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Data Sheet

PDE4D Cell-Based Activity Assay Kit

Catalog #60505

Description

Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cGMP signaling. PDE4D has 3',5'-cyclic-AMP phosphodiesterase activity and degrades cAMP. Inhibition of PDE4D activity by its inhibitors leads to an elevated intracellular level of cAMP. The PDE4D gene encodes at least 9 different isoforms, and has been linked to stroke, asthma, arrhythmia, and cardiac myopathy, making it an important therapeutic target.

The PDE4D cell-based activity assay is designed for screening inhibitors of PDE4D7 in cultured cells. The assay is based on transfecting cells with the CRE luciferase reporter. CRE reporter contains the firefly luciferase gene under the control of cAMP response element (CRE). Elevation of intracellular cAMP activates CRE binding protein (CREB) to bind CRE and induce the expression of luciferase. Forskolin is commonly used to raise the intracellular level of cAMP in cell physiology studies. When cells transiently transfected with CRE reporter are activated by forskolin, the intracellular level of cAMP is upregulated, which induces the expression of CRE luciferase reporter. However, when cells are co-transfected with PDE4D7 expression vector and CRE reporter, the level of forskolin-induced cAMP is reduced, resulting in lower expression level of luciferase. When cells are treated with PDE4D inhibitor to inhibit PDE4D7 activity, cAMP level is restored, resulting in higher luciferase activity.

The kit includes CRE luciferase reporter (premixed with constitutively-expressing *Renilla* (sea pansy) luciferase vector that serves as an internal control for transfection efficiency), PDE4D7 expression vector, and forskolin.

Applications

- Screen PDE4D inhibitors for drug discovery.
- Monitor cAMP/PDE4D signaling pathway activity

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	CRE luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 μl (60 ng DNA/ μl)	-20°C
PDE4D7 expression vector (Component B)		250 μl (80 ng DNA/ μl)	-20°C
Forskolin	10 mM, in DMSO	25 μl	-20°C

These vectors are designed for transient transfection. They are NOT suitable for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture-treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.]
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Dual luciferase assay system:
  Dual-Glo® Luciferase Assay System (Promega #E2920): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
  OR
  Dual-Luciferase® Reporter Assay System (Promega #E1910): This system reqired cell lysis step. It is ideal for luminometer with automated injectors.
- Luminometer

Assay Protocols

The following procedure is designed for transfection of HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000, follow the manufacture's recommended transfection protocol. Transfection condition should be optimized according to the cell type.

All amounts and volumes in the following protocol are provided on a per well basis.

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1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, transiently transfect the cells with CRE reporter and PDE4D7 expression vectors. For each well, prepare complexes as follows:
   a. Dilute 1 µl of Reporter (component A) and 0.5 µl of PDE4D7 expression vector (component B) in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently.
   b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.
   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.
   d. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate. Incubate cells at 37°C in a CO_2 incubator for 6 hours.

Note: we recommend setting up the assay in at least triplicate for each treatment. To minimize pipetting errors, prepare a master mix of sufficient transfection cocktail for multiple wells.

3. After ~6 hours, remove cell medium from transfected cells and replace with 50 µl of fresh growth medium containing PDE4D inhibitor. The final DMSO concentration should not exceed 0.3%. Incubate cells overnight at 37°C in a CO_2 incubator.

4. After ~22-24 hours, add forskolin (final concentration 10 µM) in 5 µl of growth medium to stimulated wells (cells treated with forskolin, with or without inhibitor). Add 5 µl of growth medium with 1% DMSO to the unstimulated control wells (cells without inhibitor and forskolin, for determining the basal activity). Add 55 µl of growth medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

5. Incubate at 37°C in a CO_2 incubator for 5-6 hours.

6. Perform dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 50 µl of Luciferase reagent per well. Rock gently at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Add 50 µl of Stop & Glo™ reagent per well, rock at room temperature for ~15 minutes, then measure *Renilla* luminescence.

7. To obtain the normalized luciferase activity of CRE reporter, subtract background luminescence, then calculate the ratio of firefly luminescence from the CRE reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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Figure 1. PDE4D7 reduces the level of cAMP following forskolin stimulation. This effect is reversed by Rolipram, a PDE4 inhibitor. The data are shown as fold induction of normalized CRE luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without forskolin treatment.

