B'SYS GmbH

CHO TREX BK$\alpha_1\beta_2$ Cell Line

Specification Sheet

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1. BACKGROUND

1.1. Big conductance (BK) 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. K_{Ca} channels consist of four alpha subunits (KCNMA1) forming a transmembrane core allowing a highly selective ion passage. In addition, the voltage-dependent and calcium modulated BK channel can bind up to four beta subunits (KCNMB1-4) resulting in a conduction modulation. Binding of calcium by the BK channel is mediated by a cytoplasmic carboxyl domain occurring in the alpha channel subunit. The cytosolic domain is comprised of two RCK (regulator of K^+ conductance) domains, RCK1 and RCK2. These domains contain two high affinity Ca^{2+} binding sites; one in the RCK1 domain and the other one in a region called the "Ca^{2+} bowl" which is located in the RCK2 domain. Binding of calcium results in conformational changes that are in turn responsible for the channel`s activities.

1.2. BK Beta subunit 2 (KCNMB2)

There are four different beta subunits (β_{1-4} encoded by the genes KCNMB1-4) with two transmembrane segments connected with a large extracellular segment. BK channel beta subunits are a family of small double-pass membrane proteins (20 – 30 kDa). They display different and complex effects on apparent calcium and voltage sensitivities, macroscopic current kinetics, and pharmacological sensitivities. The four known BK beta subunits vary in their sequence similarity between 53 to 20%. The β_2 subunit influences the calcium sensitivity.

2. PRODUCT SHIPMENT

2.1. Product Format

CHO cells stably transfected with human BKα$_1$β$_2$ channel:

- 1 x 0.5 mL aliquots of frozen cells at 1.6 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO

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 BKA1B2 CELLS

3.1. Electrophysiology

$BK\alpha_1\beta_2$ 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, Mg-ATP 2, $CaCl_2$ 2.8, $MgCl_2$ 1, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a Gigaohm seal between the patch electrodes and individual $BK\alpha_1\beta_2$ 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 BK currents were measured by applying voltage ramps from -80 mV to +40 mV every 2 s in the presence of the agonist NS19504 (10 µM). All solutions applied to cells were continuously perfused and maintained at room temperature.

3.2. Pharmacology

In previous experiments a concentration-response curve with Paxilline was established in BK channels expressing the alpha1 subunit only with a measured IC_{50} value of 48.80 nM (Hill coefficient 1.11, n=5, +40 mV). The Paxilline- sensitive $BK\alpha_1$ channel showed a remaining current of 3.6% ± 4.2% at 1000 nM Paxilline (n = 4). In order to analyze the pharmacology of cells expressing the alpha subunit and the beta subunit 2, 50 nM Paxilline was applied to cells expressing $BK\alpha_1\beta_2$ channels. A partial block of approx. 50% was also observed for the cells expressing the $BK\alpha_1\beta_2$ channel.

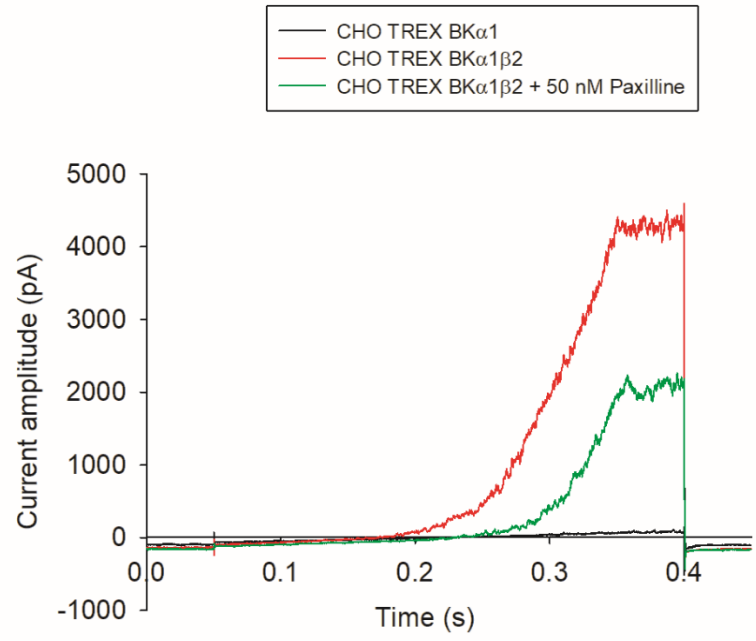

Fig.1: Representative current recordings for CHO TREX $BK\alpha_1\beta_2$ cells in bath solution (red line), treated with 50 nM Paxilline (green line) and as a reference measurement the host cell line CHO TREX in bath solution (black line).

4. CELL CULTURE CONDITIONS

4.1. General

CHO BKα1β2 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 Hygromycin, 20 µg/mL Blasticidin and 2 µg/mL Puromycin. The CHO BKa1 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 80% - 90% confluency at 1:5 to 1:7 ratio.

