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Data Sheet

hTRPC3-HEK293 Recombinant Cell line

catalog #: 90130

Product Description

Recombinant HEK293 cell line expressing human TRPC3 (transient receptor potential cation channel, subfamily C, member 3, accession number NM_003305).

Format

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

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

TRPC3 channel belongs to the transient receptor potential channel (TRP) superfamily which is divided into seven main subfamilies. TRP channels share a similar architecture of six-transmembrane domains and function as tetramers. Most channels are permeable to calcium and monovalent cations (Vassort et al, 2009).

Specifically, TRPC3 channel belongs to a canonical TRP (TRPC) subfamily that is involved in calcium entry. TRPC3 is activated through phospholipase C-linked receptors. When expressed in HEK293 cells, TRPC3 behaves as a receptor activated channel with constitutive activity that cannot be further increased by store depletion (Trebak et al, 2002).

Functional Validation

N' terminal FLAG tagged human TRPC3 channel has been stably expressed in HEK293 cell line and its expression was confirmed by western blotting.

The function of TRPC3 was characterized by calcium assay. TRPC3 produces a constitutive Ca^{2+} entry in HEK293 cells. Addition of methacholine, an activator of phospholipase C-linked muscarinic receptors, significantly activates TRPC3.

These data show the stable expression of TRPC3 channel in HEK293 cells.

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Figure 1. TRPC3 produced constitutive Ca^{2+} entry in the presence of 5μM Gd^{3+} when expressed in HEK293. A) WT-HEK293 cells; B) TRPC3-HEK293 cells.

Cells were incubated in the absence of added Ca^{2+}, and 2mM Ca^{2+} was added where indicated. To block endogenous store depletion-induced Ca^{2+} entry, 5μM Gd^{3+} was present throughout. The calcium measurements were performed using calcium indicator, Fluo-8 (excited at 485/20nm and emission at 528/20nm).

