6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

TMEM16B (ANO2) - HEK293 Recombinant Cell line
Cat #: 90332

Description
Recombinant HEK293 cell line expressing human TMEM16B (transmembrane protein 16B, also called as anoctamin 2, calcium-activated chloride channel (ANO2), accession number NM_001278596).

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

Storage
Immediately upon receipt, store vials 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
Calcium-activated chloride channels (CaCCs) are involved in a variety of physiological functions including smooth muscle contraction and olfaction. TMEM16B (ANO2) has been identified as a CaCC that is activated by intracellular Ca^{2+} and Ca^{2+}-mobilizing stimuli. It has eight putative transmembrane segments and is permeable to monovalent anions.

Functional validation
Human TMEM16B channel has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting.

The CaCC activity of TMEM16B was characterized by an assay based on a halide-sensitive yellow fluorescent protein (YFP) mutant whose fluorescence is quenched by increasing halide concentration. When TMEM16B-expressed HEK293 cells were stimulated with ionomycin to raise the intracellular level of Ca^{2+}, TMEM16B produced I^- influx in HEK293, triggering a rapid decrease of fluorescence by the transfected YFP mutant. The ionomycin-induced I^- influx through TMEM16B was blocked by niflumic acid, a CaCC channel blocker.

This data shows the stable expression of TMEM16B channel in HEK293 cells.

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Figure 1. TMEM16B expressed in HEK293 produced I^- influx after extracellular addition of I^- with ionomycin. A) TMEM16B-HEK293; B) parental HEK293 cells.

TMEM16B-HEK293 or parental HEK293 cells were transiently transfected with halide-sensitive YFP-H148Q/I152L mutant, then treated with I^- (100 mM) saline solution (arrow) with (black) or without (pink) ionomycin (1 µM). I^- influx was measured by YFP fluorescence (excited at 485±10 nm and emission at 528±10 nm). Results showed that following iodide addition, YFP fluorescence declined rapidly with ionomycin treatment in TMEM16B-HEK293 cells (but not parental HEK293 cells) due to I^- influx through the TMEM16B channel.

