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Data Sheet

PDE7B-HEK293 Recombinant Cell Line

Catalog #: 60412

Description

Recombinant HEK293 cell line expressing human PDE7B (phosphodiesterase 7B, accession number NM_018945).

Format

Each vial contains 1 X 10⁶ cells in 1 ml of 10% DMSO.

Applications

- Monitor cAMP and PDE7B signaling pathway activity.
- Screen activators or inhibitors of PDE7B

Storage

Store cells in liquid nitrogen upon arrival. Avoid freeze/thaw cycles.

Mycoplasma testing

The cell line has been screened using the PCR-based VenorGeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Introduction

Phosphodiesterases (PDEs) regulate the intracellular levels of cAMP and cGMP by hydrolyzing cAMP and cGMP to their inactive 5' monophosphates. These cyclic nucleotides play an important role as second messengers in diverse physiological functions. PDE7 is a cAMP-specific enzyme and two PDE genes (PDE7A and PDE7B) have been identified. PDE7B is widely expressed in various tissues including brain, heart, liver, skeletal muscle, and pancreas. Inhibition of PDE7 activity by its inhibitors leads to elevated intracellular level of cAMP.

Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Complete Growth Medium: Thaw Medium 1 (BPS Cat. #60187) plus 400 µg/ml of Geneticin (G418) (Life technologies #11811031) to ensure maintenance of recombinant PDE7B.

Cells should be grown at 37°C with 5% CO₂ using complete growth medium. It may be required to adjust the percentage of CO₂ in the incubator depending on the NaHCO₃ level in the basal medium. PDE7B-HEK293 cells exhibit a typical cell division time of 24 hours.

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To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin**), spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 (**no Geneticin**). Transfer resuspended cells to a T25 flask and culture in a 37°C CO₂ incubator. At first passage switch to complete growth medium (**contains Geneticin**). Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, and add complete growth medium. Transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly.

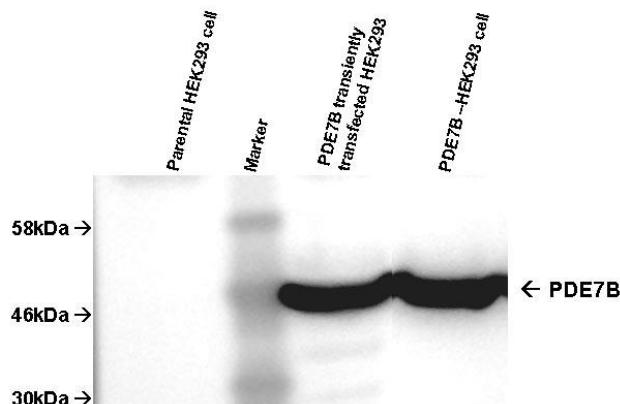
Functional validation

Human PDE7B has been stably expressed in a human embryonic kidney (HEK293) cell line. PDE7B expression was confirmed by Western blotting. The function of expressed PDE7B was validated by cAMP detection assay.

The regulation of intracellular level of cAMP by PDE7B in PDE7B stably-expressed HEK293 cells was characterized by a cell-based reporter assay using pCRE-luc reporter vector. pCRE-luc contains the luciferase gene that is under the control of cAMP response element (CRE). Elevation of intracellular cAMP activates cAMP response element binding protein (CREB) to bind CRE and induces the expression of luciferase.

Forskolin is commonly used to raise the intracellular level of cAMP in cell physiology studies. Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing cAMP levels. When cells transiently transfected with pCRE-luc reporter were activated by forskolin, the level of cAMP was upregulated in parental HEK293 cells inducing the expression of the luciferase reporter whereas hPDE7B-HEK293 cells showed reduction in the level of cAMP that resulted in lowered expression of luciferase.

These data show the stable expression of PDE7B in HEK293 cells.



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Figure 1: Western blot of PDE7B in PDE7B-HEK293 cells, probed with anti-PDE7B antibody.

