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IKCA1 (KCNN4) - HEK293 Recombinant Cell Line Cat. 90330

Product description:

Recombinant HEK293 cell line expressing human IKCA1, also known as KCNN4 (Intermediate conductance calcium-activated potassium channel protein 4), IK1, hKCa4, and hSK4), Genbank Accession No. NM_002250.

Format:

Each vial contains 2×10^6 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:

IKCA1 is part of a potentially heterotetrameric voltage-independent potassium channel that is activated by intracellular calcium. Activation is followed by membrane hyperpolarization, which promotes calcium influx. The encoded protein may be part of the predominant calcium-activated potassium channel in T-lymphocytes.

Functional validation:

N-terminal FLAG-tagged human IKCA1 has been stably expressed in HEK293 cell line and its expression was confirmed by western blotting.

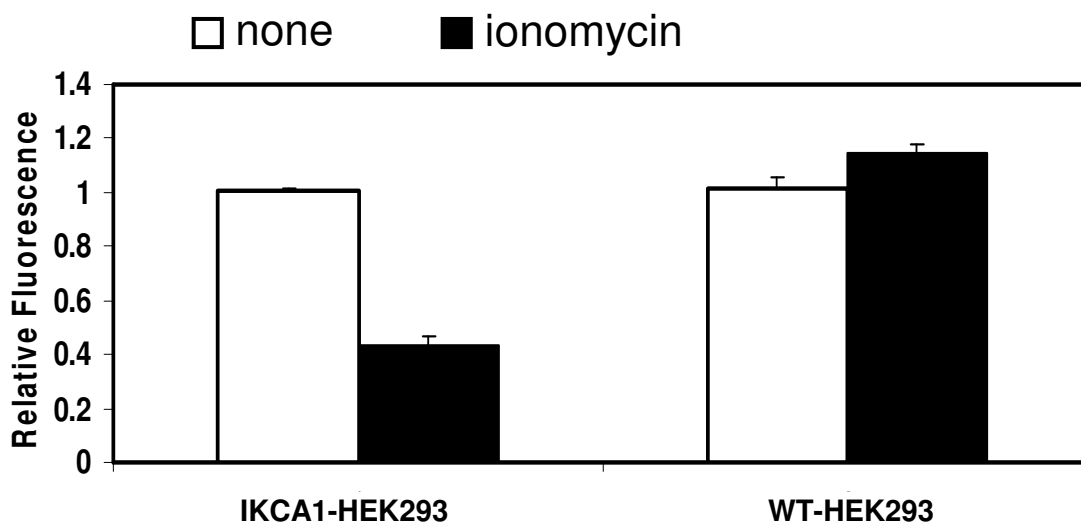
Functional analysis of IKCA1 channel in the stable recombinant HEK293 was accomplished by using the membrane potential-sensitive fluorescence dye DiBAC4(3). When IKCA1-expressed HEK293 cells were stimulated with calcium-specific ionophore ionomycin to raise the intracellular level of Ca^{2+} , IKCA1 channel was activated, leading to hyperpolarization and a decrease in cell fluorescence in the functional fluorescence assay with DiBAC4(3). This decrease of cell fluorescence was blocked by the IKCA1 channel inhibitor, clotrimazole (CLT).

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These data show the stable expression of IKCA1 in HEK293 cells.

Figure 1. Ionomycin-induced increase of intracellular calcium level activated IKCA1 channel expressed in recombinant HEK293 cells.

