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Phosphodiesterase 1B (PDE1B) ACTOne™ Stable Cell Line

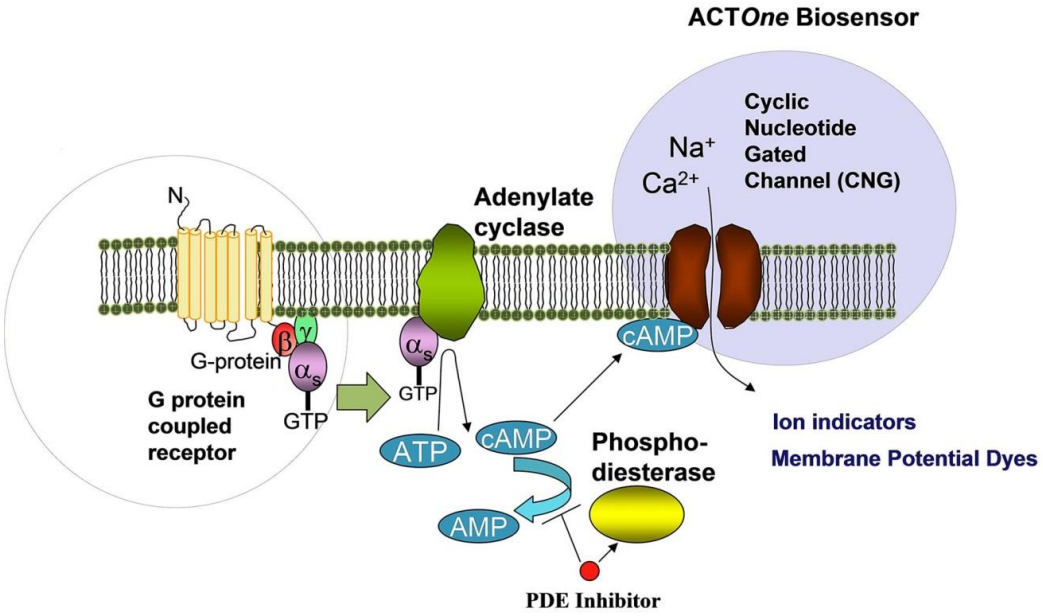
CATALOG NUMBER: CL-03-PDE1B

Introduction

The protein encoded by PDE1B gene belongs to the cyclic nucleotide phosphodiesterase (PDE) family, and PDE1 subfamily. Members of the PDE1 family are calmodulin-dependent PDEs that are stimulated by a calcium-calmodulin complex. This PDE has dual-specificity for the second messengers, cAMP and cGMP, with a preference for cGMP as a substrate. cAMP and cGMP function as key regulators of many important physiological processes.

Description

Human PDE1B ACTOne™ is a HEK293-CNG-Gs cell line that expresses human PDE1B. 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-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-Gs cells (originally developed by BD Biosciences by introducing Gs-GPCR in HEK-293 CNG cells) (Cat# CL-03-PC10)

Gene/Enzyme

PDE1B (Genebank Accession No. NP_000915.1)

Applications

- cAMP dependent human PDE1B cell based assay
- cell based high-throughput screening of human PDE1B inhibitors

Functional Test

- this cell line has been tested positive for PDE1B 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)
- Parental 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

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 resuspend 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.

Data Analysis

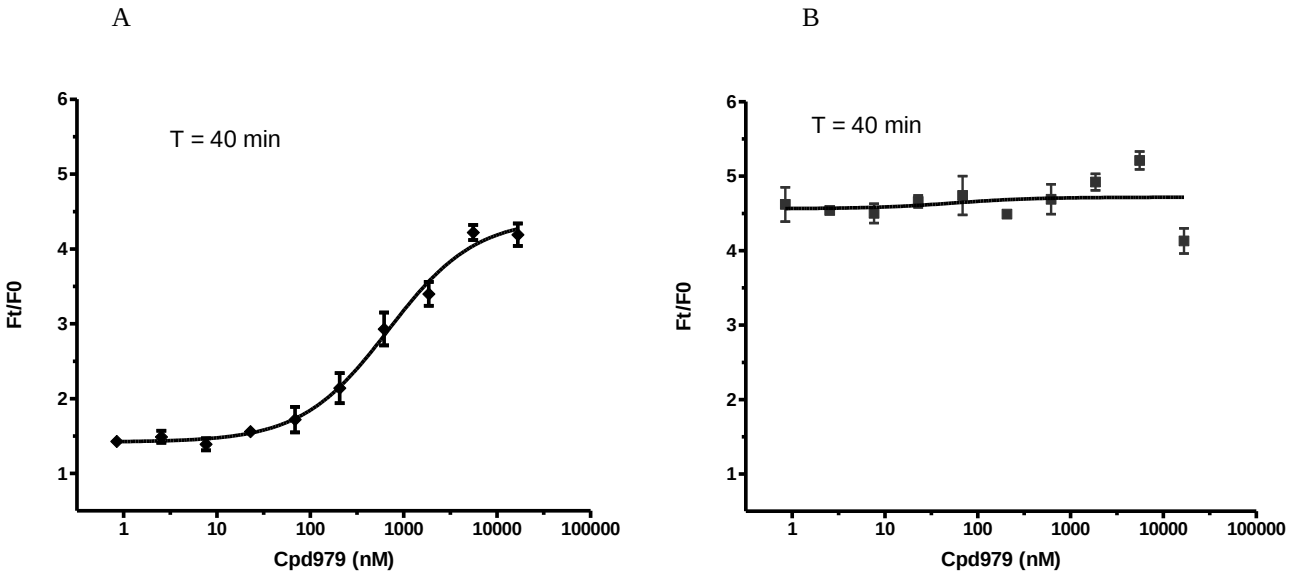


Figure 1. Response of ACT*One*™ PDE1B cell line & parental cell line to Cpd979.

ACTOne™ PDE1B cells and parental cells (Cat# CL-03-PC10) 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 (Cat# 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 (with 0.5 µM Forskolin) were added to the cell plate, and the data was recorded 40 minutes (Ft) after drug addition. Dose response curves were generated by Prism.

A. **Dose response curve of Cpd979 in ACT*One*™ PDE1B cell line. IC50 = 684 nM in the presence of 0.1µM of Forskolin**
B. **Parental cells do not respond to Cpd979 in the presence of 0.1 µM of Forskolin**

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.