B'SYS GmbH

CHO K_V1.3 Cell Line

Specification Sheet

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1. PRODUCT SHIPMENT

1.1. Product Format

CHO cells stably transfected with recombinant K_V1.3 potassium channel:

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

1.2. 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.

2. VALIDATION OF CHO KV1.3 CELLS

2.1. Electrophysiology

K_v1.3 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, CaCl2 1.8, MgCl2 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, MgCl2 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual K_V1.3 stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established K_V1.3 currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to voltages between -120 mV and +60 mV in 20 mV increments of 800 ms duration (Fig. 1). K_V1.3 tail currents were elicited upon a voltage step to 50 mV for 100 ms. The voltage pulses were run at intervals of 5 s.

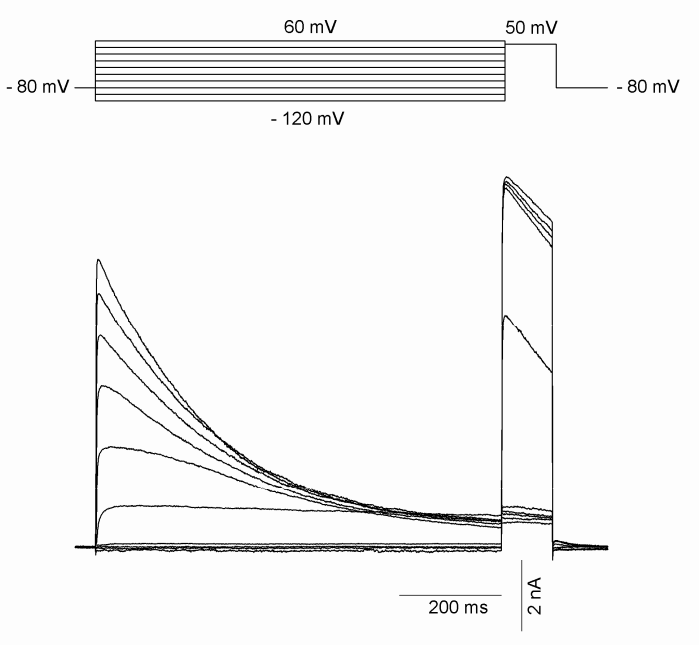

Fig. 1: Representative K_V1.3 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -120 mV and +60 mV in 20 mV increments (800 ms). Voltage pulses to 50 mV (100 ms) elicited KV1.3 tail currents.

2.2. Current Activation

The K_V1.3 channel is known as an outwardly rectifying potassium channel displaying very fast activation and slow inactivation kinetics. To verify the functional properties of B'SYS's cloned CHO K_V1.3 cells, both the inactivation plot and IV curve were constructed. The inactivation plot of K_V1.3 peak currents was best fitted with a Boltzmann function with half-maximal activation at **-19.7 mV** and a slope of **6.7** (Fig. 2, n=4 cells).

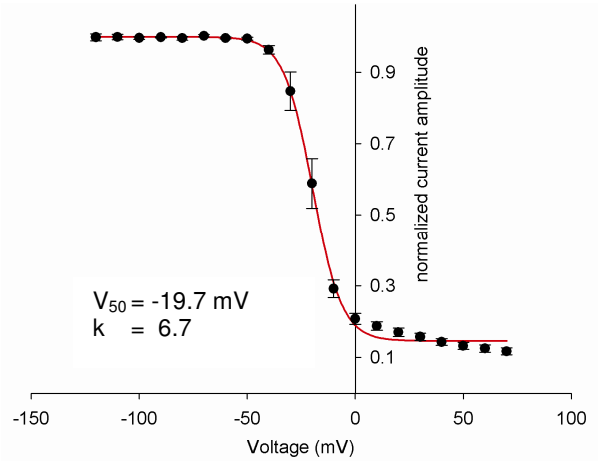

Fig. 2: Inactivation plot of K_V1.3 tail currents fitted to Boltzmann function with V_{50} = -19.7 mV, k = 6.7 (n= 4 cells).

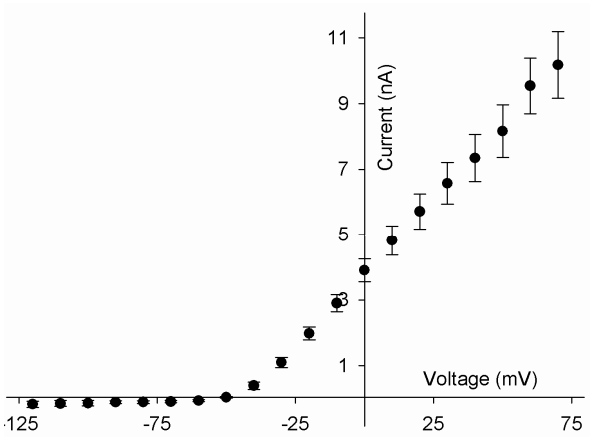

Fig. 3: IV curve of K_V1.3 activation currents. Voltages between -120 and +70 mV were applied.

