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Data Sheet

TRPC7-HEK293 Recombinant Cell line
Cat #: 90030

Description
Recombinant HEK293 cell line expressing human TRPC7 (transient receptor potential cation channel, subfamily C, member 7, accession number NM_020389).

Host cell
HEK293 cells

Format
Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Introduction
TRPC7 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.

Specifically, TRPC7 channel belongs to a canonical TRP (TRPC) subfamily that is involved in calcium entry. TRPC7 is activated via diacylglycerol (DAG) after receptor-mediated activation of PLC.

Applications
• Monitor TRPC7 calcium channel activity
• Screen for activators or inhibitors of TRPC7 calcium channel

Functional validation
N´- terminal FLAG tagged human TRPC7 channel has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting.

The function of TRPC7 was characterized by calcium assay. In TRPC7-HEK293 cells, addition of methacholine, an activator of phospholipase C-linked muscarinic receptors, significantly activates TRPC7, resulting Ca^{2+} entry in the cells.

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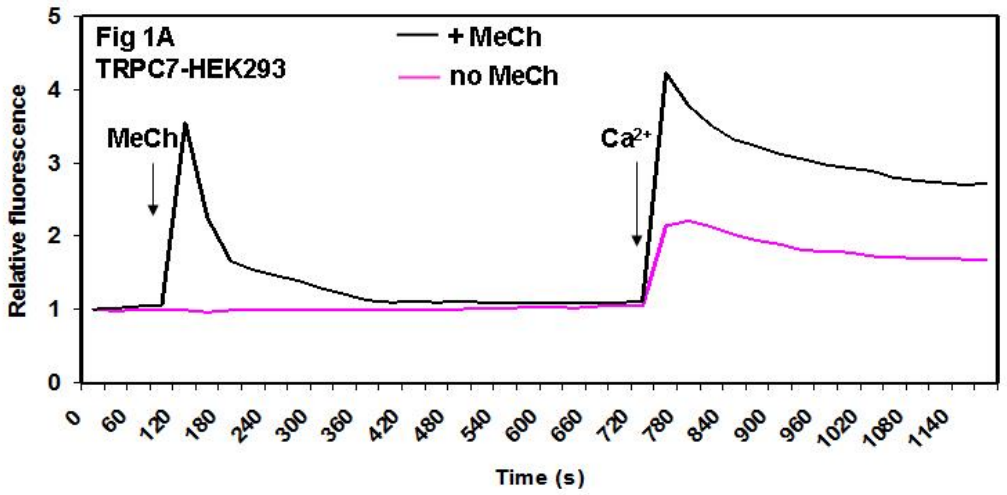
These data show the stable expression of TRPC7 channel in HEK293 cells.
Figure 1. Methacholine-activated Ca^{2+} entry in TRPC7 expressed HEK293 cells.

A) TRPC7-HEK293 cells; B) WT-HEK293 cells.

Cells were loaded with fluorescent Ca^{2+} indicator Fluo-8 and incubated in the absence of extracellular Ca^{2+}. Cell were treated with 300 μM methacholine (MeCh) (black line), followed by 2 mM of Ca^{2+} was added where indicated. Cells without methacholine (pink line) were included as a control. 5 μM Gd^{3+} was maintained throughout the experiment, to block endogenous store depletion-induced Ca^{2+} entry. The calcium influx was measured by Fluo-8 fluorescence (excitation 485±20 nm and emission 528±20 nm).



