



6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

PDE1B-HEK293 Recombinant Cell line Cat. # 60402

Product description

Recombinant HEK293 cell line expressing human PDE1B (phosphodiesterase 1B, accession number NM_000924).

Format

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

Introduction

PDE1B plays a role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. PDE1B hydrolyzes both cAMP and cGMP to nucleoside 5'-monophosphate. Although it prefers cGMP at low substrate levels it has a V_{max} that is approximately equal for both cGMP and cAMP (Polli, JW and Koncaid, RL, 1994; Yu J. *et al.*, 1997).

Functional validation

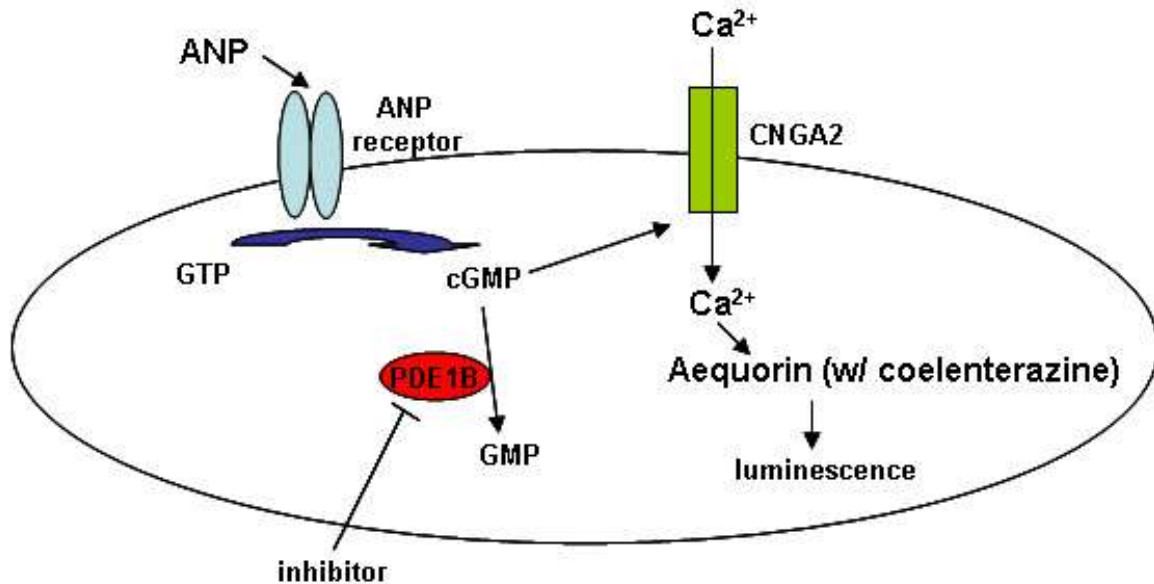
N-terminal His-tagged human PDE1B has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting.

The function of PDE1B was characterized by a cell-based cGMP assay. In this assay system, cells co-express atrial natriuretic peptide (ANP) receptor A (a guanylate cyclase), cyclic nucleotide-gated cation channel CNGA2, and the photoprotein aequorin. Intracellular cGMP level is monitored via aequorin luminescence induced by Ca^{2+} influx through CNGA2, acting as the intracellular cGMP sensor. A schematic presentation of this assay is shown in figure 1.

Figure 1 Schematic presentation of cGMP assay used to characterize the function of PDE1B.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**
Or you can Email us at: info@bpsbioscience.com
Please visit our website at: www.bpsbioscience.com



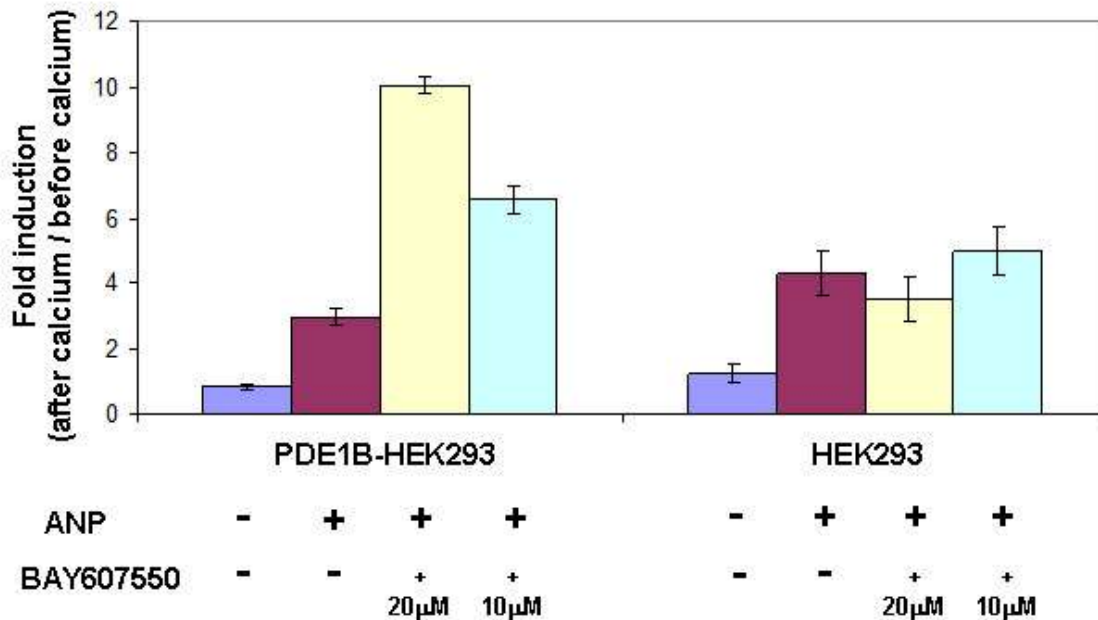
When PDE1B-HEK293 or parental HEK293 cells transiently transfected with ANP receptor, CNGA2 and aequorin, were activated by ANP in the presence or in the absence of PDE1B inhibitor BAY607550, BAY607550 significantly potentiated the ANP-induced cGMP level in PDE1B-HEK293 cells but not in parental HEK293 cells, resulting in calcium influx that stimulated luminescence signals.

