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## **hTMEM16A (ANO1) - HEK293 Recombinant Cell line**

**catalog #: 90230**

### **Product description**

Recombinant HEK293 cell line expressing human TMEM16A (transmembrane protein 16A, also called as anoctamin 1, calcium-activated chloride channel (ANO1), accession number NM\_018043).

### **Format**

Each vial contains  $1 \times 10^6$  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**

Calcium-activated chloride channels (CaCCs) are major regulators in numerous physiological processes including sensory transduction, epithelial secretion, cardiac and neuronal excitation, and smooth muscle contraction.

TMEM16A (ANO1), a member of a family of putative plasma membrane proteins, is identified as a CaCC that is activated by intracellular  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ -mobilizing stimuli. It has eight putative transmembrane segments without domains evidently involved in calcium regulation. The relative permeability of TMEM16A to monovalent anions is  $\text{NO}_3^- > \text{I}^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$ .

### **Functional validation**

N<sup>7</sup> terminal FLAG tagged human TMEM16A channel has been stably expressed in HEK293 cell line and its expression was confirmed by western blotting.

The CaCC activity of TMEM16A was characterized by an assay based on halide-sensitive yellow fluorescent protein (YFP) mutant whose fluorescence is quenched by increasing halide concentration. When TMEM16A-expressed HEK293 cells were stimulated with ionomycin to raise the intracellular level of  $\text{Ca}^{2+}$ , TMEM16A produced  $\text{I}^-$  influx in HEK293 that triggered the rapid decrease of fluorescence of transfected YFP mutant. The ionomycin-induced  $\text{I}^-$  influx through TMEM16A was blocked by niflumic acid, a CaCC channel blocker.

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These data shows the stable expression of TMEM16A channel in HEK293 cells.

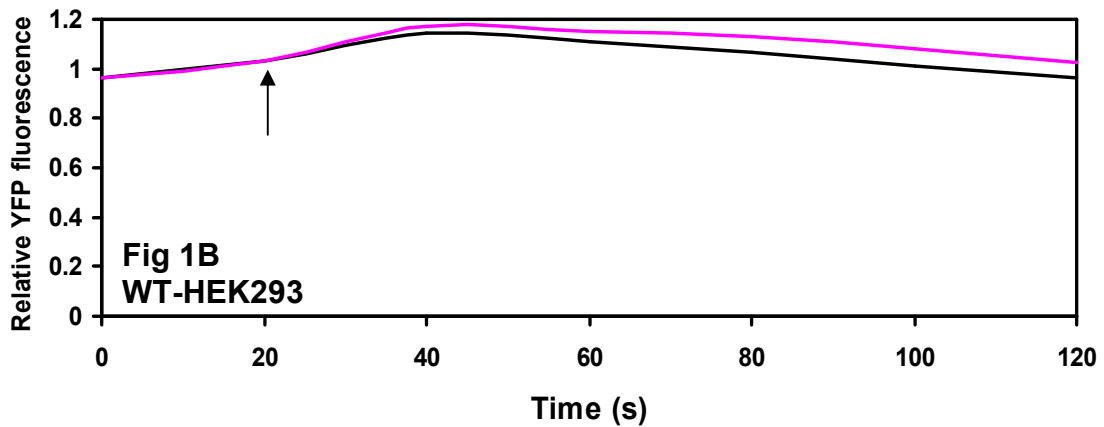
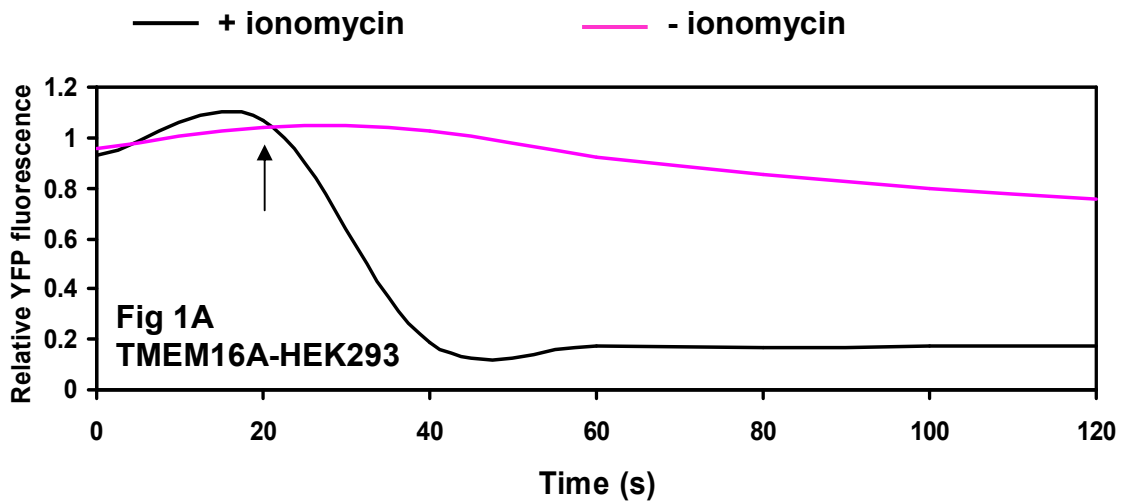
Figure 1 TMEM16A expressed in HEK293 produced  $\Gamma$  influx after extracellular addition of  $\Gamma$  with ionomycin. A) TMEM16A-HEK293 cells; B) WT-HEK293 cells.

