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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<sup>6</sup> 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<sup>7</sup> 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<sup>2+</sup> entry in HEK293 cells. Addition of methacholine, an activator of phospholipase C-linked muscarinic receptors, significantly activates TRPC3.

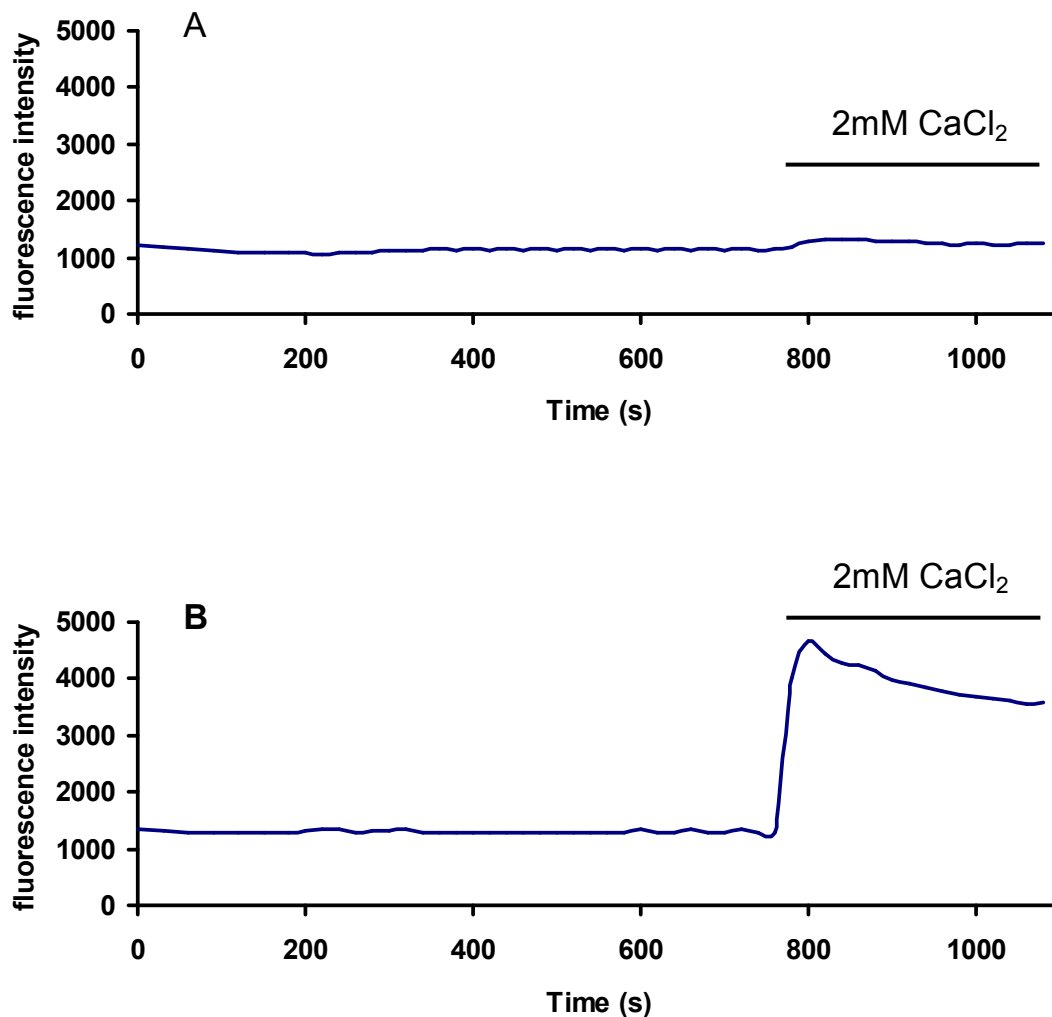
These data show the stable expression of TRPC3 channel in HEK293 cells.

