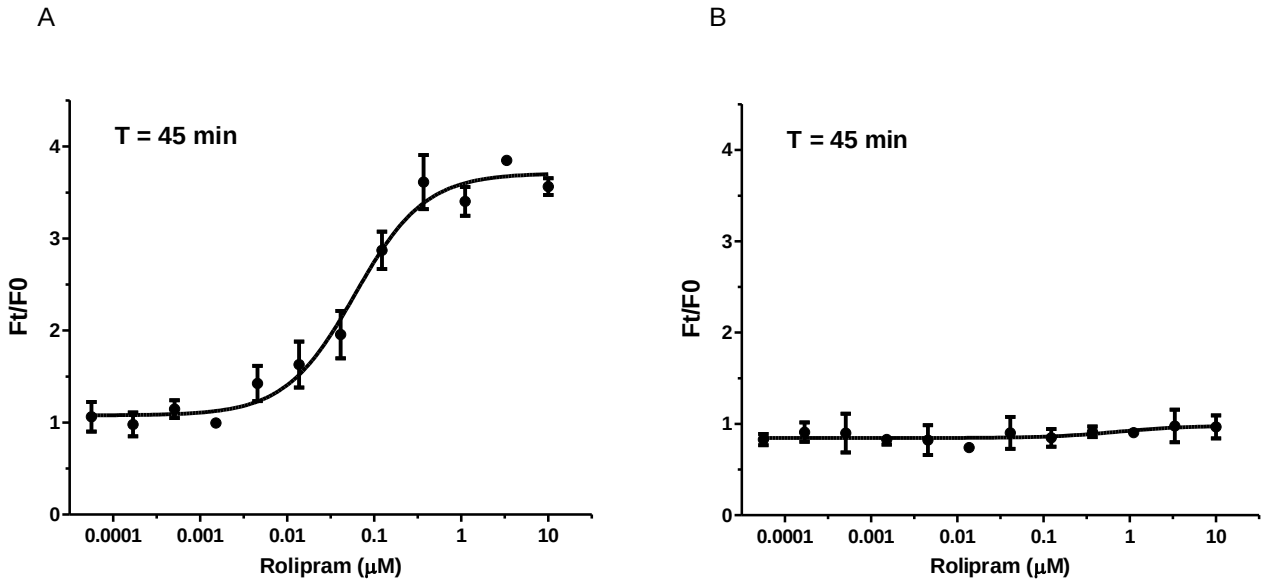


Figure 1. Response of ACT*One*™ cAMP-PDE4B cell line & parental cell line to Rolipram

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 ACT*One*™ 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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