SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Rat PDE7A - HEK293 Recombinant Cell line

Catalog Number: 60408

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

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.

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 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Cells should be grown at 37°C with 7% CO_2 using complete growth medium.

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 complete growth medium, 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 complete growth medium 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 HEK293cells 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 resensitizes 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

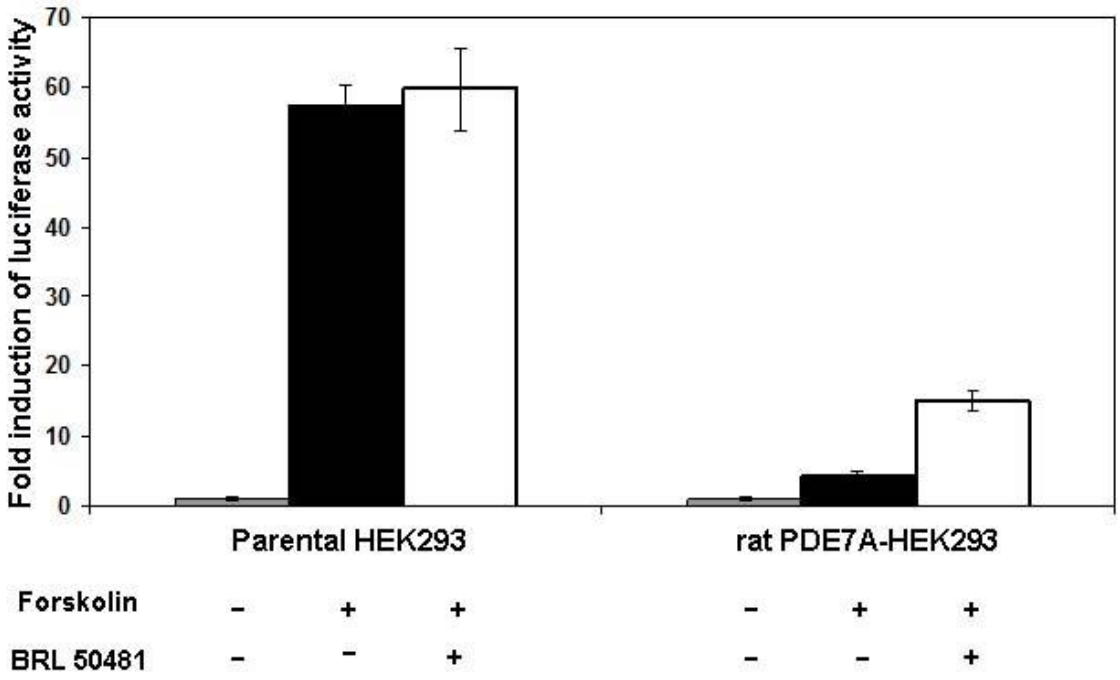

Figure 1: PDE7A overexpression reduced the level of cAMP following forskolin stimulation in rat PDE7A-HEK293 cells. This effect was reversed by BRL 50481, a PDE7A inhibitor.

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 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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
MDYKDDDDKEVCYQLPVLPLDRPVPQHVLSRRGAISFSSSSALFGCPHPRQLSQRRG
AISYDSSDQTALYIRMLGDVRVRSRAGFETERRGSHPYIDFRIFHAQSEIEASVSARNIR
RLLSFQRYLRSSRFFRGATVCRSLNILDEDYNGQAKCMLEKVGNWNFDIFLFDRLTNG
NSLVSLTFHLFSLHGLIEYFHLDMVKLRRFLVMIQEDYHSQNPYHNAVHAADVTQAMH
CYLKEPKLANSVTPWDILLSLIAAATHDLDHPGVNQPFLIKTNHYLATLYKNTSVLENHH
WRSAVGLLRESGLFSHLPLESRHEMEAQIGALILATDISRQNEYLSLFRSHLDKGDLHL
DDGRHRHLVLQMALKCADICNPCRNWELSKQWSEKVTEEFFHQGDIEKKYHLGVSPL
CDRQTESIANIQIGFMTYLVEPLFTEWARFSDTRLSQTMLGHVGLNKASWKGLQRQQP
SSEDASAAFELNSQLLTQENRLS

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

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees**;** contact *sales@bpsbioscience.com* for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.