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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Phosphodiesterase 4D (PDE4D) ACTOne™ Stable Cell Line

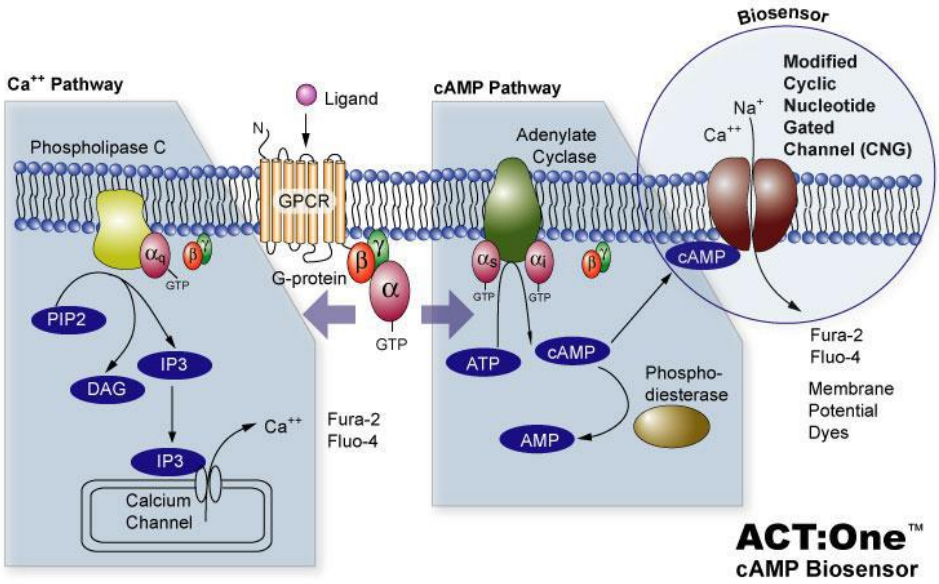
CATALOG NUMBER: CL-02-PDE4D

Introduction

The PDE4D receptor, also known as the Phosphodiesterase 4D 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 PDE4D ACTOne™ is a CHO-K1 CNG cell line that expresses recombinant 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 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 (Cat# CL-02-PC30)

Gene/Enzyme Introduced

UniProtKB/Swiss-Prot: Q08499.2

Applications

- cAMP dependent human PDE4D cell based assay
- cell based high-throughput screening of human PDE4D agonists/antagonists

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Functional Tests

- 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: 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

- Growth medium (**for PDE4D Cells**): DMEM-F12 plus 10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin and 5 µg/ml blasticidin
- Growth medium (**for Control Cells**): DMEM-F12 plus 10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin and 300 µg/ml hygromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

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
Blasticidin	

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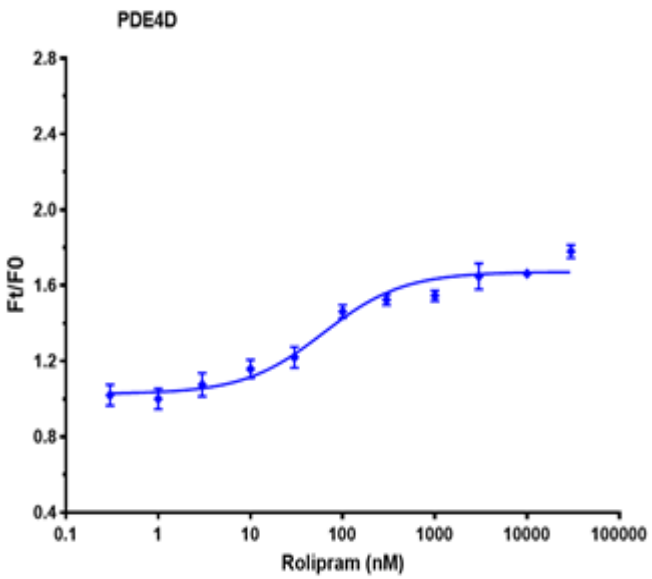
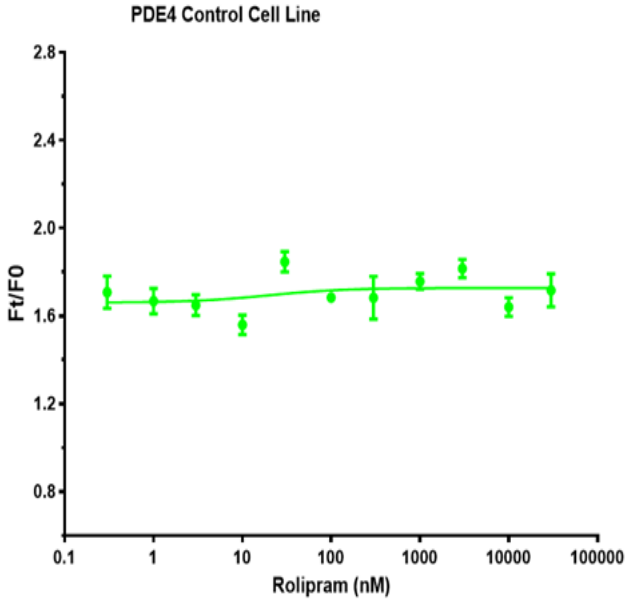
DATA EXAMPLE