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
Hygromycin B (50 mg/mL)	Gibco	10687010
Puromycin (10 mg/mL)	Gibco	A1113803
Blasticidin S HCl	Gibco	R21001
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438

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 dH2O to achieve a stock concentration of 15 mg/mL, filter sterilized and stored frozen.

For the preparation of tetracycline, the powder is diluted in dH2O 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!)

IMPORTANT: To induce the expression of BKα1β2 proteins 2.5 µg/mL Tetracycline has to be added at least 16 h to 24 h before experimentation.

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 BKα1β2 cells, 100 µg/mL Hygromycin, 20 µg/mL Blasticidin and 2 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-75 culture flask containing 10 mL complete medium.
- Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 10 mL complete medium & antibiotics.
- Incubate cells and check them daily until 80% - 90% confluency is reached.

4.5. Splitting Cells

- When cells are 80% - 90% confluent remove medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate cells for 3 min at 37°C.
- Detach cells, add complete medium and pipette up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:5 to 1:7 ratio.

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.
- Wash cells once with 1x PBS to remove excess medium.

- Add 1x Trypsin/EDTA and incubate cells for 3 min at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells with centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.5 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 BKα$_1$β$_2$

To induce the expression of BKα$_1$β$_2$ ion channels, 2.5 µg/mL Tetracycline has to be added at least 16 h - 24 h before experimentation.

5. BKA1B2 (KCNMA1 / KCNMB2) SEQUENCE

CHO cells have been stably transfected with codon optimized cDNA encoding for the human subunits by non-viral lipofection.

5.1. Human KCNMA1 Acession Number: NP_002238.2 (isoform b)

Cloned cDNA sequence encodes for KCNMA1

```
MANGGGGGGGSSGGGGGGGGSSLRMSSNIHANHLSLDASSSSSSSSSSSSSSSSSSSSSVHEPKMDALIIPVTMEVPCDSRGQRMWWA
FLASSMVTFFGGLFIILLWRTLKYLWTVCCHCGGKTKEAQKINNGSSQADGTLKPVDEKEEAVAAEVGWMTSVKDWAGVMISAQTLTGR
VLVVLVFALSIGALVIYFIDSSNPIESCQNFYKDFTLQIDMAFNVFFLLYFGLRFIAANDKLWFWLEVNSVVDFFTVPPVFVSVYLNRS
WLGLRFLRALRLIQFSEILQFLNILKTSNSIKLVNLLSIFISTWLTAAGFIHLVENSGDPWENFQNNQALTYWECVYLLMVTMSTVGYG
DVYAKTTLGRLFMVFFILGGLAMFASYVPEIIELIGNRKKYGGSYSAVSGRKHIVVCGHITLESVSNFLKDFLHKDRDDVNVEIVFLHN
ISPNLELEALFKRHFTQVEFYQGSVLNPHDLARVKIESADACLILANKYCADPDAEDASNIMRVISIKNYHPKIRIITQMLQYHNKAHL
LNIPSWNWKEGDDAICLAELKLGFIAQSCLAQGLSTMLANLFSMRSFIKIEEDTWQKYYLEGVSNEMYTEYLSSAFVGLSFPTVCELCF
VKLKLLMIAIEYKSANRESRILINPGNHLKIQEGTLGFFIASDAKEVKRAFFYCKACHDDITDPKRIKKCGCKRLEDEQPSTLSPKKKQ
RNGGMRNSPNTSPKLMRHDPLLIPGNDQIDNMDSNVKKYDSTGMFHWCAPKEIEKVILTRSEAAMTVLSGHVVVCIFGDVSSALIGLRN
LVMPLRASNFHYHELKHIVFVGSIEYLKREWETLHNFPKVSILPGTPLSRADLRAVNINLCDMCVILSANQNNIDDTSLQDKECILASL
NIKSMQFDDSIGVLQANSQGFTPPGMDRSSPDNSPVHGMLRQPSITTGVNIPIITELVNDTNVQFLDQDDDDDPDTELYLTQPFACGTA
FAVSVLDSLMSATYFNDNILTLIRTLVTGGATPELEALIAEENALRGGYSTPQTLANRDRCRVAQLALLDGPFADLGDGGCYGDLFCKA
LKTYNMLCFGIYRLRDAHLSTPSQCTKRYVITNPPYEFELVPTDLIFCLMQFDHNAGQSRASLSHSSHSSQSSSKKSSSVHSIPSTANR
QNRPKSRESRDKQKYVQEERL
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5.2. Human KCNMB2 Acession Number: NP_001265840.1

Cloned cDNA sequence encodes for KCNMB2

```
MFIWTSGRTSSSYRHDEKRNIYQKIRDHDLLDKRKTVTALKAGEDRAILLGLAMMVCSIMMYFLLGITLLRSYMQSVWTE
ESQCTLLNASITETFNCSFSCGPDCWKLSQYPCLQVYVNLTSSGEKLLLYHTEETIKINQKCSYIPKCGKNFEESMSLVN
VVMENFRKYQHFSCYSDPEGNQKSVILTKLYSSNVLFHSLFWPTCMMAGGVAIVAMVKLTQYLSLLCERIQRINR
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