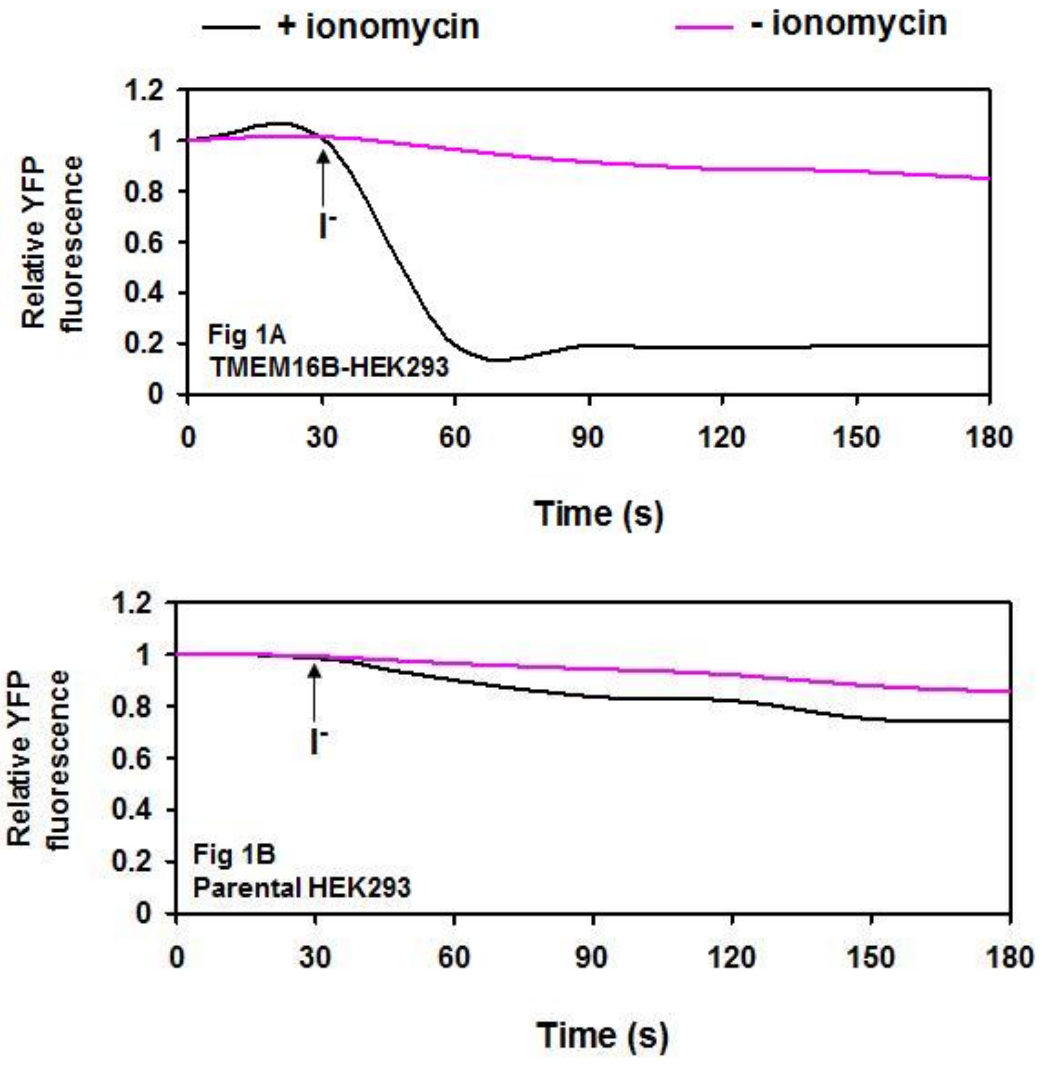

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Figure 2. Ionomycin-induced I^- influx in TMEM16B-HEK293 cells was blocked by niflumic acid, a CaCC channel blocker. A) TMEM16B-HEK293; B) parental HEK293 cells.

Cells were transiently transfected with halide-sensitive YFP-H148Q/I152L mutant, then treated with I^- (100 mM) saline solution plus ionomycin (1 µM) (arrow) with (green) or without (black) pre-treatment of niflumic acid (100 µM). I^- influx was measured by YFP fluorescence (excited at 485±10 nm and emission at 528±10 nm). Results showed that quenching of YFP fluorescence by ionomycin-induced I^- influx through TMEM16B was blocked by niflumic acid.

