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Data Sheet

TRPM2-HEK293 Recombinant Cell line
Cat #: 90331

Description
Recombinant HEK293 cell line expressing tetracycline-inducible human TRPM2 (transient receptor potential cation channel, subfamily M, member 2, accession number NM_003307).

Host cell
HEK293 cells, tetracycline-inducible

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
TRPM2 channel belongs to the transient receptor potential channel (TRP) superfamily which is divided into seven main subfamilies. TRPM2 channel is a nonselective, calcium-permeable cation channel highly expressed in the brain, vascular smooth muscle, endothelial cells and immune cells. It is activated by oxidative stressors such as hydrogen peroxide, cyclic ADP ribose, and NADP, leading to an increase in the intracellular free calcium concentration and cell death. TRPM2 is a potential drug target in a variety of human diseases, including cardiovascular and neurodegenerative diseases.

Applications
• Monitor TRPM2 calcium channel activity
• Screen for activators or inhibitors of TRPM2 calcium channel

Functional validation
N´- terminal FLAG tagged human TRPM2 channel has been stably integrated intoHEK293 cellsand its expression can be induced by tetracycline (doxycycline). The tetracycline-inducible expression of TRPM2 was confirmed by Western blotting.

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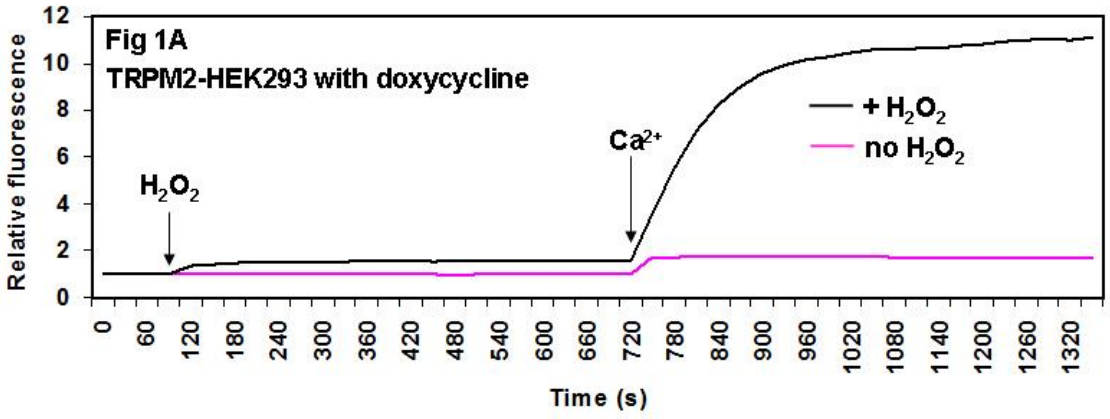
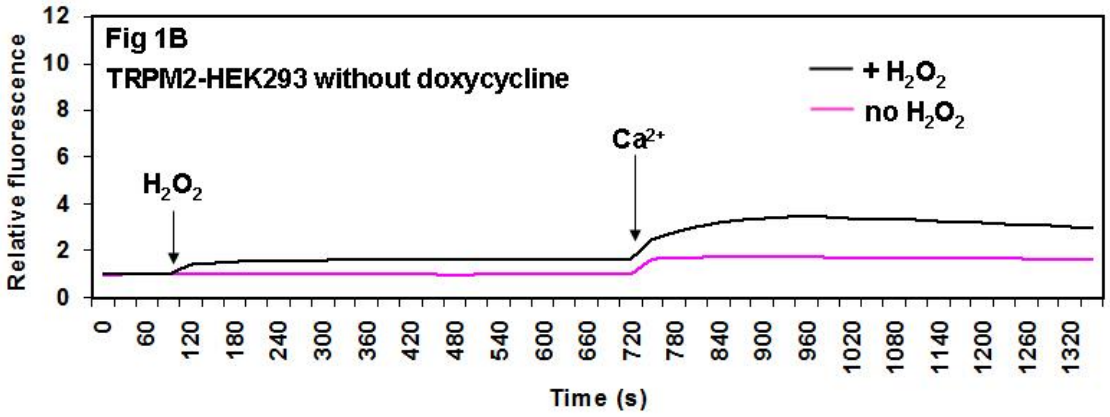
The function of TRPM2 was characterized by calcium assay. TRPM2-HEK293 cells were pre-treated with doxycycline to induce the expression of TRPM2, then stimulated with H_2O_2, and calcium influx was observed. The H_2O_2-induced calcium influx via TRPM2 could be blocked by N-(p-Amylcinnamoyl) anthranilic Acid (ACA), a TRP channel blocker.

Figure 1. TRPM2 expression in HEK293 cells produced calcium influx after extracellular addition of H_2O_2.