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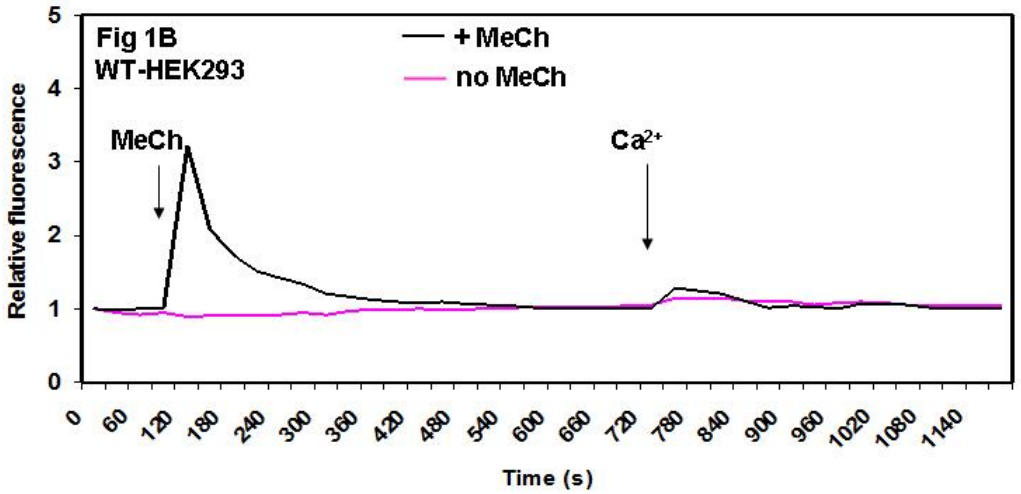

Culture Conditions

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)

Complete Growth Medium: Thaw Medium 1 (BPS Cat. #60187) plus 400 μg/ml of Geneticin (Life Technologies #11811031)

Cells should be grown at 37°C with 5% CO_2 using complete growth medium. It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

To thaw the cells, 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 Thaw Medium 1 **(no Geneticin)**, spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**. Transfer resuspended cells to a T25 flask and culture in a 37°C CO_2 incubator. At first passage, switch to complete growth medium **(contains Geneticin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, and add Thaw Medium 1 containing Geneticin. 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.

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Vector and sequence

N´-terminal FLAG tagged human TRPC7 was cloned into pIRES-neo expression vector.
Polylinker: CMV-EcoRV-NheI-**TRPC7**-BamHI-NotI-BstXI-IRES-neomycin^R

hTRPC7 sequence (accession number NM_020389)
MLRNSTFKNMQRRHTTLREKGRRQAIRGPAYMFNEKGTSLTPEEERFLDSAEYGNIPV
VRKMLEESKTLNFNCVDYMGQNALQLAVGNEHLEVTELLLKKENLARVGDALLLAISKG
YVRIVEAILNHPAFAQGQRLTLSPLEQELRDDDFYAYDEDGTRFSHDITPIILAAHCQEYE
IVHILLLKGARIERPHDYFCKCNECTEKQRKDSFSHSRSRMNAYKGLASAAYLSLSSED
PVLTALELSNELARLANIETEFKNDYRKLSMQCKDFVVGVLDLCRDTEEVEAILNGDVNF
QVWSDHHRPSLSRIKLAIKYEVKKFVAHPNCQQQLLTMWYENLSGLRQQSIAVKFLAVF
GVSIGLPFLAIAYWIAPCSKLGRTLRSPFMKFVAHAVSFTIFLGLLVVNASDRFEGVKTLP
NETFTDYPKQIFRVKTTQFSWTEMLIMKWVLGMIWSECKEIWEEGPREYVLHLWNLLD
FGMLSIFVASFTARFMAFLKATEAQLYVDQHVQDDTLHNVSLPPEVAYFTYARDKWWP
SDPQIISEGLYAIAVVLSFSRIAYILPANESFGPLQISLGRTVKDIFKFMVIFIMVFVAFMIG
MFNLYSYYRGAKYNPAFTTVEESFKTLFWSIFGLSEVISVVLKYDHKFIENIGYVLYGVY
NVTMVVVLLNMLIAMINNSYQEIEEDADVEWKFARAKLWLSYFDEGRTLPAPFNLVPSP
KSFYYLIMRIKMCLIKLCKSKAKSCENDLEMGMLNSKFKKTRYQAGMRNSENLTANNTL
SKPTRYQKIMKRLIKRYVLKAQVDRENDEVNEGELKEIKQDISSLRYELLEEKSQATGEL
ADLIQQLSEKFGKNLNKDHLRVNKGKDI

References

1. Moran MM *et al* (2011) Transient receptor potential channels as therapeutic targets. *Nat Rev Drug Discov*. **10(8)**:601-620.
2. 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.
3. 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.

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