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Data Sheet

PDE7A - HEK293 Recombinant Cell Line (Rat)

Catalog Number: 60408

Description:

Recombinant HEK293 cell line expressing rat PDE7A (phosphodiesterase 7A, accession number NM_031080).

Format:

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

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. PDE7 is widely expressed in various tissues, with PDE7A found primarily in skeletal muscle, T lymphocytes, and pancreas, while high levels of PDE7B are detected in brain, heart, and liver. Inhibition of PDE7 activity by its inhibitors leads to elevated intracellular level of cAMP.

Culture Conditions:

Thaw Medium 1 (BPS Bioscience #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)

Growth Medium 1B (BPS Bioscience #79531): 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% CO₂ using Growth Medium 1B.

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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 Growth Medium 1B, spin down cells, resuspend cells and transfer to a T25 flask. Cells should be split before they reach complete confluency.

To passage the cells, pre-wash cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA (Hyclone #SH30236.01), add Growth Medium 1B and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.

Functional validation:

N-terminal FLAG-tagged rat PDE7A has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting.

The regulation of intracellular level of cAMP by rat PDE7A in rat PDE7A stably-expressed HEK293 cells was characterized by a cell-based reporter assay using pCRE-luc reporter vector. pCRE-luc contains a luciferase gene that is under the control of the cAMP response element (CRE). Elevation of intracellular cAMP activates cAMP response element binding protein (CREB) to bind CRE, inducing the expression of luciferase.

Forskolin is commonly used to raise the intracellular level of cAMP in cell physiology studies. Forskolin re-sensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing cAMP level. 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 luciferase reporter whereas rat PDE7A-HEK293 cells showed reduction in the level of cAMP, resulting in lowered levels of luciferase expression. 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 rat PDE7A in HEK293 cells.

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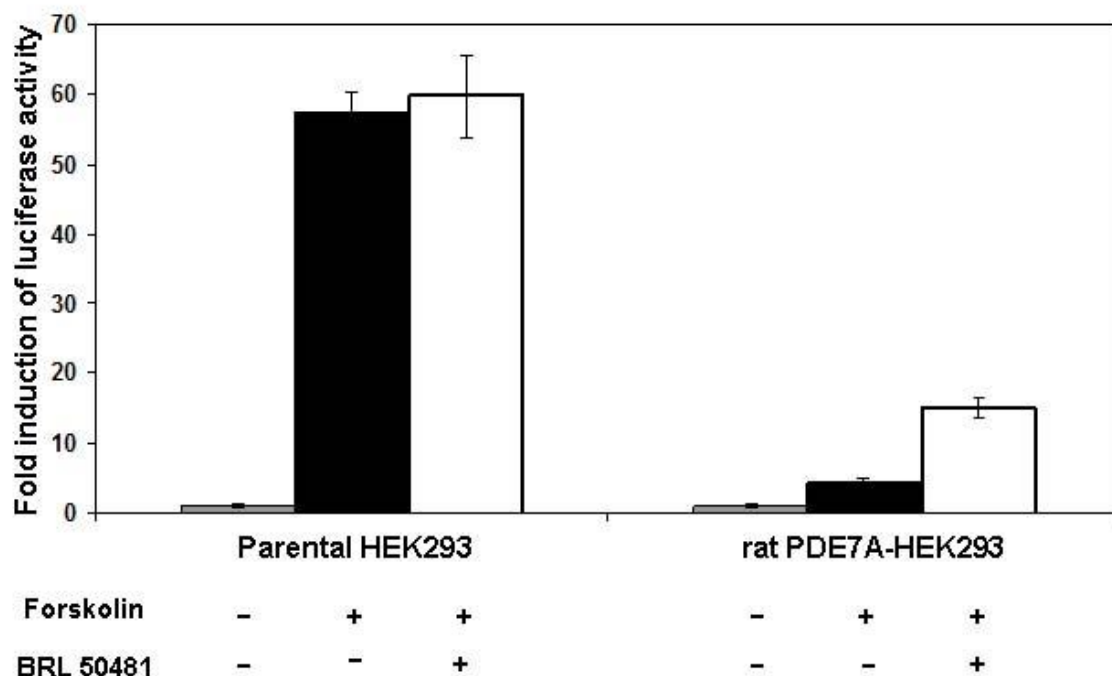


Figure 1: PDE7A overexpression reduced the level of cAMP following forskolin stimulation in rat PDE7A-HEK293 cells. This effect was reversed by BRL 50481, a PDE7A inhibitor.

Rat 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 μ M). 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 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 rat PDE7A (accession number NM_031080) was cloned into pcDNA3.1 vector (Invitrogen).

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

FLAG-rat PDE7A sequence

MDYKDDDDKEVCYQLPVLPLDRPVPQHVLSSRGAISSSSALFGCPHPRQLSQRRGAISYDS
SDQTALYIRMLGDVVRVRSRAGFETERRGSHPYIDFRIFHAQSEIEASVSARNIRLLSFQRYLRS
SRFFRGATVCRSLNILDYNGQAKCMLEKVGNNWFDIFLFDRLTNGNSLVSLTFHLFSLHGLI
EYFHLDMMVKLRRFLVMIQEDYHSQNPYHNAVHAADVTQAMHCYLKEPKLANSVTPWDILLSLIA
AATHDLDPGVNQPFLLIKTNHYLATLYKNTSVLENHHWRSVAVGLLRESGLFSHLPLESRHEME
AQIGALILATDISRQNEYLSLFRSHLDKGDHLDDGRHRHLVLQMALKCADICNPCRNWELSKQ
WSEKVTEEFFHQGDIEKKYHLGVSPLCDRQTESIANYIGFMTYLVEPLFTEWARFSDTRLSQT
MLGHVGLNKASWKGLQRQQPSSSEDASAAFELNSQLLTQENRLS

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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