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Data Sheet

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 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
Calcium-activated chloride channels (CaCCs) are major regulators in numerous physiological processes including sensory transduction, epithelial secrection, cardiac and neutonal 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 Ca^{2+} and 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 NO_3^- > I^- > Br^- > Cl^- > F^-.

Functional validation
N' 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 Ca^{2+}, TMEM16A produced I^- influx in HEK293 that triggered the rapid decrease of fluorescence of transfected YFP mutant. The ionomycin-induced I^- influx through TMEM16A was blocked by niflumic acid, a CaCC channel blocker.

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

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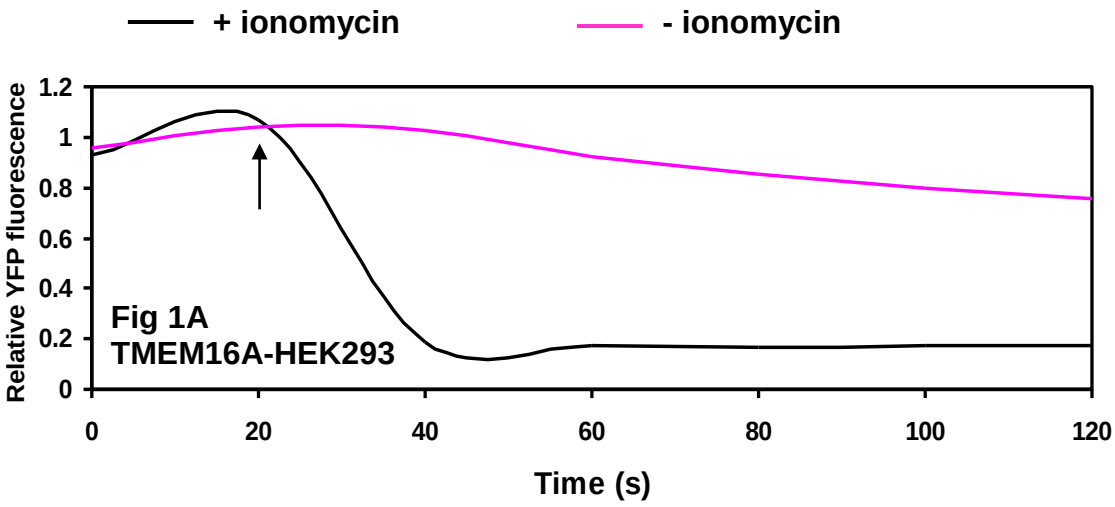
Figure 1. TMEM16A expressed in HEK293 produced I^- influx after extracellular addition of I^- with ionomycin. A) TMEM16A-HEK293 cells; B) WT-HEK293 cells.