A



	PDE4D
Bottom	1.027
Top	1.672
LogEC50	1.792
EC50	61.89
Span	0.6452

B



	PDE4B-shRNA
Bottom	1.661
Top	1.727
LogEC50	1.299
EC50	19.93
Span	0.06684

Figure 1. Response of ACTOne PDE4D cells & parental cells to Rolipram

ACTOne PDE4D cells and parental cells were plated overnight in 20 µl culture medium on a BD Biocoat 384 well plate. The next day, cells were dye-loaded with 20 µl/well of 1X Dye-loading solution (Membrane Potential Assay Kit # 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 PDE4D cell line 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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TROUBLESHOOTING GUIDE

1. **Low survival rate of cells after thawing**
   - Cell vials could have thawed accidentally. Store cell vials in liquid nitrogen immediately after receiving and keep frozen at all time.
   - Leaving the vial at 37°C for too long during thawing will lower the survival rate. Place the vial at 37°C until cells are just thawed.
   - Handle the cells gently. Don't tap the vial or pipette the cells too many times before plating the cells.
   - Replace the medium four hours after thawing or when the cells have settled to remove DMSO.

2. **Slow growth rate of cells**
   - Do not split cells before they have completely recovered from thawing and reach at least 50% confluence**.**
   - Do not dilute cells excessively while splitting.
   - Spit cells before they reach 80 – 90% confluence.
   - Use Trypsin-EDTA solution to dissociate cells.
   - Cells may not be able to recover to an optimal stage if trypsin- free dissociation buffer is used.

3. **High baseline fluorescent signal**
   - Inspect the cell density and morphology under a microscope. High cell density or unhealthy cells can result in high baseline signal.
   - Do not remove serum-containing medium from cell plates before dye-loading. If a serum-free environment is required, use DPBS buffer containing 0.2 to 0.5% BSA to replace medium.

4. **Response to agonist is lower than expected**
   - Check the overall health of cells.
   - Cell density is too high or too low. Cell number titration may be necessary.
   - Keep cells growing in medium with proper drug selection.
   - Check settings of fluorescence readers.

5. **High well-to-well variations.**
   - Cells should be evenly distributed among wells. Before plating, microscopically examine the culture to be sure that they have been dissociated into single cells. Leave the cell plates at room temperature for 30 minutes prior to transferring the plates to a cell culture incubator.
   - Check the liquid handling system for dispensing accuracy. Optimize the settings of liquid handling system so that cell monolayer is not disturbed by dye and compound addition.
   - Check settings of fluorescence readers.

6. **Response from cells after the addition of buffer containing only DMSO**
   - Keep the final DMSO concentration below 1%.

Notice to Purchaser

1. 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.
2. Refer to the license agreement for details on the usage restrictions.