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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 X 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).

cAMP is an important signal carrier necessary for the proper biological response of cells to hormones and other extracellular signals. It is required for cell communication in the hypothalamus/pituitary gland axis and for the feedback control of hormones (Alewijnse A.E. *et al.,* 1997).

Culture conditions

Cells should be grown at 37°C with 7% CO_2 using MEM (Hyclone) medium supplemented with 10% FBS, 1% non-essential amino acid, 1 mM Na-pyruvate, plus 400 μg/ml of Geneticin (Invitrogen) to ensure the recombinant expression is maintained. PDE1B-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 10ml growth medium, spin down cells, resuspend cells and transfer to T25 flask. Cells should be split before they reach complete confluency.

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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 cAMP detection assay.

In general, forskolin is commonly used to raise levels of cyclic AMP (cAMP). Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing the intracellular levels of cAMP.

When 293 cells are activated by forskolin, cAMP levels are upregulated in parental cells whereas cells overexpressing PDE1B show reduction in cAMP accumulation.

The data provided below represents effect of PDE1B overexpression on cAMP accumulation after forskolin stimulation in 293 cells.

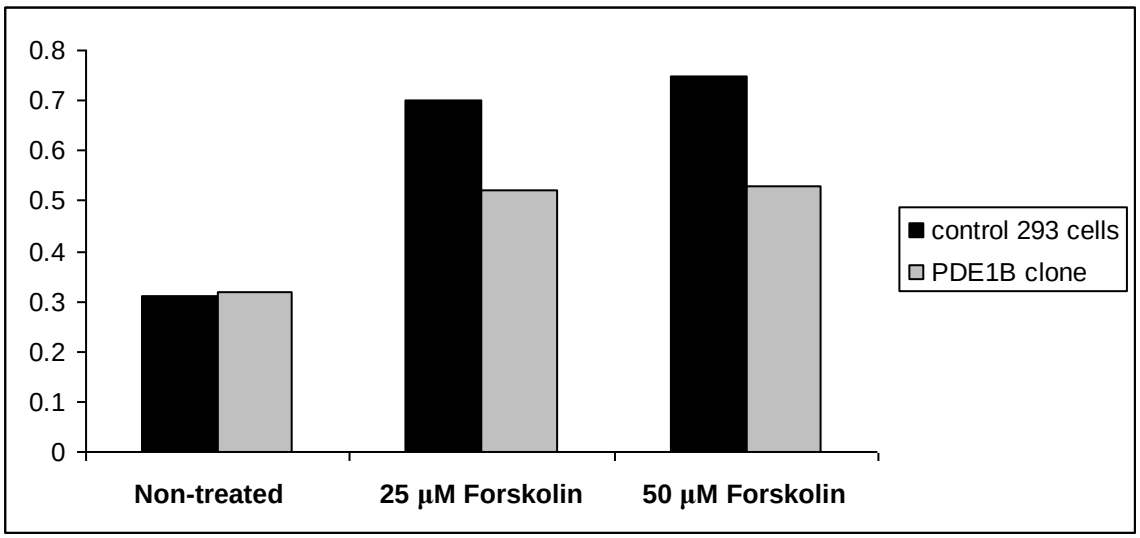

Figure 1. PDE1B overexpression reduces cAMP accumulation after forskolin stimulation.
Cells were incubated in PBS buffer for 15 min and consequently stimulated with 0, 25, or 50 □M forskolin for 20 min. cAMP production was measured by fluorescent quenching detection method using a 96-well assay kit (Mediomics).

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Vector and sequence

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

MHHHHHHELSPRSPPEMLEESDCPSPLELKSAPSKKMWIKLRSLLRYMVKQLENGEIN
IEELKKNLEYTASLLEAVYIDETRQILDTEDELQELRSDAVPSEVRDWLASTFTQQARAK
GRRAEEKPKFRSIVHAVQAGIFVERMFRRTYTSVGPTYSTAVLNCLKNLDLWCFDVFS
LNQAADDHALRTIVFELLTRHNLISRFKIPTVFLMSFLDALETGYGKYKNPYHNQIHAAD
VTQTVHCFLLRTGMVHCLSEIELLAIIFAAAIHDYEHTGTTNSFHIQTKSECAIVYNDRSV
LENHHISSVFRLMQDDEMNIFINLTKDEFVELRALVIEMVLATDMSCHFQQVKTMKTAL
QQLERIDKPKALSLLLHAADISHPTKQWLVHSRWTKALMEEFFRQGDKEAELGLPFSP
LCDRTSTLVAQSQIGFIDFIVEPTFSVLTDVAEKSVQPLADEDSKSKNQPSFQWRQPSL
DVEVGDPNPDVVSFRSTWVKRIQENKQKWKERAASGITNQMSIDELSPCEEEAPPSP
AEDEHNQNGNLD.

References

1) 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.

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

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

4) Alewijnse, AE *et al.* (1997). “Modulation of forskolin-mediated adenylyl cyclase activation by constitutively active G_s-coupled receptors”. *FEBS Letters* **419** (2-3): 171-174.

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