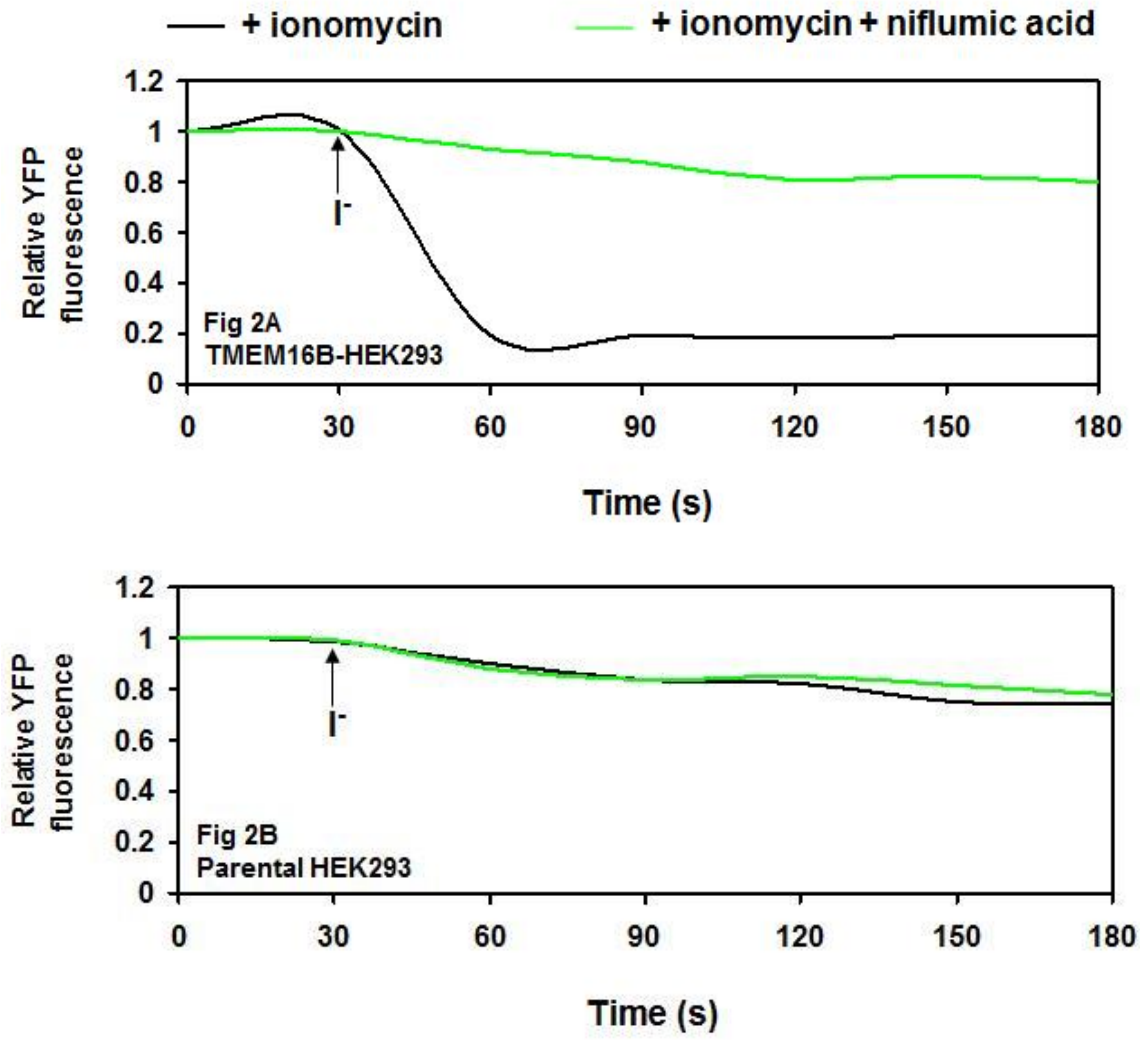

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Figure 4: Whole- cell patch clamp recordings from HEK-hTMEM16B cells.

A, lack of current response in cell with $[Ca^{2+}]I$ clamped at 0 mV. Voltage protocol used a holding potential of -70mV with 1s steps to voltages between - 90mV and +90mV in 20mV increments. B, increasing $[Ca^{2+}]I$ to 590nM reveals a rapidly activating and inactivating, outwardly rectifying current. Addition of the chloride channel blocker CaCCinhA01 at 100μM effectively inhibited the current activated by elevation of $[Ca^{2+}]i$. C, current-voltage relationships (I-V curves) for the recordings shown in A. Image provided by Enterprise Therapeutics, Brighton, U.K