2.3. Specific KV1.3 channel Blocker Margatoxin

Margatoxin, which is known as a selective K_V1.3 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.3 cells. The effect of Margatoxin was first measured at 1 nM, which blocked K_V1.3 peak currents almost completely (24.32 % relative peak current amplitude 1 cells). When the cells were exposed to 0.1, 0.3, 1.0 and 3.0 nM Margatoxin, the steady-state level relative peak current amplitudes revealed values of 77.65 ± 3.98%, 44.03 ± 3.82%, 20.48 ± 2.98%, 5.63 ± 0.90%, respectively (mean ± SEM of 3 cells). The inhibition curve (Fig. 5) was best fitted with an IC_{50} value of **268.7 pM**, Hill Coefficient: 1.15.

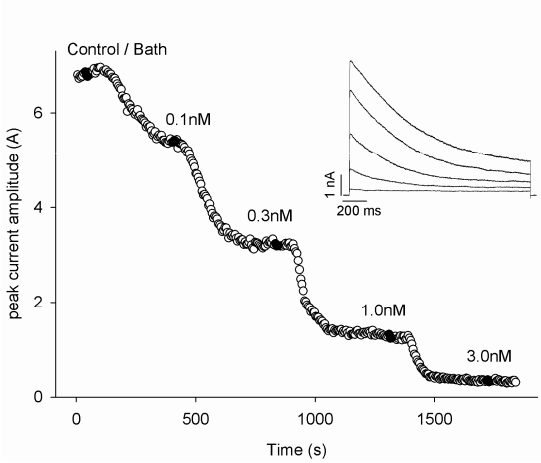

Fig. 4: Long time course of a CHO K_V1.3 cell. Peak current amplitude was reduced by increasing concentrations of Margatoxin.

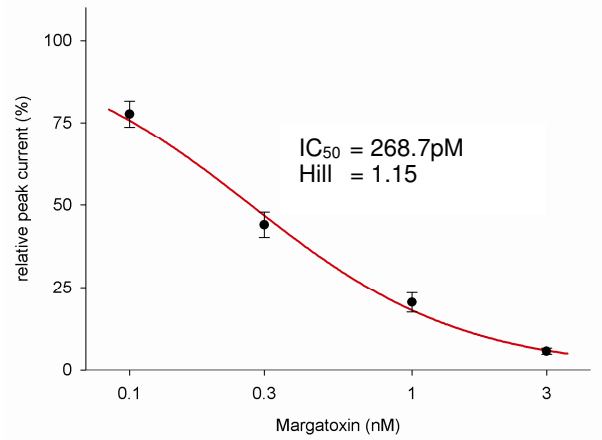

Fig. 5: K_V1.3 peak current inhibition curve of Margatoxin. The inhibition curve was best fitted with an IC_{50} value of 268.7 pM, Hill Coefficient: 1.15 (n=4)

2.4. Patch-clamp Success Rates

The patch-clamp properties of the CHO K_V1.3 cell line were elucidated at typical working passage numbers (passage 5 - 12). A total of 14 cells were analyzed. Success for establishment of on-cell configuration was defined as follows: > 1 GΩ. The whole-cell configuration was not accepted if the membrane resistance was below 1 GΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **100%** (n=14)
- Whole-cell successful: **86%** (n=14)
- Recording (20-30 min) successful: **79%** (n=14)

3. CELL CULTURE CONDITIONS

3.1. General

CHO K_V1.3 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 F12 (HAM) medium supplemented with 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 100 µg/mL Geneticin. The CHO K_V1.3 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% - 80% confluence at 1:3 to 1:5 ratio.

3.2. Recommended Complete Medium

- F12 (HAM) with GlutaMAX I
- 9% FBS
- 0.9% Penicillin/Streptomycin

3.3. Antibiotics

- CHO K_V1.3 clones were selected under 500 µg/mL Geneticin antibiotic pressure.
- To cultivate CHO K_V1.3 cells, a reduced antibiotic pressure (100 µg/mL) must be used.
- To separate CHO K_V1.3 cells from untransfected cells, use 500 µg/mL Geneticin.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

3.4. 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.
- Antibiotics: 100 µg/mL Geneticin
- Incubate cells and check them daily until 70% - 80% confluence is reached.

3.5. Splitting Cells

- When cells are 70% - 80% confluent remove medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 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 1:3 to 1:5 ratio.
- Use remaining suspension for counting the cells.

3.6. Freezing Cells

- Cells should have 80% - 90% confluence prior to freezing.
- Remove the complete medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Resuspend Cells in complete medium to reach a density of approximately1.0E+06 cells per mL.
- Add sterile DMSO to reach 10% DMSO in the complete medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Mix DMSO and cell suspension by pipetting up and down several times
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

3.7. Stability of CHO KV1.3 cells

CHO K_V1.3 cells stably express functionally active K_V1.3 potassium channels over 40 passages. Under recommended cell culture conditions no variation in current density was observed over 40 cell splitting cycles.

4. KV1.3 SEQUENCE

4.1. Accession Number NM_002232

Cloned cDNA sequence of K_V1.3 channel was error-free and identical with NM_002232 sequence:

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