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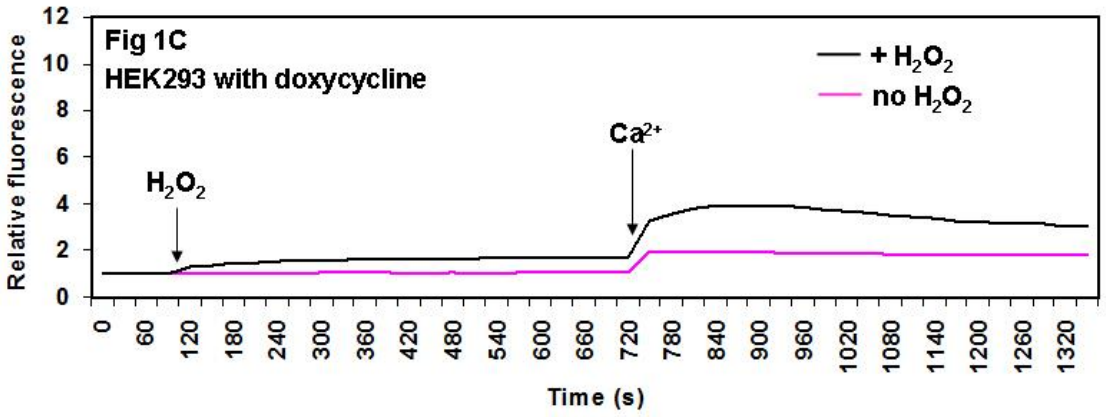

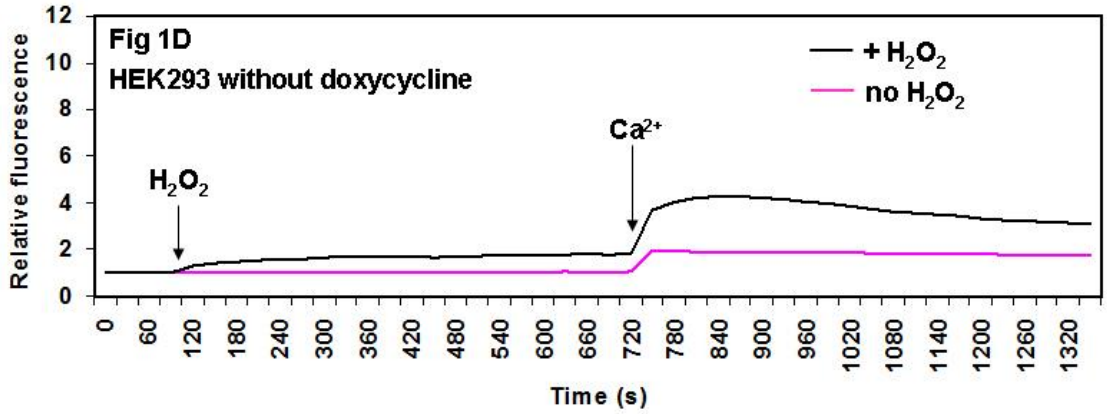

A) TRPM2-HEK293 cells with doxycycline treatment; B) TRPM2-HEK293 cells without doxycycline treatment; C) Control HEK293 cells (tetracycline-inducible) with doxycycline treatment; D) Control HEK293 cells (tetracycline-inducible) without doxycycline treatment.

TRPM2-HEK293 or parental HEK293 cells (tetracycline-inducible) cells were pre-treated with doxycycline (A and C) or without doxycycline (B and D) for 24 hours. The next day cells were loaded with the fluorescent Ca^{2+} indicator Fluo-8 and incubated in the absence of extracellular Ca^{2+}, then treated with (black) or without (pink) H_2O_2 (~ 6.7 mM). Following this, Ca^{2+} (final 10 mM) was added to cells where indicated. The calcium influx was measured by Fluo-8 fluorescence (excitation 485±10nm and emission 528±10nm).