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



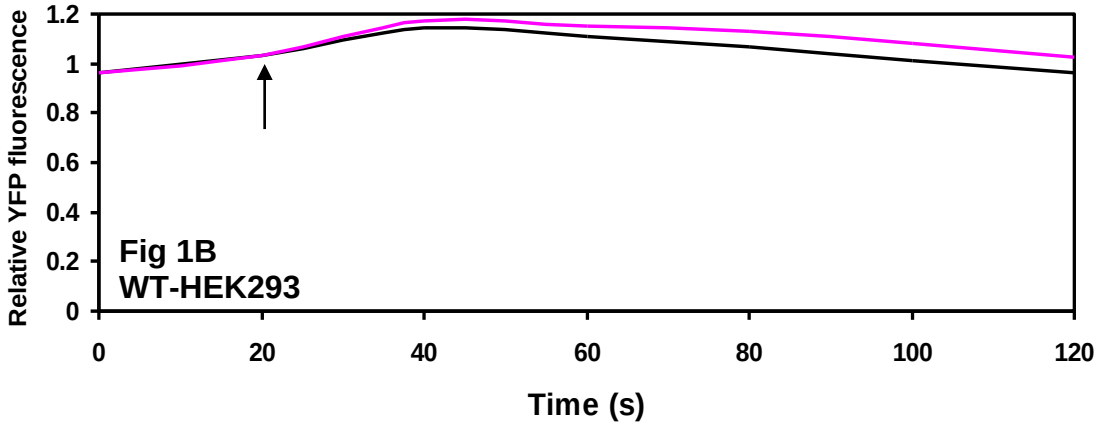


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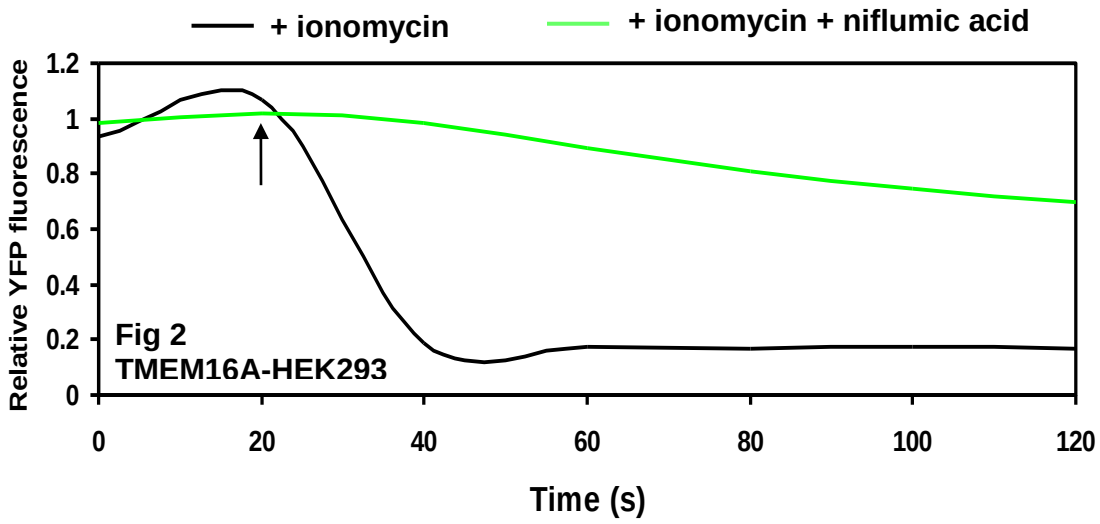
Figure 2. Ionomycin-induced I^- influx in TMEM16A-HEK293 cells was blocked by niflumic acid, a CaCC channel blocker.

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



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.

Cells should be grown at 37° with 7% CO2 using Growth Medium 1B. It may be required to adjust the percentage of CO2 in the incubator depending on the NaHCO3 level in the basal medium. TMEM16A-HEK293 cells should exhibit a typical cell division time of 24 hours.

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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, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**, transfer resuspended cells to T25 flask and culture in 37° CO_2 incubator. At first passage switch to Growth Medium 1B **(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, 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.

Storage
Immediately upon receipt, store in liquid nitrogen.

Vector and sequence
Human TMEM16A was cloned into pIRES-neo vector (Clontech).
Polylinker: CMV-EcoRV-NheI-**TMEM16A**-EcoRI-BamHI-NotI-BstXI-IRES-neomycin^R

hTMEM16A sequence
MRVNEKYSTLPAEDRSVHIINICAIEDIGYLPSEGTLLNSLSVDPDAECKYGLYFRDGRR
KVDYILVYHHKRPSGNRTLVRRVQHSDTPSGARSVKQDHPLPGKGASLDAGSGEPPM
DYHEDDKRFRREEYEGNLLEAGLELERDEDTKIHGVGFVKIHAPWNVLCREAEFLKLK
MPTKKMYHINETRGLLKKINSVLQKITDPIQPKVAEHRPQTMKRLSYPFSREKQHLFDLS
DKDSFFDSKTRSTIVYEILKRTTCTKAKYSMGITSLLANGVYAAAYPLHDGDYNGENVEF
NDRKLLYEEWARYGVFYKYQPIDLVRKYFGEKIGLYFAWLGVYTQMLIPASIVGIIVFLYG
CATMDENIPSMEMCDQRHNITMCPLCDKTCSYWKMSSACATARASHLFDNPATVFFS
VFMALWAATFMEHWKRKQMRLNYRWDLTGFEEEEEAVKDHPRAEYEARVLEKSLKK
ESRNKEKRRHIPEESTNKWKQRVKTAMAGVKLTDKVKLTWRDRFPAYLTNLVSIIFMIA
VTFAIVLGVIIYRISMAAALAMNSSPSVRSNIRVTVTATAVIINLVVIILLDEVYGCIARWLTK
IEVPKTEKSFEERLIFKAFLLKFVNSYTPIFYVAFFKGRFVGRPGDYVYIFRSFRMEECAP
GGCLMELCIQLSIIMLGKQLIQNNLFEIGIPKMKKLIRYLKLKQQSPPDHEECVKRKQRYE
VDYNLEPFAGLTPEYMEMIIQFGFVTLFVASFPLAPLFALLNNIIEIRLDAKKFVTELRRPV
AVRAKDIGIWYNILRGIGKLAVIINAFVISFTSDFIPRLVYLYMYSKNGTMHGFVNHTLSSF
NVSDFQNGTAPNDPLDLGYEVQICRYKDYREPPWSENKYDISKDFWAVLAARLAFVIVF
QNLVMFMSDFVDWVIPDIPKDISQQIHKEKVLMVELFMREEQDKQQLLETWMEKERQK
DEPPCNHHNTKACPDSLGSPAPSHAYHGGVL

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3. Yang Y.D. *et al*. (2008) TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* **455**: 1210-1215
4. Schroeder B.C. *et al*. (2008) Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* **134**: 1019-1029

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