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Data Sheet
PDE4B Cell-Based Activity Assay Kit
Catalog #79526

Description

Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cGMP signaling. PDE4B has 3',5'-cyclic-AMP phosphodiesterase activity and degrades cAMP. Inhibition of PDE4B activity by its inhibitors leads to an elevated intracellular level of cAMP. The PDE4B gene encodes at least 4 different isoforms, and has been linked to inflammation in monocytes by its regulation of the toll-receptor pathway. It is also highly expressed in the central nervous system, and has been targeted as a potential treatment for autism, depression, schizophrenia, and other conditions.

The PDE4B cell-based activity assay kit is designed for screening inhibitors of PDE4B1 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 PDE4B1 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 PDE4B inhibitor to inhibit PDE4B1 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), and a PDE4B1 expression vector.

Applications

- Screen PDE4B inhibitors for drug discovery.
- Monitor cAMP/PDE4B 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
PDE4B1 expression vector (Component B)		250 µl (40 ng DNA/ 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)
- Forskolin (Sigma Aldrich #F3917)
- TWO-step luciferase assay system:
  TWO-Step Luciferase (Firefly & Renilla) Assay System (BPS Bioscience #60683): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
- 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 manufacturer'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 PDE4B1 expression vectors. For each well, prepare complexes as follows:
   a. Dilute 1 µl of Reporter (component A) and 0.5 µl of PDE4B1 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 PDE4B 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 TWO-step luciferase assay using TWO-Step Luciferase (Firefly & Renilla) Assay System (BPS Bioscience #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and 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. PDE4B1 reduces the level of cAMP following forskolin stimulation.

This effect is reversed by Apremilast, 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.

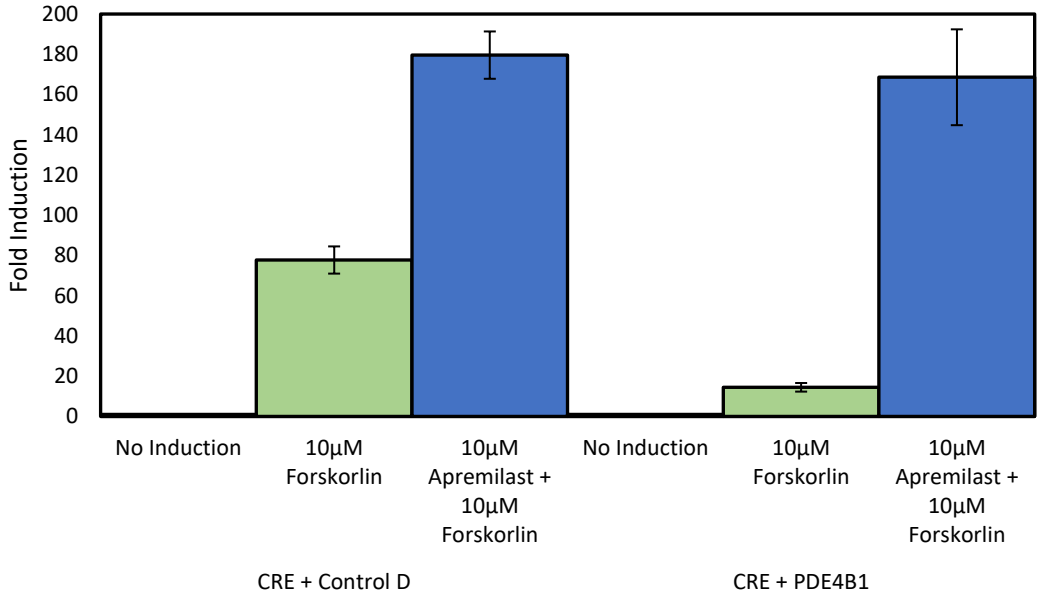

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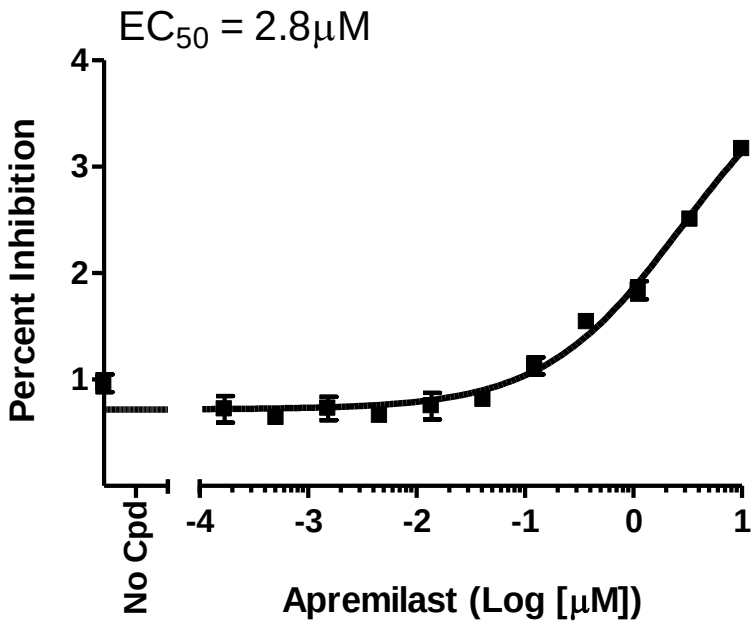
Figure 2. Apremilast dose response in PDE4B1-transfected HEK293.

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 Apremilast. The inhibition of PDE4B1 in cells induces the luminescence, so the inhibitory effects of the compounds on PDE4B1 activity is expressed as EC_{50}. The EC_{50} of Apremilast is ~2.8 μM.



References

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Fox, David, Alex B. Burgin, and Mark E. Gurney. 2014. "Structural Basis for the Design of Selective Phosphodiesterase 4B Inhibitors." *Cellular Signaling* **26 (3):** 657–663. PMC. Web. 14 Aug. 2018.

Zhang, Chong et al. 2017. "Comparison of the Pharmacological Profiles of Selective PDE4B and PDE4D Inhibitors in the Central Nervous System." *Scientific Reports* **7:** 40115.

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Related Products

Product	Cat. #	Size
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.
PDE4B1 Assay Kit	79558	96 rxns.
PDE4B2 Assay Kit	60343	96 rxns.
PDE4B3 Assay Kit	79574	96 rxns.
PDE4B (Dog) Assay Kit	79573	96 rxns.
PDE4B (Rat) Assay Kit	79571	96 rxns.
PDE4B1, GST-Tag	60041	10 µg
PDE4B2, GST-Tag	60042	10 µg
PDE4B3, GST-Tag	11675	5 µg
PDE4B (Dog), GST-Tag	60049	5 µg
PDE4B (Rat), GST-Tag	60055	5 µg
PDE4D2, GST-Tag	60048	5 µg
PDE4D3, GST-Tag	60046	5 µg
PDE4D7, GST-Tag	60047	5 µg
PDE4A1A, GST-Tag	60040	10 µg
PDE4B1, GST-Tag	60041	10 µg
PDE4C1, GST-Tag	60044	10 µg
Transfection Collection™	79286	500 rxns.
PDE4D Transient Pack		