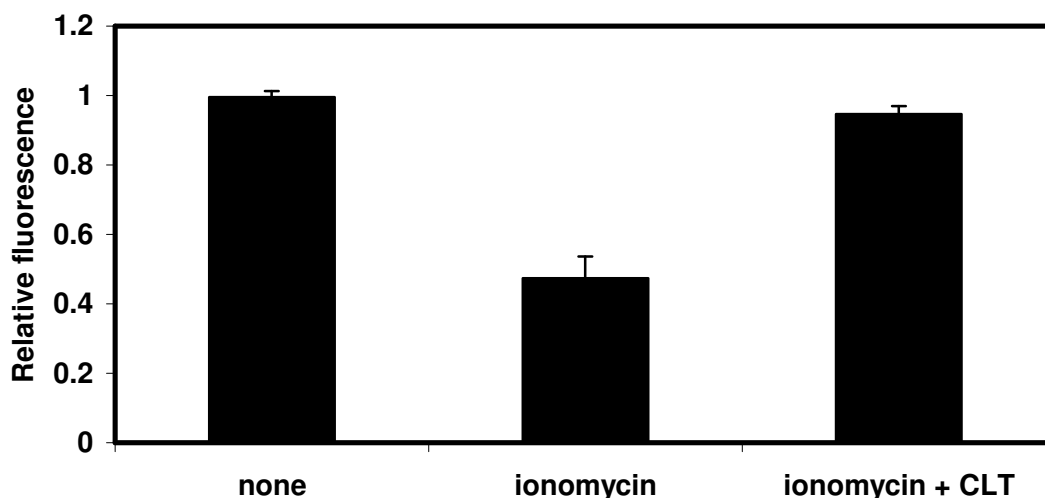


IKCA1 stably-expressed HEK293 or WT-HEK293 cells were pre-incubated with DiBAC4(3), then treated with ionomycin (1 μ M). Channel activation was monitored by measuring cell fluorescence (excitation 485 ± 10 nm, emission 528 ± 10 nm). Results showed that ionomycin-treated IKCA1-HEK293 cells exhibited decreased cell fluorescence due to hyperpolarization induced by channel activation. (Values are presented as cell fluorescence after addition of ionomycin / cell fluorescence before addition of ionomycin.)

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Figure 2. Ionomycin-induced IKCA1 activation in IKCA1-HEK293 cells was blocked by clotrimazole (CLT), an IKCA1 channel inhibitor.



IKCA1-HEK293 cells were pre-incubated with DiBAC4(3) in the presence of CLT (1 μ M) or control DMSO, then treated with ionomycin (1 μ M). Channel activation was monitored by measuring cell fluorescence (excitation 485 ± 10 nm, emission 528 ± 10 nm). Results showed that the ionomycin-induced cell fluorescence decrease in IKCA1-HEK293 cells was blocked by CLT. (Values are presented as cell fluorescence after addition of ionomycin / cell fluorescence before addition of ionomycin.)

Culture conditions:

Cells should be grown at 37°C with 7% CO₂ using MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) medium supplemented with 10% FBS (Hyclone #SH30070.03), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na-pyruvate (Hyclone #SH30239.01), plus 400 μ g/ml of Geneticin (Invitrogen #11811031) to ensure the recombinant expression is maintained. IKCA1-HEK293 cells typically exhibit a cell division time of ~24 hours.

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

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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 IKCA1 was cloned into pIRESneo3 vector (Clontech).

Polylinker: CMV-EcoRV-NheI-**IKCA1**-EcoRI-BamHI-NotI-BstXI-IRES-neomycin^R

Human IKCA1 sequence (accession number NM_002250)

MGGDLVLGLGALRRRKRLLEQEKSLAGWALVLAGTGIGLMVLHAEMLWFGGCSWALYLF
LVKCTISISTFLLCLIVAFHAKEVQLFMTDNGLRDWRVALTGRQAAQIVLELVVCGH
PAPVRGPPCVQDLGAPLTSQPWPGFLGQGEALLSLAMLLRLYLVPRAVLLRSGVLLNA
SYRSIGALNQVRFRRHWFVAKLYMNTHPGRLLLGLTLGLWLTAWVLSVAERQAVNATGH
LSDTLWLIPITFLTIGYGDVVPGTMWGKIVCLCTGVMGVCCTALLVAVVARKLEFNKAE
KHVHNFMMDIQYTKEMKESAARVLQEAWMFYKHTRRKESHAARRHQKLLAAINAFRQV
RLKHRKLREQVNSMVDISKMHMILYDLQQNLSSSHRALEKQIDTLAGKLDALTELLSTA
LGPRQLPEPSQQSK

References:

Ghanshani S *et al.* (2000) Up-regulation of the IKCa1 potassium channel during T-cell activation. *J Biol Chem.* **275(47)**: 37137-37149

Hoffman JF *et al.* (2003) The hSK4 (KCNN4) isoform is the Ca²⁺-activated K⁺ channel (Gardos channel) in human red blood cells. *PNAS* **100 (12)**: 7366-7371

de-Allie FA *et al.* (1996) Characterization of Ca(2+)-activated ⁸⁶Rb+ fluxes in rat C6 glioma cells: a system for identifying novel IKCa-channel toxins. *Br J Pharmacol.* **117(3)**:479-487

Terstappen GC *et al.* (2003) The antidepressant fluoxetine blocks the human small conductance calcium-activated potassium channels SK1, SK2 and SK3. *Neurosci Lett.* **346(1-2)**:85-88

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