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Phosphodiesterase 4 (PDE4) ACTOne™ Stable Cell Line

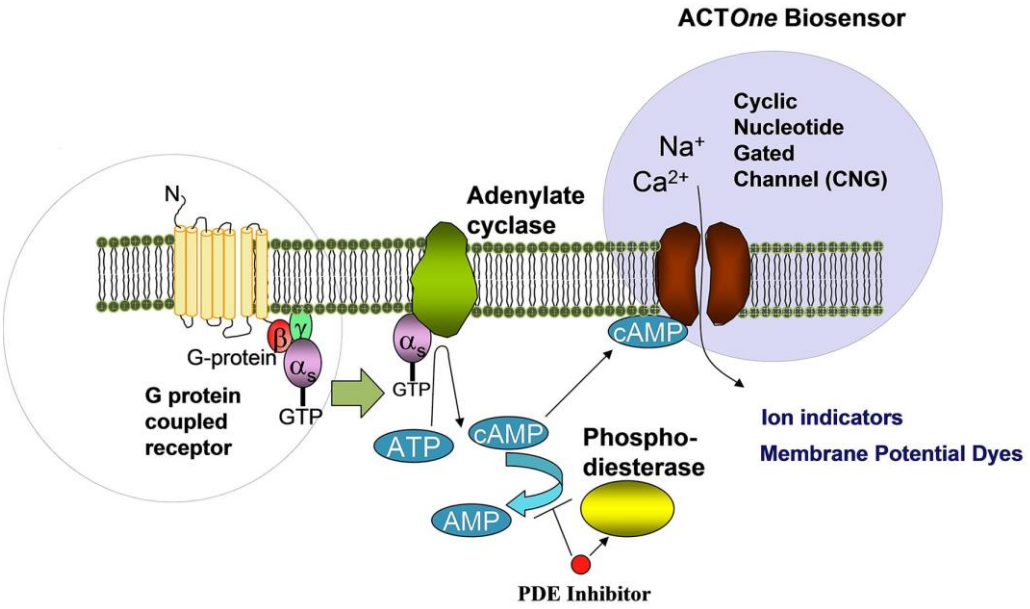
CATALOG NUMBER: CL-03-PDE4

Introduction

Phosphodiesterase 4 belongs to the cyclic nucleotide phosphodiesterase (PDE) family. 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 PDE4 ACTOne™ is a HEK293-CNG cell line that expresses endogenous human PDE4 (mainly 4B&4D). HEK293-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-M145). 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 cells (originally developed by BD Biosciences) (Cat# CL-03-PC20)

Gene Introduced

Constitutively active Gs coupled GPCR gene

Applications

- cAMP dependent human PDE4 cell based assay
- cell based high-throughput screening of endogenous human PDE4 inhibitors

Functional Test

- this cell line has been tested positive for PDE4 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-03-PC10): 1 mL (1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)
- Parent cells (CL-03-PC20): 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

Subculturing Procedure

1. Thaw the frozen cryovial of cells within 1-2 min by gentle agitation in a37 ^{o}C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% ethanol and transfer into a 75 cm^2 flask with 20 ml of complete DMEM growth medium.
2. Remove and discard culture medium next day, and then add fresh DMEM complete medium.
3. Monitor cell density daily. Cells should be passaged (1:3) when the culture reaches 90% confluence. Expected cell yield is between 1.5 x 10^5 and 2x 10^5 viable cells/cm^2.
4. Add 2.0 to 3.0 mL of 0.25% (w/v) trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 15 to 20 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.
5. Transfer cell suspension to a 15mL centrifuge tube and spin at approximately 250 x *g* for 5 to 10 minutes.
6. Discard supernatant and re-suspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 4 to 6 x 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C (5% CO_2).

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.

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Data Analysis

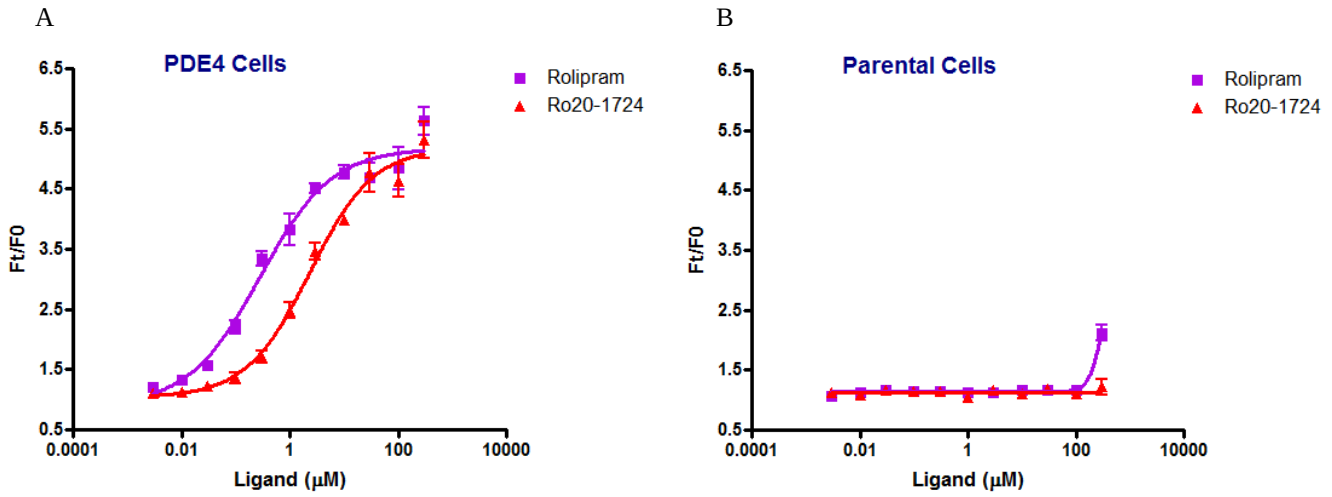

Figure 1. Response of ACT*One*™ PDE4 cell line & parental cell line to Rolipram and Ro20-1724.

ACTOne™ PDE4 cells and parental cells (CL-003-PC20) 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 (CA-M145). After 2 hour 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 curves of Rolipram and Ro20-1724 in ACT*One*™ PDE4 cell line. EC50 = 0.28 μM for Rolipram and EC50 = 2.34 μM for Ro20-1724.

B. Parental cells do not respond to Rolipram or Ro20-1724.

Notice to Purchaser

This cell line is to be used for research purposes only. It may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of eEnzyme LLC.