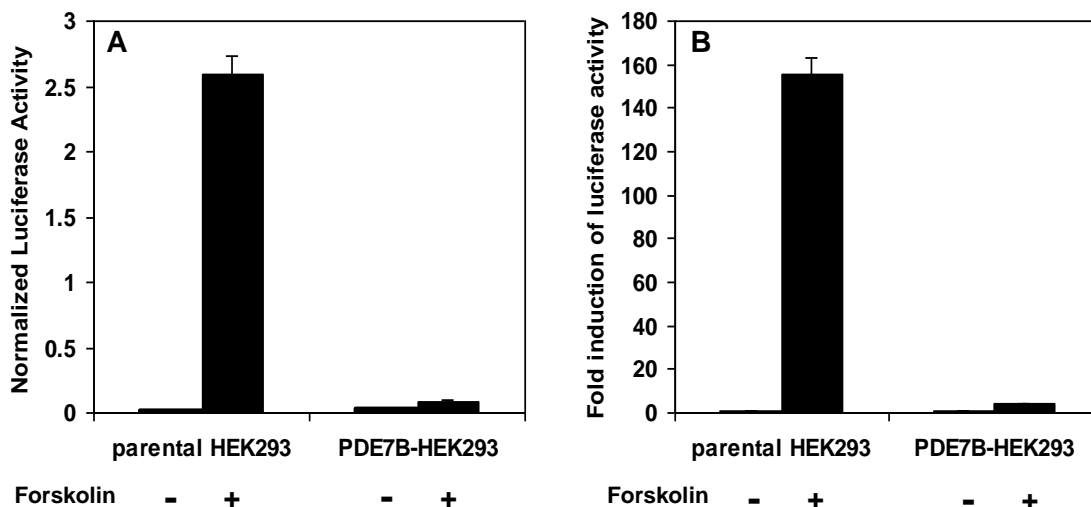


Figure 2: PDE7B overexpression reduced the level of cAMP following forskolin stimulation in PDE7B-HEK293 cells.

PDE7B-HEK293 or parental HEK293 cells were transiently transfected with pCRE-luc reporter and control Renilla luciferase reporter. The next day, cells were stimulated with forskolin (1 μ M) for 6 hours. The luciferase activity was measured using Dual-Glo luciferase reagent (Promega) and the activity values were normalized to control Renilla luciferase activity. Data are shown as normalized luciferase activity (Fig. 2A) and fold induction of luciferase activity (determined by comparing values against the mean value for control cells without forskolin) (Fig. 2B). Results showed that PDE7B reduced the level of intracellular cAMP, resulting in lowered luciferase activity.

Vector and sequence

human PDE7B (NM_018945) was cloned into pcDNA3.1 vector (Invitrogen).

Polylinker: CMV-HindIII-KpnI-BamHI-**PDE7B**-XhoI-XbaI-ApaI-----SV40-neomycin^R

Human PDE7B sequence:

MSCLMVERCGEILFENPDQNAKVCMLGDIRLRGQTGVRAERRGSYPFIDFRLLNSTTYSGEI
 GTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVGMWDFDIFLFDRLTN
 GNSLVTLCHLNFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNPYHNAVHAADVTQAMHCYLK
 EPKLASFLTPLDIMLGLLAAAHDVDHPGVNQPFLIKTNHHLANLYQNMSVLENHHWRSTIGML

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RESRLLAHLPKEMTQDIEQQLGSLILATDINRQNEFLTRLKAHLHNKDLRLEDAQDRHFMLQIAL
KCADICNPCRIWEMSKQWSERVCEEFYRQGELEQKFELEISPLCNQQKDSIPSIIQIGFMSYIVE
PLFREWAHFTGNSTLSENMLGHLAHNKAQWKSLLPRQHRSRGSSGSGPDHHDHAGQGTESEE
QEGDSP

References:

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

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