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

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

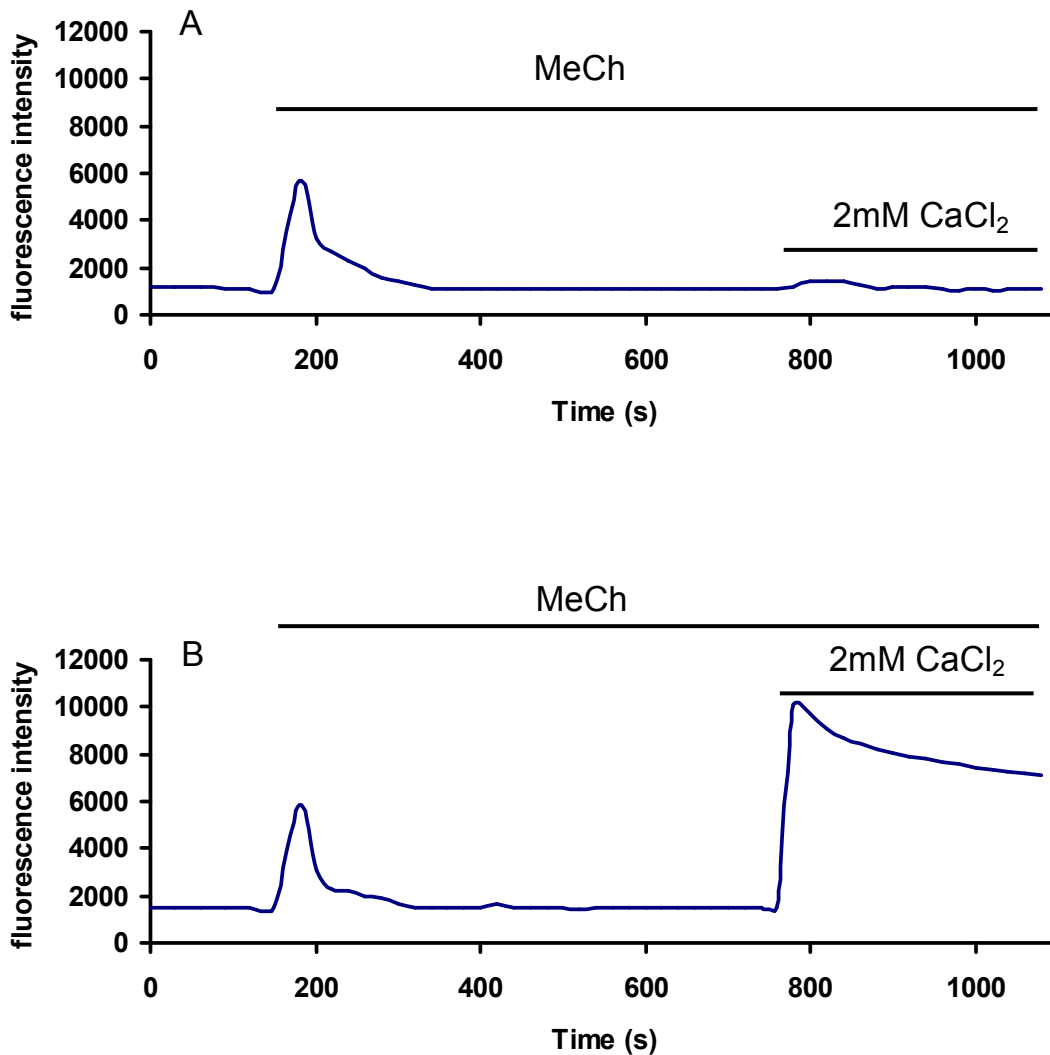


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**Figure 2.** Methacholine activated  $\text{Ca}^{2+}$  entry in TRPC3 expressed HEK293 cells. A) WT-HEK293 cells; B) TRPC3-HEK293 cells.

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



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### Culture conditions

Cells should be grown at 37° with 7% CO<sub>2</sub> 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 (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 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. 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<sup>R</sup>

hTRPC3 sequence (accession number NM\_003305)

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MEGSPSLRRMTVMREKGRQAVRGPAPFMFNDRTSLTAEERFLDAAEYGNIPVVRKML  
EESKTLNVNVCVDYMGQNALQLAVGNEHLEVTELLKKNLARIGDALLAISKG YVRIV  
EAILNHGFAASKRLTLSPCEQELQDDDFYAYDEGTRFSPDITPIILAAHCQKYE VVH  
MLLMKGARIERPHDYFCKCGDCMEKQRHDSFHSRSRINAYKGLASPAYLSLSEDPVL  
TALELSNELAKLANIEKEFKNDYRKL SMQCKDFVVGVLDCRDSEEVEAILNGDLESAE  
PLEVHRHKASLSRVKLAIKYEVK KFVAHPNCQQQLLTIWYENLSGLREQTIAIKCLVVL  
VVALGLPFLAIGYWIAPCSRLGKILRSPFMKFVAHAASFIIIFLGLLVFNASDRFEGIT T  
LPNITVTDYPKQIFRVKTTQFTWTEMLIMVWVLGMMWSECKELWLEGPREYI LQLWNVL  
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SDPQIIISEGLYAI AVVLSFSRIAYILPANESFGPLQISLGR TVKDIKFVMVLFIMVFFA  
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LYGIYNVTMVVLLNMLIAMINSSYQEIEDDS DVEWKFARSKLWLSYFDDGKTLPPPF S  
LVSPKSFVYFIMRIVNFPKCRRRRLQKDIEMGMGNSK SRLNLFTQSNRVFESH SFNS  
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EELAILIHKLSEKLNPSMLRCE
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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.

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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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