SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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

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 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:
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.

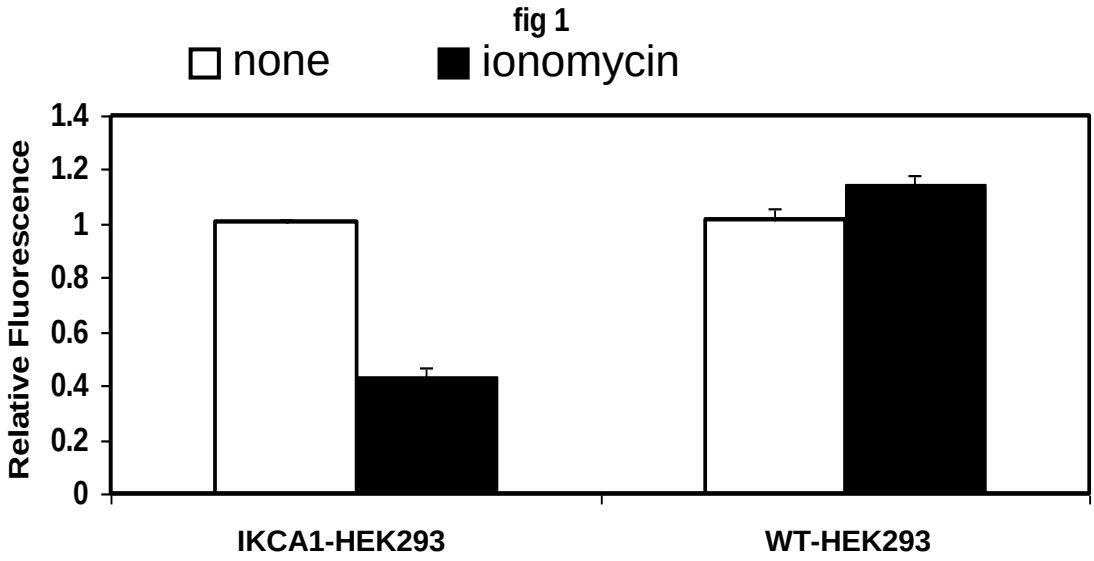
Functional validation:
N-terminal FLAG-tagged human IKCA1 has been stably expressed in HEK293 cell line and its expression was confirmed by western blotting.

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).

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

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

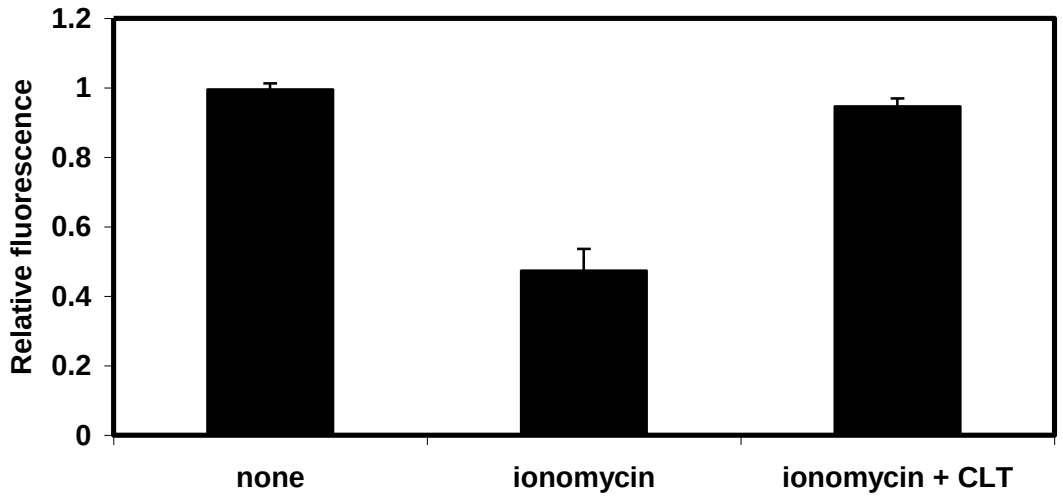
Figure 1. Ionomycin-induced increase of intracellular calcium level activated IKCA1 channel expressed in recombinant HEK293 cells.



IKCA1 stably-expressed HEK293 or WT-HEK293 cells were pre-incubated with DiBAC4(3), then treated with ionomycin (1 μM). Channel activation was monitored by measuring cell fluorescence (excitation 485±10nm, emission 528±10nm). 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.)

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

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 μM) or control DMSO, then treated with ionomycin (1 μM). Channel activation was monitored by measuring cell fluorescence (excitation 485±10nm, emission 528±10nm). 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:

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. IKCA1-HEK293 cells typically exhibit a cell division time of ~24 hours.

Cells should be grown at 37°C with 7% CO_2 using Growth Medium 1B.

Quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Growth Medium 1B, spin down cells, resuspend cells and transfer to T25 flask. Cells should be split before they reach complete confluency. To passage the

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

cells, pre-wash cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA (Hyclone #SH30236.01), 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.

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)
MGGDLVLGLGALRRRKRLLEQEKSLAGWALVLAGTGIGLMVLHAEMLWFGGCSWALY
LFLVKCTISISTFLLLCLIVAFHAKEVQLFMTDNGLRDWRVALTGRQAAQIVLELVVCGLH
PAPVRGPPCVQDLGAPLTSPQPWPGFLGQGEALLSLAMLLRLYLVPRAVLLRSGVLLN
ASYRSIGALNQVRFRHWFVAKLYMNTHPGRLLLGLTLGLWLTTAWVLSVAERQAVNAT
GHLSDTLWLIPITFLTIGYGDVVPGTMWGKIVCLCTGVMGVCCTALLVAVVARKLEFNK
AEKHVHNFMMDIQYTKEMKESAARVLQEAWMFYKHTRRKESHAARRHQRKLLAAINA
FRQVRLKHRKLREQVNSMVDISKMHMILYDLQQNLSSSHRALEKQIDTLAGKLDALTEL
LSTALGPRQLPEPSQQSK

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 Ca^{2+}-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 ^{86}Rb+ 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

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory.

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

BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees**;** contact *sales@bpsbioscience.com* for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.