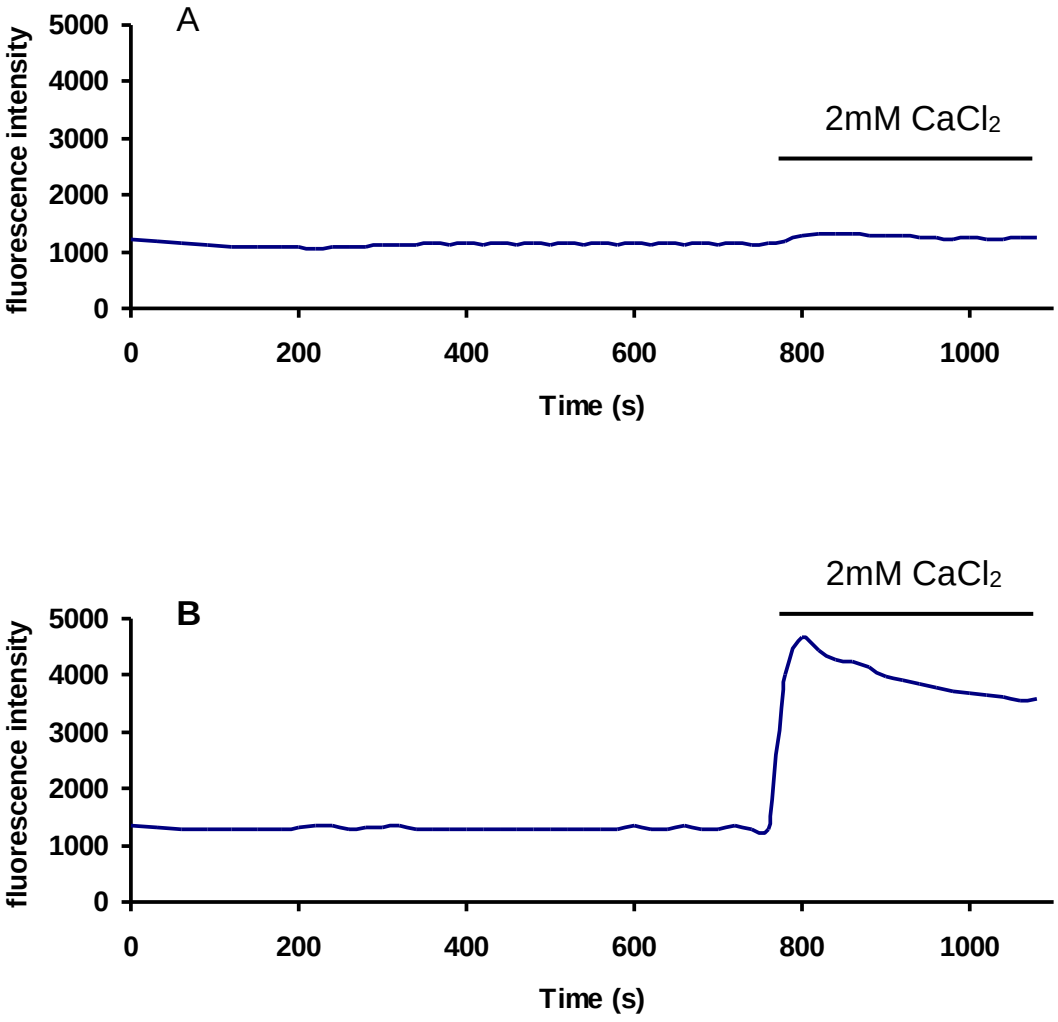

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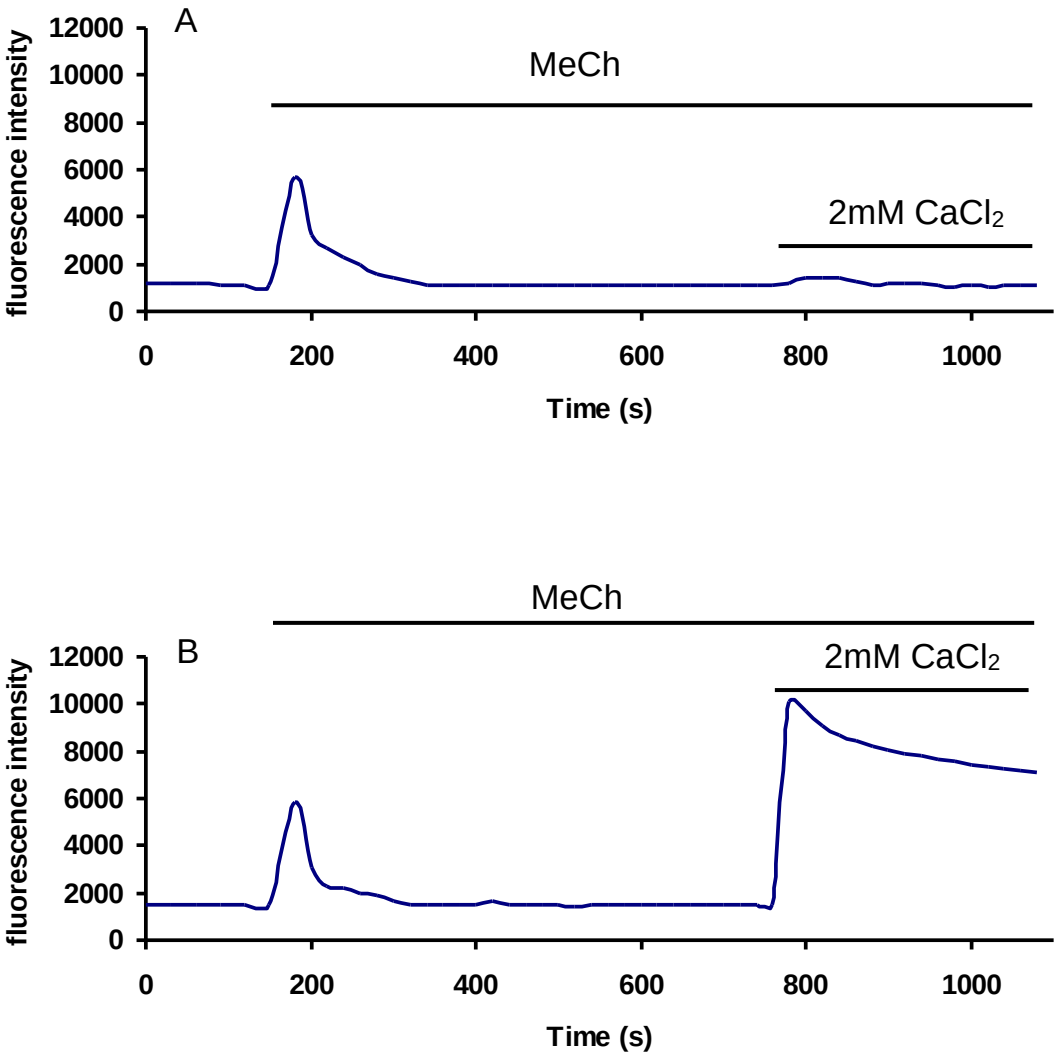
Figure 2. Methacholine activated Ca^{2+} entry in TRPC3 expressed HEK293 cells. A) WT-HEK293 cells; B) TRPC3-HEK293 cells.

