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

PDE10A-HEK293 Recombinant Cell Line

Catalog #: 60410
Lot #: 161121

Description
Recombinant HEK293 cell line expressing human PDE10A2 (phosphodiesterase 10A, accession number NM_006661).

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
PDE10A plays a key role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. PDE10A hydrolyzes both cAMP and cGMP to nucleoside 5′-monophosphate, but exhibits higher affinity for cAMP, and it utilizes cAMP more efficiently as a substrate (Fujishige K. *et al.,* 1999).

cAMP is an critical second messenger that is required for the proper biological responses of cells to hormones and other extracellular signals. It is required for cellular communication in the hypothalamus/pituitary gland axis and for the feedback control of hormones (Alewijnse A.E. *et al.,* 1997). cAMP is also involved in the activation of protein kinases and ion channel regulation.

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 (Invitrogen) to ensure maintenance of recombinant PDE10A.

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Cells should be grown at 37° with 7% CO_2 using complete growth medium.PDE10A-HEK293 cells 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 a T25 flask. Cells should be split prior to reaching complete confluence.

Functional Validation

N-terminal His-tagged human PDE10A2 has been stably expressed in a human embryonic kidney (HEK293) cell line. PDE10A2 expression was confirmed by Western blotting. The function of PDE10A2 was confirmed by cAMP detection assay.

Forskolin is commonly used to raise levels of cyclic AMP (cAMP) in the study and research of cell physiology. Forskolin re-sensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing intracellular levels of cAMP. When HEK293 cells are activated by forskolin, cAMP levels are up-regulated in parental cells whereas cells overexpressing PDE10A show reduction in cAMP accumulation. Inhibition of PDE10A activity restores cAMP levels.

The data provided below demonstrates the effect of PDE10A2 overexpression on cAMP accumulation following forskolin stimulation in HEK293 cells.

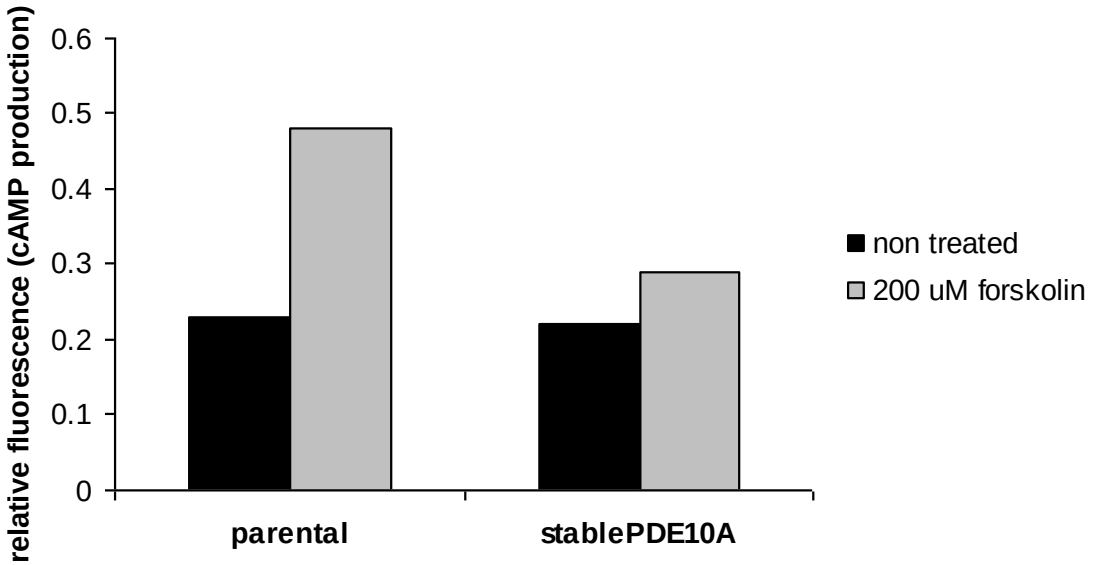

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Figure 1. PDE10A2 overexpression reduces cAMP accumulation following forskolin stimulation.

Cells were incubated in PBS buffer for 15 min. and subsequently stimulated with 200 µM of forskolin for 30 min. cAMP production was measured by detection of fluorescent quenching using a cAMP fluorescent assay kit (Mediomics, St. Louis, MO).

Addition of the pan-PDE inhibitor IBMX to the assay buffer reverses the effect of PDE10A overexpression.

