B'SYS GmbH

CHO TREX SK2 Cell Line

Specification Sheet

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1. BACKGROUND

1.1. Small conductance (SK) calcium-dependent potassium channels (K_{Ca})

Calcium-dependent potassium channels play a pivotal role in the physiology as they are broadly expressed in various mammalian cells and tissues such as neurons, skeletal muscle, and smooth muscle. Within this ion channel family, calcium-activated potassium channels share a common functional role by coupling the increase in intracellular Ca^{2+} concentration to hyperpolarization of the membrane potential. K_{Ca} channels are divided into three main subfamilies as small conductance (SK), intermediate conductance (IK) and big conductance (BK) channels, according to their single-channel conductance. There are three different small conductance channels (SK1-3) which can be present as heteromers in cells. SK2 and SK1 K_{Ca} channels consist of four alpha subunits (encoded by the gene KCNN2 and KCNN1, respectively) forming a transmembrane core allowing a highly selective ion passage. SK channels do not bind Ca^{2+} directly, but rather detect intracellular calcium based on calmodulin, which is constitutively bound to the cytoplasmic C-terminal region.

2. PRODUCT SHIPMENT

2.1. Product Format

CHO cells stably transfected with human SK2 channels:

- 1 x 0.75ml aliquots of frozen cells at 1.6 E+06 cells/ml
- Cells are frozen in complete medium with 10% DMSO
- Cells are frozen at passage number, see vial

2.2. Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

3. VALIDATION OF CHO SK2 CELLS

3.1. Electrophysiology

SK2 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $CaCl_2$ 8.75, $MgCl_2$ 1, HEPES 10, EGTA 10, pH (KOH) 7.20. After formation of a Gigaohm seal between the patch electrodes and individual SK2 stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. Cells were held at a holding potential of -80 mV, and SK2 currents were measured by applying voltage ramps from -120 mV to +60 mV every 5 s. All solutions applied to cells were continuously perfused and maintained at room temperature.

3.2. Pharmacology

In order to determine the concentration-response relationship of Apamin, the antagonist was applied to the cells in a concentration range between 0.0001 nM and 10 nM. A concentration-dependent effect of current inhibition could be observed with an IC_{50} value of 0.13 nM (Hill coefficient: 1.16, n=6, +60 mV). At the highest concentration of Apamin tested (10 nM), 22.70 ± 3.50% of the SK2 current was still remaining, which is in line with the literature (Dale et al., 2002).

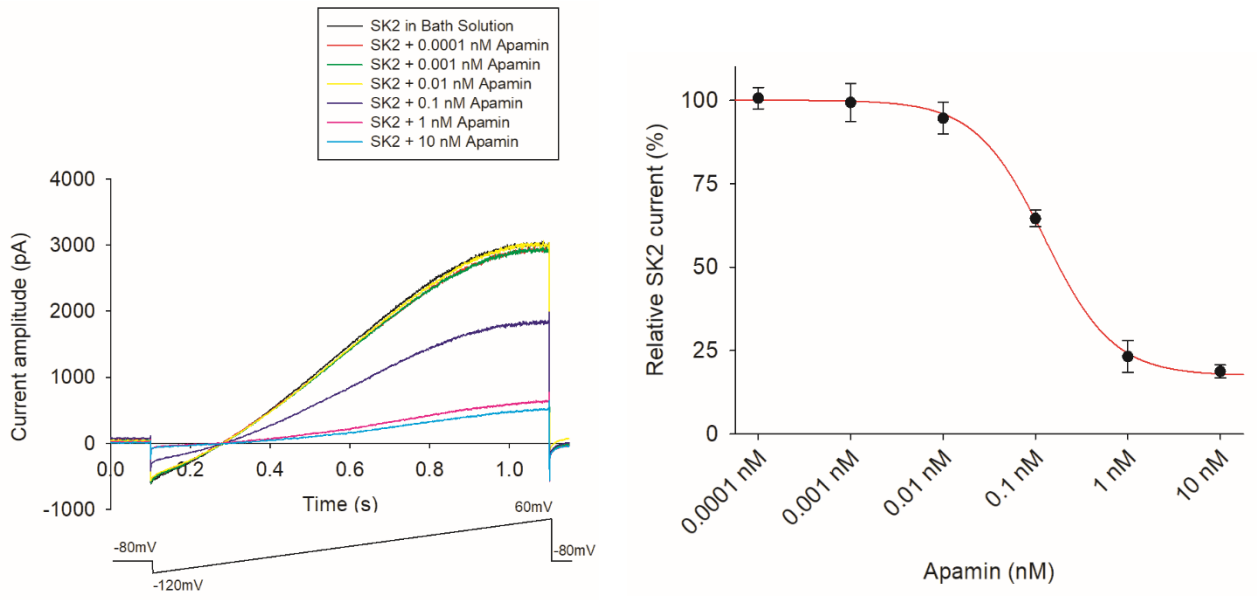

Fig. 1: Representative current recordings for CHO TREX SK2 cells treated with 0.0001 nM, 0.001 nM, 0.01 nM, 0.1 nM, 1.0 nM and 10 nM Apamin (left) and corresponding concentration-response curve (right). IC_{50}: 0.13 nM Apamin, Hill coefficient: 1.16.