Cells were incubated in the absence of added Ca^{2+}, and 300μM methacholine (MeCh), followed by 2mM Ca^{2+} was added where indicated. 5μM Gd^{3+} was present throughout. The calcium measurements were performed using calcium indicator, Fluo-8 (excited at 485/20nm and emission at 528/20nm).



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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)

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) plus 400 μg/ml of Geneticin (invitrogen #11811031) to ensure the recombinant expression is maintained. TRPC3-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 Growth Medium 1B, 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 Growth Medium 1B and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly.

Vector and sequence

Human TRPC3 was cloned into pIRES-neo vector (Clontech).
Polylinker: CMV-EcoRV-NheI-**TRPC3**-EcoRI-BamHI-NotI-BstXI-IRES-neomycin^R

hTRPC3 sequence (accession number NM_003305)

MEGSPSLRRMTVMREKGRRQAVRGPAFMFNDRGTSLTAEEERFLDAAEYGNI
PVVRKMLEESKTLNVNCVDYMGQNALQLAVGNEHLEVTELLLKKENLARIGDAL
LLAISKGYVRIVEAILNHPGFAASKRLTLSPCEQELQDDDFYAYDEDGTRFSPDI
TPIILAAHCQKYEVVHMLLMKGARIERPHDYFCKCGDCMEKQRHDSFSHSRSRI
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KSFVYFIMRIVNFPKCRRRRLQKDIEMGMGNSKSRLNLFTQSNSRVFESHSFNS
ILNQPTRYQQIMKRLIKRYVLKAQVDKENDEVNEGELKEIKQDISSLRYELLEDK
SQATEELAILIHKLSEKLNPSMLRCE

References

Albert A.P., Saleh S.N., and Large W.A. (2009) Identification of canonical transient receptor potential (TRPC) channel proteins in native vascular smooth muscle cells. *Current medicinal chemistry* 16: 1158-1165.

Lievremont J., Bird G.J., and Putney, Jr. J.W. (2004) Canonical transient receptor potential TRPC7 can function as both a receptor- and store-operated channel in HEK-293 cells. *Am J Physiol Cell Physiol* 287: C1709-C1716.

Trebak M., Bird G.J., McKay R.R., Putney, Jr. J.W. (2002) Comparison of human TRPC3 channels in receptor-activated and store-operated modes. *Journal of Biological Chemistry* 277: 21617-21623.

Vassort G. and Alvarez J. (2009) Transient receptor potential: a large family of new channels of which several are involved in cardiac arrhythmia. *Can. J. Physiol. Pharmacol.* 87: 100-107.

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