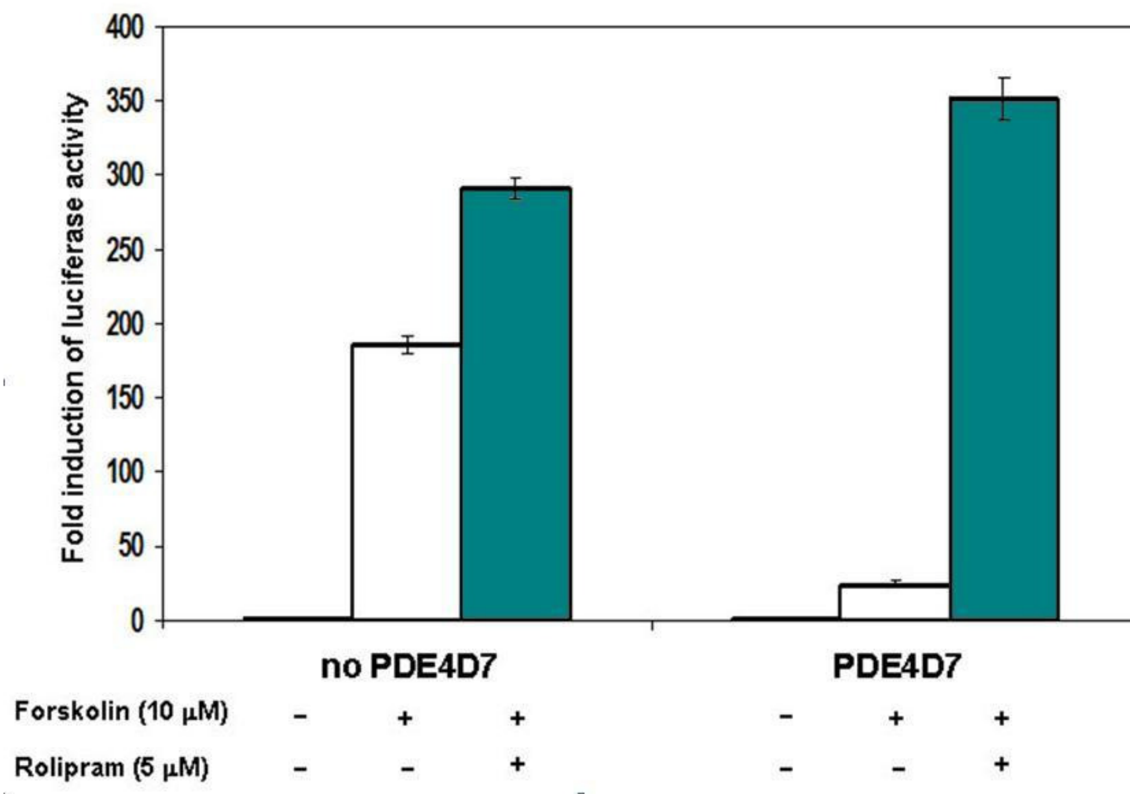

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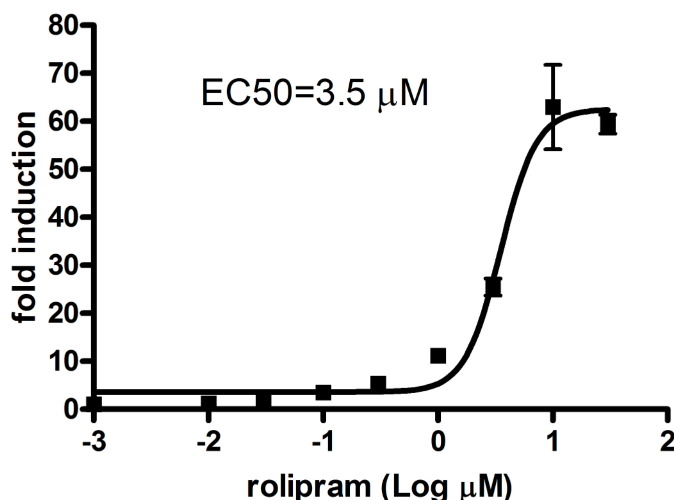
Figure 2. Rolipram dose response in PDE4D7-transfected HEK293.
The results were shown as fold induction of CRE reporter activity. Fold induction was determined by comparing values against the mean value for cells stimulated with forskolin in the absence of rolipram. The inhibition of PDE4D7 in cells induces the luminescence, so the inhibitory effects of the compounds on PDE4D7 activity is expressed as EC_{50}. The EC_{50} of rolipram is ~ 3.5 μM.



References

Montminy MR *et al.* (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature* **328(6126):**175-178

Fan Chung, K. (2006) Phosphodiesterase inhibitors in airways disease. *Eur. J. Pharmacol.* **533(1-3):**110-117

Malik, R. *et al.* (2008) Cloning, stable expression of human phosphodiesterase 7A and development of an assay for screening of PDE7 selective inhibitors. *Appl. Microbiol. Biotechnol.* **77 (5):** 1167-1173

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