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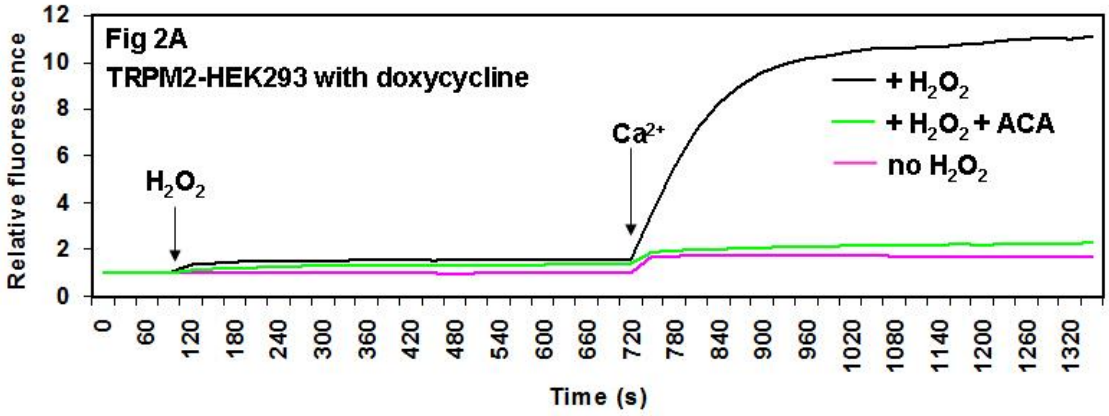
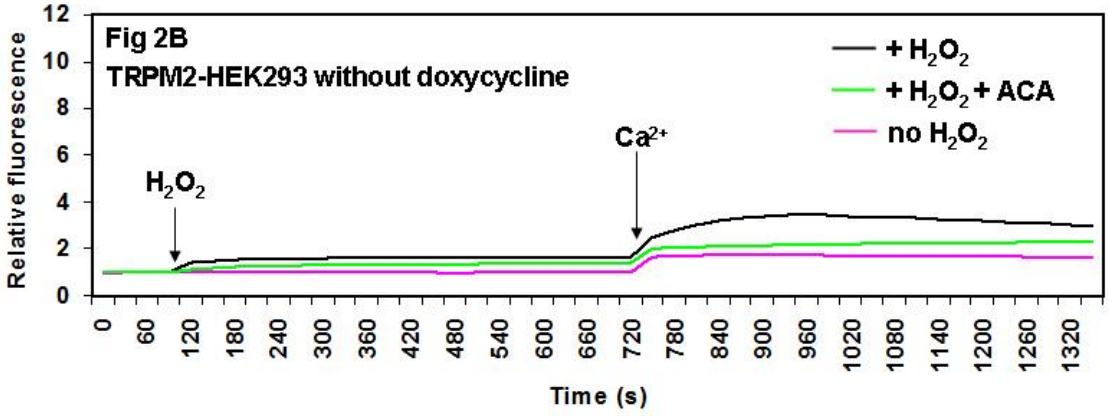
Figure 2. H_2O_2-induced Ca^{2+} influx in doxycycline-treated TRPM2-HEK293 cells was blocked by N-(p-Amylcinnamoyl) anthranilic Acid (ACA), a TRP channel blocker.
A) TRPM2-HEK293 cells with doxycycline treatment; B) TRPM2-HEK293 cells without doxycycline treatment;

TRPM2-HEK293 cells were pre-treated with (A) or without (B) doxycycline for 24 hours. The next day cells were loaded with the fluorescent Ca^{2+} indicator Fluo-8 and incubated with or without ACA (10 μM) in the absence of extracellular Ca^{2+}, then treated with or without H_2O_2 (~ 6.7 mM). Following this, Ca^{2+} (final 10 mM) was added to cells where indicated. The calcium influx was measured by Fluo-8 fluorescence (excitation 485±10nm and emission 528±10nm).





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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 5 μg/ml Blasticidin (Life Technologies # R210-01), and 400 μg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium (Thaw Medium 1, Blasticidin, and Geneticin). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium. Cells should exhibit a typical cell division time of ~24 hours.

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 Blasticidin and Geneticin)**, spin down the cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Blasticidin and Geneticin)** in a T25 flask, and seed appropriate aliquots of cell suspension into new culture vessels. Culture at 37°C in a CO_2 incubator. At first passage switch to complete growth medium **(contains Blasticidin and 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 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 ratio: 1:10 weekly.

Induction of the target protein expression
Induce cells in MEM medium, 10% FBS, 1% non-essential amino acids, 1 mM Na-pyruvate, 1% Penicillin/Streptomycin, 0.2 μg/ml Doxycycline (MP Biomedicals #0219504401) for 24 hours before cell harvesting or assay.

Vector and sequence
N´-terminal FLAG tagged human TRPM2 was cloned into a tetracycline regulated expression vector.
Polylinker: CMV-tetracycline operator (x 2)-BamHI-**TRPM2**-XhoI---SV40-neomycin^R

hTRPM2 sequence (accession number NM_003307)
MEPSALRKAGSEQEEGFEGLPRRVTDLGMVSNLRRSNSSLFKSWRLQCPFGNNDKQ
ESLSSWIPENIKKKECVYFVESSKLSDAGKVVCQCGYTHEQHLEEATKPHTFQGTQWD
PKKHVQEMPTDAFGDIVFTGLSQKVKKYVRVSQDTPSSVIYHLMTQHWGLDVPNLLISV

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NLLKCGMEVYKGYMDDPRNTDNAWIETVAVSVHFQDQNDVELNRLNSNLHACDSGAS
IRWQVVDRRIPLYANHKTLLQKAAAEFGAHY

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1. Moran, M.M., *et al.* (2011) Transient receptor potential channels as therapeutic targets. *Nat. Rev. Drug Discov*. **10(8):**601-620
2. Song, Y., *et al.* (2008) Development and validation of a cell-based high-throughput screening assay for TRPM2 channel modulators. *J. Biomol. Screen*. **13(1):**54-61
3. Fonfria, E., *et al.* (2004) TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase. *Br. J. Pharmacol.* **143(1):**186-192

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