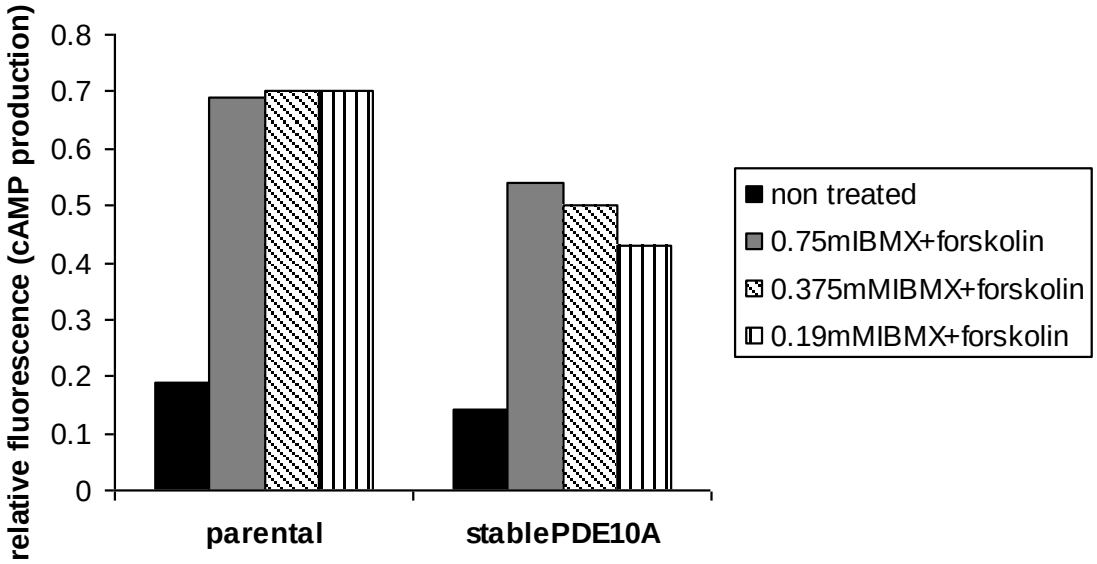

Figure 2. Presence of IBMX, a pan-PDE inhibitor, affects PDE10A2 activity and restores accumulation of cAMP.
Cells were incubated in assay buffer with different concentration of IBMX for 15 min and consequently stimulated with 200 µM of forskolin for 30 min. cAMP production was measured by detection of fluorescent quenching (Mediomics).

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Vector and Sequence

Human His-PDE10A2 cloned in pcDNA3.1 (accession number NM_006661)

His-PDE10A sequence:
MHHHHHHRIEERKSQHLTGLTDEKVKAYLSLHPQVLDEFVSESVSAETVEKWLKRKN
NKSEDESAPKEVSRYQDTNMQGVVYELNSYIEQRLDTGGDNQLLLYELSSIIKIATKAD
GFALYFLGECNNSLCIFTPPGIKEGKPRLIPAGPITQGTTVSAYVAKSRKTLLVEDILGDE
RFPRGTGLESGTRIQSVLCLPIVTAIGDLIGILELYRHWGKEAFCLSHQEVATANLAWAS
VAIHQVQVCRGLAKQTELNDFLLDVSKTYFDNIVAIDSLLEHIMIYAKNLVNADRCALFQ
VDHKNKELYSDLFDIGEEKEGKPVFKKTKEIRFSIEKGIAGQVARTGEVLNIPDAYADPR
FNREVDLYTGYTTRNILCMPIVSRGSVIGVVQMVNKISGSAFSKTDENNFKMFAVFCAL
ALHCANMYHRIRHSECIYRVTMEKLSYHSICTSEEWQGLMQFTLPVRLCKEIELFHFDI
GPFENMWPGIFVYMVHRSCGTSCFELEKLCRFIMSVKKNYRRVPYHNWKHAVTVAHC
MYAILQNNHTLFTDLERKGLLIACLCHDLDHRGFSNSYLQKFDHPLAALYSTSTMEQHH
FSQTVSILQLEGHNIFSTLSSSEYEQVLEIIRKAIIATDLALYFGNRKQLEEMYQTGSLNL
NNQSHRDRVIGLMMTACDLCSVTKLWPVTKLTANDIYAEFWAEGDEMKKLGIQPIPMM
DRDKKDEVPQGQLGFYNAVAIPCYTTLTQILPPTEPLLKACRDNLSQWEKVIRGEETAT
WISSPSVAQKAAASED.

References

1. Fujishige K, *et al.* (1999). "Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A)." *J. Biol. Chem.* **274** (26): 18438–45.
2. 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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