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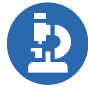 Laborgeräte & Service

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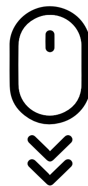

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Data Sheet

PDE7A-HEK293 Recombinant Cell line

Catalog #: 60407

Description
Recombinant HEK293 cell line expressing human PDE7A (phosphodiesterase 7A, accession number NM_002603).

Format
Each vial contains 1 X 10^6 cells in 1 ml of 10% DMSO.

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

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. PDE7A is widely expressed in various tissues including skeletal muscle, T lymphocytes, brain and pancreas. Inhibition of PDE7 activity by its inhibitors leads to elevated intracellular level of cAMP.

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.

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) (Invitrogen #11811031) to ensure maintenance of recombinant PDE7A.

Cells should be grown at 37°C with 7% CO_2 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. hPDE7A-HEK293 cells exhibit a typical cell division time of 24 hours.

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, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**, transfer resuspended cells to T25 flask and culture in 37° 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, add complete growth medium and 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
N-terminal FLAG-tagged human PDE7A has been stably expressed in a human embryonic kidney (HEK293) cell line. PDE7A expression was confirmed by Western blotting.

The regulation of intracellular level of cAMP by PDE7A in PDE7A stably-expressed HEK293cells 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 hPDE7A-HEK293 cells showed reduction in the level of cAMP that resulted in lowered expression of luciferase. Inhibition of PDE7A activity by BRL 50481, a PDE7A inhibitor, restored the cAMP level, resulting in higher luciferase activity.

These data show the stable expression of PDE7A in HEK293 cells.

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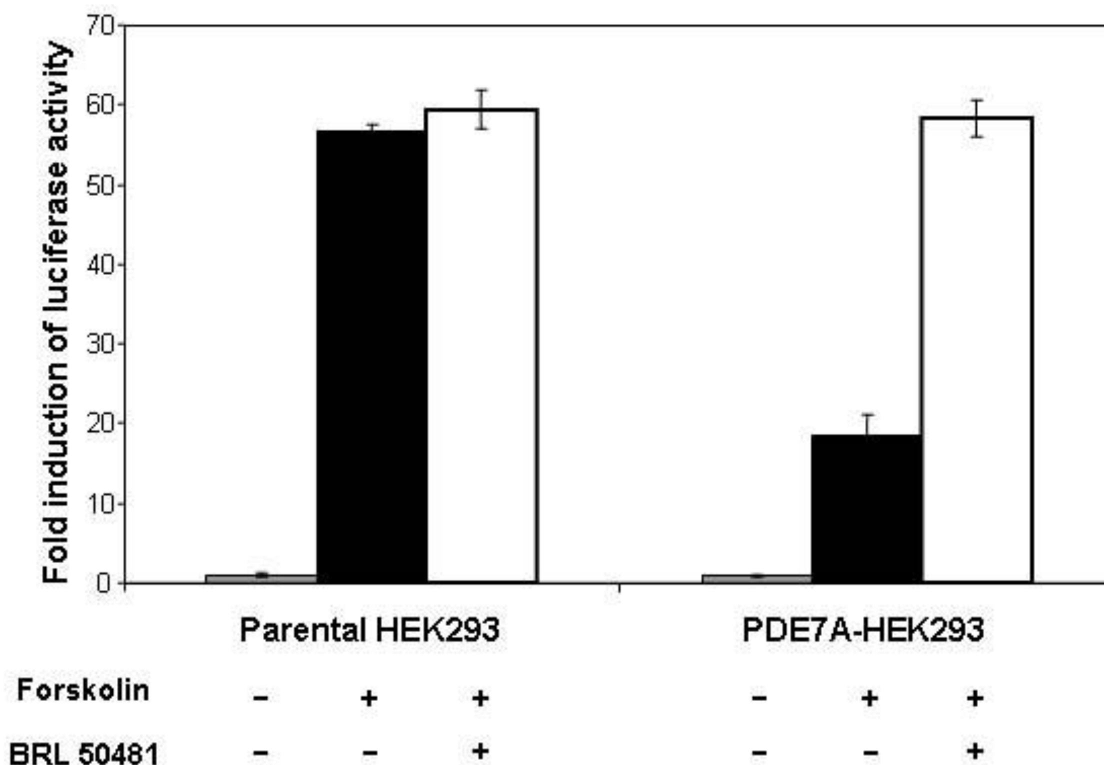

Figure 1: PDE7A overexpression reduced the level of cAMP following forskolin stimulation in PDE7A-HEK293 cells. This effect was reversed by BRL 50481, a PDE7A inhibitor.

PDE7A-HEK293 or parental HEK293 cells were transiently transfected with pCRE-luc reporter and control Renilla luciferase reporter, then treated overnight with BRL 50481 (20 µM). The next day, cells were stimulated with forskolin (1 µM) 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 fold induction of luciferase activity, determined by comparing values against the mean value for control cells without forskolin and BRL 50481 treatment. Results showed that PDE7A reduced the level of intracellular cAMP, resulting in lowered luciferase activity. Inhibition of PDE7A activity by BRL 50481 restored the cAMP level, resulting in higher luciferase activity.

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Vector and sequence
N-terminal FLAG-tagged human PDE7A was cloned into pcDNA3.1 vector (Invitrogen).

Polylinker: CMV-HindIII-KpnI-BamHI-**PDE7A**-XhoI-XbaI-ApaI------SV40-neomycin^{R}

FLAG-hPDE7A sequence:
MDYKDDDDKGITLIWCLALVLIKWITSKRRGAISYDSSDQTALYIRMLGDVRVRSRAGFESERR
GSHPYIDFRIFHSQSEIEVSVSARNIRRLLSFQRYLRSSRFFRGTAVSNSLNILDDDYNGQAKCM
LEKVGNWNFDIFLFDRLTNGNSLVSLTFHLFSLHGLIEYFHLDMMKLRRFLVMIQEDYHSQNPY
HNAVHAADVTQAMHCYLKEPKLANSVTPWDILLSLIAAATHDLDHPGVNQPFLIKTNHYLATLYK
NTSVLENHHWRSAVGLLRESGLFSHLPLESRQQMETQIGALILATDISRQNEYLSLFRSHLDRG
DLCLEDTRHRHLVLQMALKCADICNPCRTWELSKQWSEKVTEEFFHQGDIEKKYHLGVSPLCD
RHTESIANIQIGFMTYLVEPLFTEWARFSNTRLSQTMLGHVGLNKASWKGLQREQSSSEDTDA
AFELNSQLLPQENRLS

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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