Description

IKCA1 (KCNN4) HEK293 Cell Line are engineered HEK293 cells expressing human IKCA1, also known as KCNN4 (Intermediate conductance calcium-activated potassium channel protein 4), IK1, hKCa4, and hSK4), Genbank Accession No. NM_002250. This cell line has been validated in a cellular assay using a membrane potential-sensitive fluorescence dye (DiBAC4(3)) and by Western blot for IKCA1 expression.

Background

IKCA1, also known as Intermediate conductance calcium-activated potassium channel protein 4), IK1, hKCa4, and hSK4, are part of a potentially heterotetrametric 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, but it is also present in endothelial cells and cardiac fibroblasts. They contribute to vascular smooth muscle cell proliferation, migration and cardiac fibrosis. IKCA1 was found to be present at high levels in breast cancer (BC) cells, and when depleted lead to decreased tumorigenesis. IKCA1 can be modulated with small molecules, and these may provide a therapeutical opportunity in the treatment of BC and cardiovascular diseases.

Application

Screen and validate agonists and antagonists of IKCA1 during research and drug discovery.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1B (BPS Bioscience #79531):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin.

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Functional Data

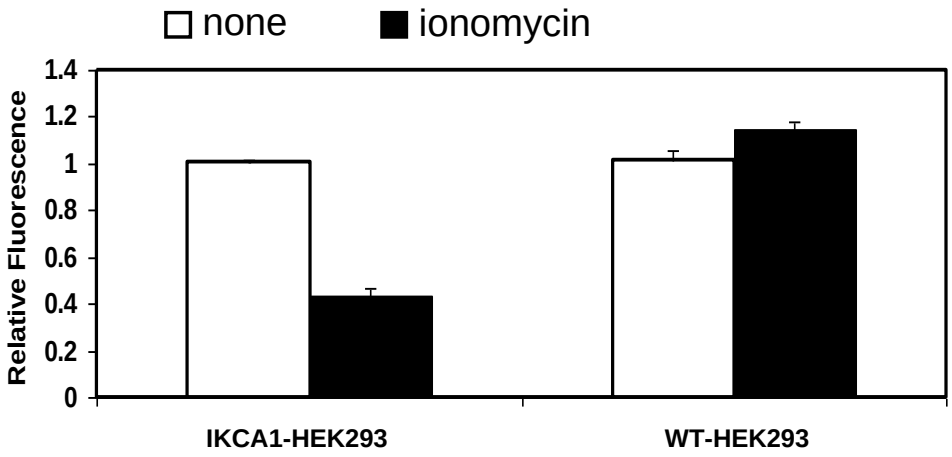

Figure 1. Ionomycin-induced increase of intracellular calcium level activates IKCA1 channel expressed in IKCA1 (KCNN4) HEK293 Cell Line.
IKCA1 (KCNN4) HEK293 cells and wild-type HEK93 cells were pre-incubated with DiBAC4(3), then treated with ionomycin (1 μM). Channel activation was monitored by measuring cell fluorescence (λexcitation 485±10nm, λemission 528±10nm). Results indicate that ionomycin-treated IKCA1-HEK293 cells exhibited a decrease in fluorescence due to the hyperpolarization induced by channel activation. Values are presented as cell fluorescence after addition of ionomycin/cell fluorescence before addition of ionomycin.

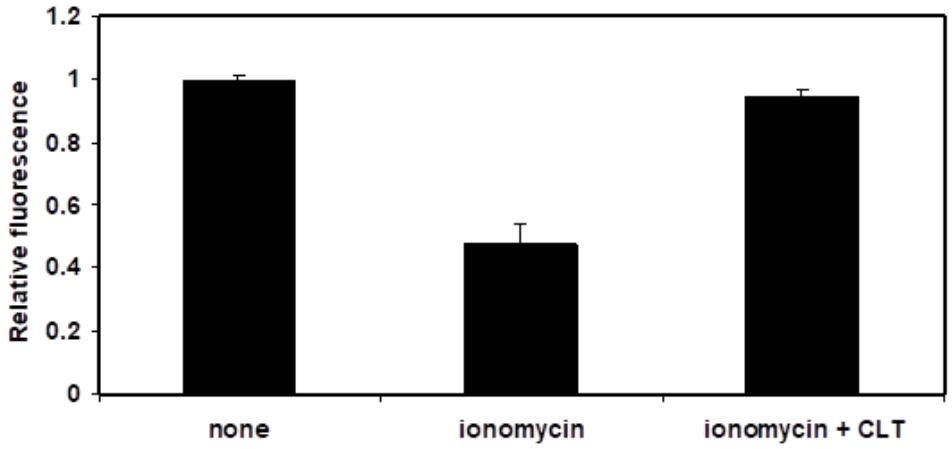

Figure 2. Ionomycin-induced IKCA1 activation in the IKCA1 (KCNN4) HEK293 Cell Line is blocked by clotrimazole (CLT), an IKCA1 channel inhibitor.
IKCA1 (KCNN4) HEK293 cells were pre-incubated with DiBAC4(3) in the presence of CLT (1 μM) or DMSO, then treated with ionomycin (1 μM). Channel activation was monitored by measuring cell fluorescence (λexcitation 485±10nm, λemission 528±10nm). Results indicate that the ionomycin-induced cell fluorescence decrease in IKCA1-HEK293 cells is blocked by CLT. Values are presented as cell fluorescence after addition of ionomycin/cell fluorescence before addition of ionomycin.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*.*

Sequence

Human IKCA1 sequence (accession number NM_002250)

MGGDLVLGLGALRRRKRLLEQEKSLAGWALVLAGTGIGLMVLHAEMLWFGGCSWALYLFLVKCTISISTFLLLCLIVAFHAKEVQL
FMTDNGLRDWRVALTGRQAAQIVLELVVCGLHPAPVRGPPCVQDLGAPLTSPQPWPGFLGQGEALLSLAMLLRLYLVPRAVLL
RSGVLLNASYRSIGALNQVRFRHWFVAKLYMNTHPGRLLLGLTLGLWLTTAWVLSVAERQAVNATGHLSDTLWLIPITFLTIGYG
DVVPGTMWGKIVCLCTGVMGVCCTALLVAVVARKLEFNKAEKHVHNFMMDIQYTKEMKESAARVLQEAWMFYKHTRRKESH
AARRHQRKLLAAINAFRQVRLKHRKLREQVNSMVDISKMHMILYDLQQNLSSSHRALEKQIDTLAGKLDALTELLSTALGPRQLPE
PSQQSK

References

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
ULK1, FLAG-Tag Recombinant	40099	10 µg
ULK1 Kinase Assay Kit	78362	96 reactions
CTFR HEK293 Recombinant Cell Line	60506	2 vials
KCTD3, GST-tag Recombinant	80410	20 µg

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