TMEM16A-HEK293 or WT-HEK293 cells were transiently transfected with halide-sensitive YFP-H148Q/I152L mutant, then treated with  $\Gamma$  (100mM) saline solution (arrow) with (black) or without (pink) ionomycin (1 $\mu$ M).  $\Gamma$  influx was measured by YFP fluorescence (excited at 485 $\pm$ 10nm and emission at 528 $\pm$ 10nm). Results showed that following iodide addition YFP fluorescence declined rapidly with ionomycin treatment in TMEM16A-HEK293 cells only due to  $\Gamma$  influx through TMEM16A channel.

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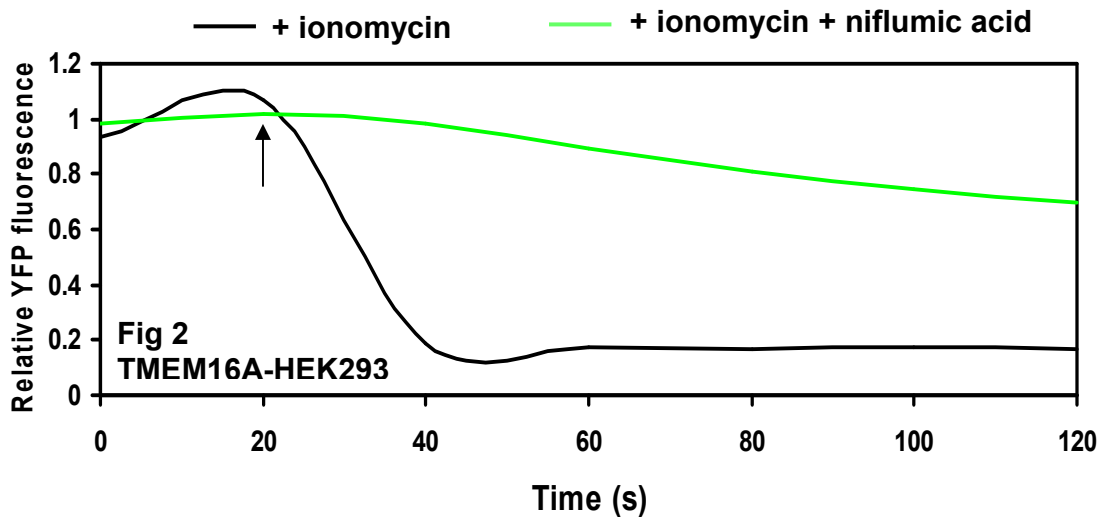


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Figure 2 Ionomycin-induced  $\Gamma$  influx in TMEM16A-HEK293 cells was blocked by niflumic acid, a CaCC channel blocker.

Cells were transiently transfected with halide-sensitive YFP-H148Q/I152L mutant, then treated with  $\Gamma$  (100mM) saline solution plus ionomycin (1 $\mu$ M) (arrow) with (green) or without (black) pre-treatment of niflumic acid (100 $\mu$ M).  $\Gamma$  influx was measured by YFP fluorescence (excited at 485 $\pm$ 10nm and emission at 528 $\pm$ 10nm). Results showed that ionomycin-induced  $\Gamma$  influx through TMEM16A that quenched YFP fluorescence was blocked by niflumic acid.



### 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  $\mu$ g/ml of Geneticin (invitrogen #11811031) to ensure the recombinant expression is maintained. TMEM16A-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

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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 TMEM16A was cloned into pIRES-neo vector (Clontech).

Polylinker: CMV-EcoRV-NheI-TMEM16A-EcoRI-BamHI-NotI-BstXI-IRES-neomycin<sup>R</sup>

hTMEM16A sequence (accession number NM\_018043)

MRVNEKYSTLPAEDRSVHIINICAIEDIGYLPSEGTLLNSLSVDPDAECKYGLYFRDGR  
RKVDYILVYHHKRPSGNRTLVRVQHS DTPSGARSVKQDHPLPGKGASLDAGSGEPPMD  
YHEDDKRFRREEYEGNLLLEAGLELERDEDTKIHG VGFVKIHAPWNVLCREAEFLKMKMP  
TKKMYHINETRGLLKKINSVLQKITDPIQPKVAEHRPQTMKRLSYPPFSREKQHLFDLSD  
KDSFFDSKTRSTIVYEILKRTTCTKAKYSMGITSLLANGVYAAAYPLHDGDYNGENVEF  
NDRKLLYE EWARYGVFYKYQPIDLVRKYFGEKIGLYFAWLGVYTQMLIPASIVGIIIVFL  
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VMALWAATFMEHWKRKQMRNLNWRDLTGFE EEEEEAVKDHPRAEY EARVLEKSLKKE SR  
NKEKRRHIPEESTNKWKQRVKTAMAGVKLTDKVKLTWRDRFPAYLTNLVSIIFMIAVTF  
AIVLGVIIYRISMAAALAMNSSPSVRSNIRVTVTATAVIINLVVILLDEVYGC IARWL  
TKIEVPKTEKSFEERLIFKAFL LKFVNSYTPIFYVAFFKGRFVGRPGDYVYIFRSFRME  
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RKQRYEVDYNLEPFAGLTPEYMEMIIQFGFVTLFVASFPLAPL FALLNNIIEIRLDAKK  
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VLAARLAFVIVFQNLVFMMSDFVDWVIPDIPKDISQQIHKEKVL MVELFMREEQDKQQL  
LETWMEKERQKDEPPCNHHNTKACPDSLGS PASHAYHGGVL

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