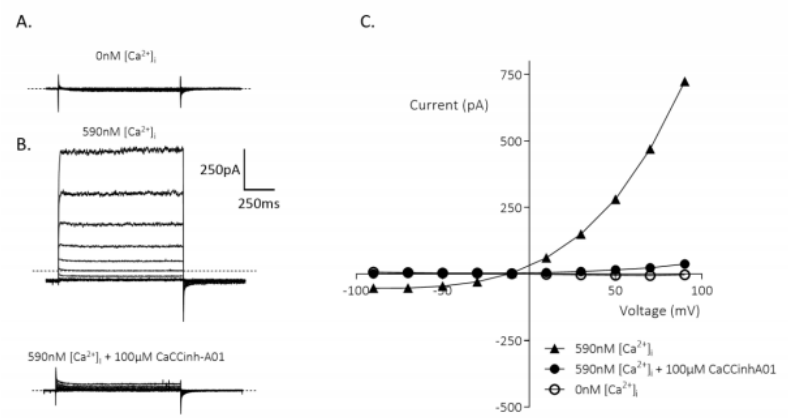
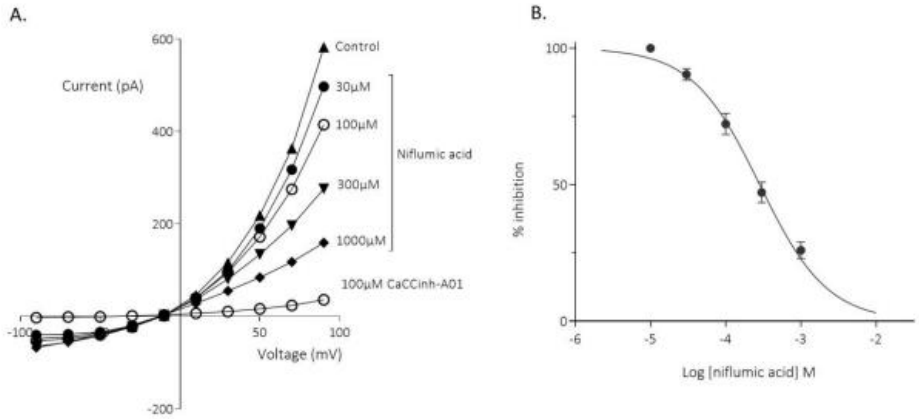


Figure 2: Niflumic acid blocks the currents in HEK-hTMEM16B cells.

A, current-voltage relationship showing the effects of increasing concentrations of niflumic acid upon the Ca2+activated currents in a single HEK-hTMEM16B cell B, concentration-response curve for inhibition of TMEM16B currents by niflumic acid – symbols are mean (15 cells) with s.e.m. indicated by the vertical bars. Image provided by Enterprise Therapeutics, Brighton, U.K



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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 1F (BPS Cat. #79540): Thaw Medium 1 (BPS Cat. #60187) plus 100 µg/ml of Hygromycin B (Hyclone #SV30070.01) to ensure the recombinant expression is maintained. It may be necessary to adjust the percentage of CO_2 in the incubator, depending on the $NaHCO_3$ level in the basal medium. TMEM16B-HEK293 cells should exhibit a typical cell division time of approximately 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 Thaw Medium 1 **(no Hygromycin B)**, spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 **(no Hygromycin B).** Transfer resuspended cells to T25 flask and culture at 37°C in a CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 1 **(no Hygromycin B)**, and continue growing the culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should reach ~60-80% confluence two days after being thawed. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1F **(contains Hygromycin B).**

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.05% Trypsin/EDTA. Add Growth Medium 1F 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 or twice a week.

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Vector and sequence
Human TMEM16B was cloned into pIRES-hyg vector (Clontech).
Polylinker: CMV-BsrGI-StuI-AflII-NaeI-BssHII-NheI-**TMEM16B**-BamHI-EcoRV-BsiWI-BstXI-IRES-hygromycin^R

hTMEM16B sequence (accession #NM_001278596, UniProtKB/Swiss-Prot #Q9NQ90-1)

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LLVDFFLKEEHEKLKLMDEPALRSPGGGDRSRSRAASSAPSGQSQLGSMMSSGSQHTNV

References
Verkman A.S. and Galietta L.J.V. (2009) Cholide channels as drug targets. *Nature Reviews* **8:**153-171.

Scudieri P. *et al.* (2012) The anoctamin family: TMEM16A and TMEM16B as calcium-activated chloride channels. *Exp. Physiol.* **97(2):**177-183.

Stöhr H. *et al.* (2009) TMEM16B, a novel protein with calcium-dependent chloride channel activity, associates with a presynaptic protein complex in photoreceptor terminals. *J. Neurosci.* **29(21):**6809-6818.

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