

## Phosphodiesterase 4D (PDE4D) ACTOne™ Stable Cell Line

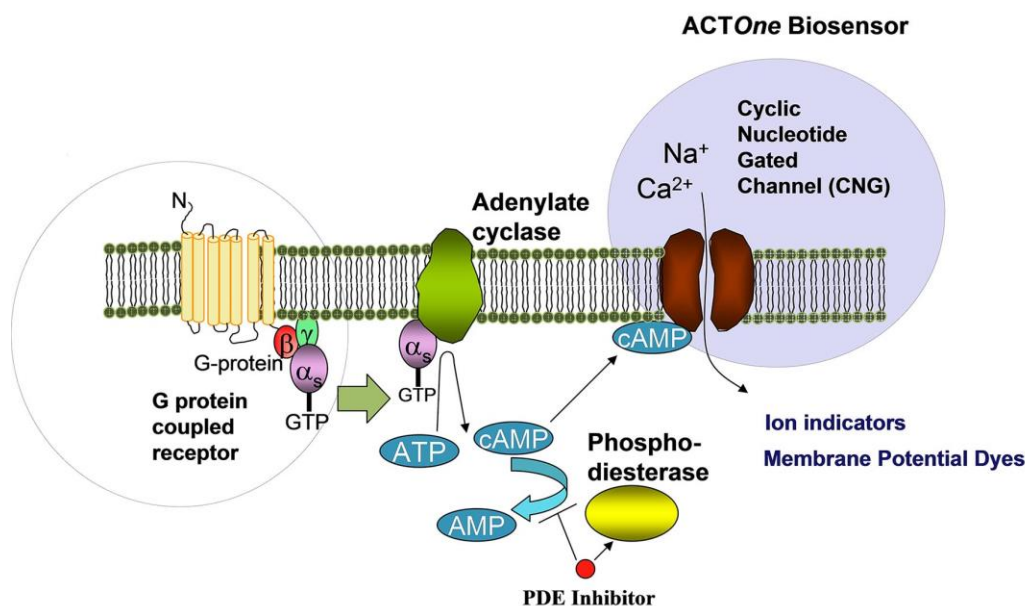
CATALOG NUMBER: CL-02-PDE4D

### Introduction

Phosphodiesterase 4D 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 PDE4D ACTOne™ is a CHO-K1-CNG cell line that expresses human PDE4D. 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

PDE4D (Genbank No. NP\_001184152.1)

### Applications

- cAMP dependent human PDE4D cell based assay
- cell based high-throughput screening of endogenous human PDE4D inhibitors

### Functional Test

- this cell line has been tested positive for PDE4D 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-PDE4D): 1 mL ( $1 \times 10^6$  cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

### Cell Culture Medium

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

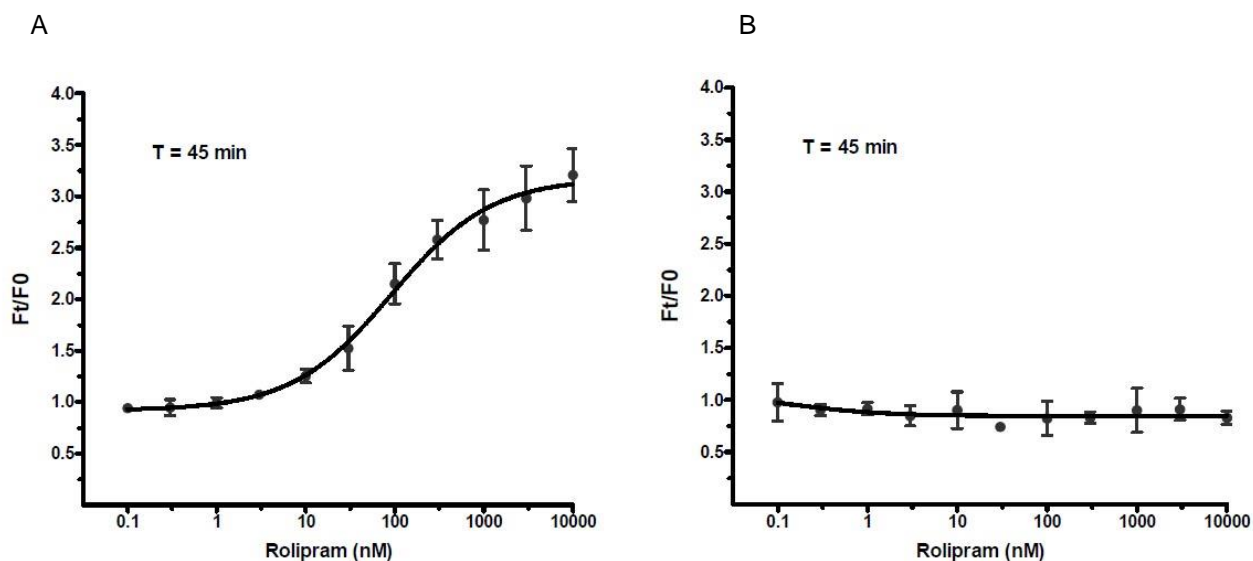
### Growth Properties

- Adherent

### Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

### Data Analysis



**Figure 1. Response of ACTOne™ cAMP-PDE4D cell line & parental cell line to Rolipram**

ACTOne™ cAMP-PDE4D cells and parental cells (Cat# CL-02-PC30) were plated overnight in 20  $\mu$ l culture medium on a 384 well microplate. The next day, cells were dye-loaded with 20  $\mu$ l/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  $\mu$ l 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.

- Dose response curve of Rolipram in ACTOne™ cAMP-PDE4D cell line. IC50 = 92 nM in the presence of 3  $\mu$ M of Forskolin
- Parental cells do not respond to Rolipram in the presence of 3  $\mu$ M of Forskolin

### Notice to Purchaser

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