4. CELL CULTURE CONDITIONS

4.1. General

CHO TREX SK2 cells are incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing DMEM medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 100 µg/mL Zeocin, 15 µg/mL Blasticidin and 1 µg/mL Puromycin. The CHO SK2 cells are passaged at a confluence of about 80%. For electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes containing complete medium.

- All solutions and equipment coming in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.
- Be sure to have frozen cell stocks at hand before starting experiments.
- Cells should be split every 2-3 days at 70% - 90% confluency at 1:5 to 1:7 ratio.

IMPORTANT: **To induce the expression of SK2 channels, 2.5 µg/mL Tetracycline has to be added at least 16 h to 24 h before experimentation.**

Table 1: Cell culture reagents

Product	Supplier	Order number
Nutrient mixture F-12 Ham (F12 Ham)	Sigma-Aldrich	N6658
Fetal Bovine Serine (FBS)	Gibco	10270-106
Penicillin / Streptomycin (100X)	Gibco	10378-016
Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+})	Sigma-Aldrich	D8537
Zeocin (100 mg/mL)	Gibco	R25001
Puromycin (10 mg/mL)	Gibco	A1113803
Blasticidin S HCl	Gibco	R21001
Detachin	Genlantis	T100T100
Trypsin EDTA (10X)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438
Distilled water (dH_2O)	Gibco	15230204
Tetracycline hydrochloride	Sigma Aldrich	T7660

For the preparation of 1X Trypsin/EDTA, the 10X solution is diluted in PBS (without Ca^{2+} and Mg^{2+}), aliquoted and stored in the freezer.

For the preparation of blasticidin, the powder is diluted in dH_2O to achieve a stock concentration of 15 mg/mL, filter sterilized and stored frozen.

For the preparation of tetracycline, the powder is diluted in dH_2O to achieve a stock concentration of 1 mg/mL, filter sterilized, aliquoted and stored in the freezer. (Do not store for more than 6 months!)

4.2. Recommended Complete Medium

- 500 mL F12 (HAM) with L-Glutamine or GlutaMax I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3. Antibiotics

- To cultivate CHO TREX SK2, 100 µg/mL Zeocin, 15 µg/mL Blasticidin and 1 µg/mL Puromycin are recommended.

4.4. Thawing Cells

- Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
- Decontaminate outside of vial with 70% Ethanol.
- Transfer cells to a T-25 culture flask containing 5 mL complete medium
- Incubate cells at 37°C for at least 4-6 hours to allow the cells to attach to the bottom of the flask
- Once cells attach to the bottom of the flask and look healthy, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 4.3)
- To check whether cells are attached properly, the flask can be gently moved while looking under the microscope
- If 48h after thawing the confluency is below 50%, replace the medium in the flask with fresh medium containing antibiotics
- Incubate cells at 37°C and check them daily until 50% to 80% confluency is reached.

4.5. Splitting Cells

- When cells are 70% to 90% confluent remove complete medium.
- Wash cells with 1xPBS to remove excess medium
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C.
- Detach cells by gently tapping the sides of the flask add complete medium and pipet up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
- Use remaining suspension for counting the cells.

4.6. Freezing Medium

- Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.
- Sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

4.7. Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium and wash cells with 1xPBS.
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C
- Detach cells by gently tapping the sides of the flask, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells at 200 g using a centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 1.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.75 mL of cell suspension into each cryovial.
- Overnight incubate cells in a polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4.8. Inducing Expression of SK2

To induce the expression of SK2 ion channels, 2.5 µg/mL Tetracycline has to be added at least 16 h - 24 h before experimentation.

5. SK2 (KCNN2) SEQUENCE

5.1. Human KCNN2 Accession Number: Q9H2S1

Cloned cDNA sequence encodes for KCNN2

MSSCRYNGGVMRPLSNLSASRRNLHEMDSEAQPLQPPASVGGGGGASSPSAAAAAAAAVSSSAPEIVVSKPEHNNSNNLALYGTGGGGSTGGGG
GGGGSGHGSSSGTKSSKKKNQNIGYKLGHRRALFEKRKRLSDYALIFGMFGIVVMVIETELSWGAYDKASLYSLALKCLISLSTIILLGLIIVYHAREIQLFM
VDNGADDWRIAMTYERIFFICLEILVCAIHPIPGNYTFTWTARLAFSYAPSTTTADVDIILSIPMFLRLYLIARVMLLHSKLFTDASSRSIGALNKINFNTRF
VMKTLMTICPGTVLLVFSISLWIIAAWTVRACERYHDQQDVTSNFLGAMWLISITFLSIGYGDMVPNTYCGKGVCLLTGIMGAGCTALVVAVVARKLEL
TKAEKHVHNFMMDTQLTKRVKNAAANVLRETWLIYKNTKLVKKIDHAKVRKHQRKFLQAIHQLRSVKMEQRKLNDQANTLVDLAKTQNIMYDMISD
LNERSEDFEKRIVTLETKLETLIGSIHALPGLISQTIRQQQRDFIEAQMESYDKHVTYNAERSRSSSRRRRSSSTAPPTSSESS

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