These data show the stable expression of PDE1B in HEK293 cells.

Figure 2 Inhibition of PDE1B activity by BAY607550 in PDE1B-HEK293 cells potentiated the ANP-induced cGMP level, resulting in calcium influx that stimulated luminescence signals.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**
Or you can Email us at: info@bpsbioscience.com
Please visit our website at: www.bpsbioscience.com



PDE1B-HEK293 stable cells or parental HEK293 cells were transiently transfected with ANP receptor, CNGA2, and aequorin and cultured for 2 days. After removal of the cell medium, cells were loaded with 5 μM of coelenterazine in Ca²⁺ free assay buffer, with or without PDE1B inhibitor BAY607550, for 3 hours. Cells were stimulated with ANP (10 nM) for 15min and aequorin luminescence was measured. Then Ca²⁺ (3 mM) was added to cells and measurement of aequorin luminescence was started immediately. Data were shown as fold induction of luminescence after addition of Ca²⁺ compared to values before addition of Ca²⁺. Results showed that BAY607550 significantly potentiated the ANP-induced cGMP level in PDE1B-HEK293 cells but not in parental HEK293 cells, resulting in calcium influx that stimulated luminescence signals.

Storage

Upon receipt, store in liquid nitrogen.

Culture conditions

Cells should be grown at 37° with 7% CO₂ using MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) medium supplemented with 10% FBS (Hyclone #SH30070.03), 1% non-essential amino acid (Hyclone #SH30238.01), 1mM Na-pyruvate (Hyclone #SH30239.01), plus 400 μg/ml of Geneticin (G418) (invitrogen #11811031) to ensure the

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**
 Or you can Email us at: info@bpsbioscience.com
 Please visit our website at: www.bpsbioscience.com



6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

recombinant expression is maintained. hPDE1B-HEK293 cells should exhibit a typical cell division time of 24 hours.

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 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.

Vector and sequence

Human His-PDE1B cloned in pcDNA3.1 (accession number NM_000924)

His-PDE1B sequence:

MHHHHHHELSPRSPPEMLESDCPSPLELKSAPSKKMWIKLRSLRYMVKQLEN
GEINIEELKKNLEYTASLLEAVYIDETRQILDTEDELQELRSDAVPSEVRDWLAST
FTQQARAKGRRAEEKPKFRSIVHAVQAGIFVERMFRRTYTSVGPTYSTAVLNCL
KNLDLWCFDVFSLNQAADDHALRTIVFELLTRHNLISRFKIPTVFLMSFLDALET
GYGKYKNPYHNQIHAADVTQTVHCFLLRGTMVHCLSEIELLAIIFAAAIHDYEHT
GTTNSFHIQTKSECAIVYNDRSVLENHHISSVFRLMQDDEMNFIFNLTKDEFVELR
ALVIEMVLATDMSCHFQQVKTMTALQQLERIDKPKALSLLLHAADISHPTKQW
LVHSRWTALMEEFFRQGDKEAELGLPFSPLCDRTSTLVAQSQIGFIDFIVEPTFS
VLTDVAEKSVQPLADEDSKSKNQPSFQWRQPSLDVEVGDPNPVVVSFRSTWVK
RIQENKQKWKERAASGITNQMSIDELSPCEEEAPPSPAEDEHNQNGNLD.

References:

Polli, JW and Kincaid RL. (1994). "Expression of a calmodulin-dependent phosphodiesterase isoform (PDE1B1) correlates with brain regions having extensive dopaminergic innervation." *J. Neurosc.* **14**: 1251-1261.

Yu, J, et al. (1997). "Identification and Characterization of a Human Calmodulin-Stimulated Phosphodiesterase PDE1B1" *Cell Signaling* **9** (7): 519-529.

Bender A.T. *et al.*, (2005). "Selective up-regulation of PDE1B2 upon monocyte-to-macrophage differentiation." *PNAS* **102** (2): 497-502.

Wunder F et al., (2009). "A novel PDE2A reporter cell line: characterization of the cellular activity of PDE inhibitors." *Mol. Pharm.* **6** (1): 326-36.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com