B'SYS GmbH

CHO K_V7.2 Cell Line

Specification Sheet

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1. BACKGROUND

1.1. The neuronal IK(M) current is associated with K_V7.2

$I_{K(M)}$ currents are observed in neuronal cells of the nociceptive pathway and contribute to cell membrane potential regulation by hyperpolarization and influencing action potentials. Inherited mutations in K_V7.2 potassium channel (KCNQ2) are a cause for benign familial neonatal convulsions (BFNC, a form of epilepsy).

1.2. B'SYS' CHO K_V7.2 Cells

As a new test system B'SYS has designed a CHO K_V7.2 cell line with constitutive expression of K_V7.2 channels. The K_V7.2 cDNA was cloned and transfected into CHO cells and then the functional properties of the K_V7.2 channels validated by means of the patch-clamp technique

2. PRODUCT SHIPMENT

2.1. Product Format

CHO cells stably transfected with recombinant human K_V7.2 channel:

- 1 x 0.8 mL aliquots of frozen cells at 2.3 E+06 cells/mL
- Cells are frozen in complete medium with 10 % DMSO
- Cells are frozen at passage number 3

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 KV7.2

3.1. Electrophysiology

K_V7.2 currents were measured by means of the patch-clamp technique in the whole-cell configuration. Access to cells was established with Amphotericin B which perforates the patched membrane. The bath solution contained (in mM) Potassium Chloride 137, Potassium Chloride 4, Calcium Chloride 1.8, Magnesium Chloride 1, HEPES 10, D-Glucose 10, pH (NaOH) 7.4. The pipette solution consisted of (in mM) Potassium Chloride 175, Magnesium Chloride 4, Mg-ATP 5, HEPES 10, Amphotericin B (0.5 mg / ml), pH (KOH) 7.4. After formation of a Gigaohm seal between the patch electrodes and individual K_V7.2 stably transfected CHO cells, the cell membrane was perforated within 5 minutes. Improvement of electrical access to the cell interior was monitored until series resistance was stable. All solutions applied to cells were continuously perfused and maintained at room temperature. K_V7.2 outward currents were measured at the end of a depolarizing pulse to +20 mV for 500 ms from a holding potential of -80 mV. K_V7.2 deactivating tail currents were elicited upon partial repolarization to -40 mV for 500 ms. The voltage pulses were run at intervals of 30 s.

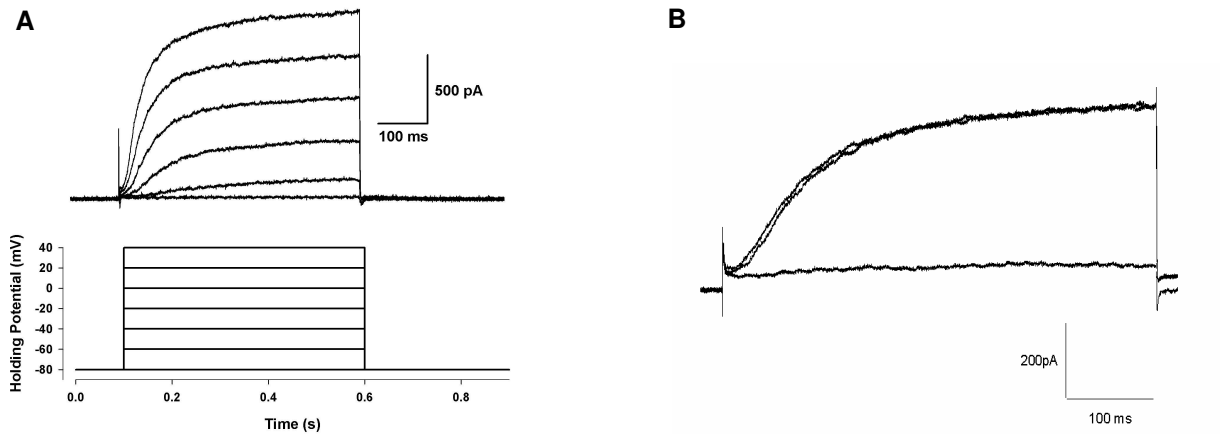

Fig.1 A) Voltage dependence of K_V7.2 currents. The voltage protocol used is shown below (voltage steps applied every 10 s). B) Application of 10 µM XE-991 (Wang et al., 1998) blocks K_V7.2 currents elicited by repeated voltage steps from -80 mV to +20 mV. Application of bath and vehicle DMSO (0.2%). Frequency of applied voltage steps: 1 / 30 s.

3.2. Positive Control XE-991

XE-991, which is known a K_V blocker, was used as positive control to validate the pharmacological profile of the CHO cells. The effect of XE-991 was first measured at 10 µM, which blocked K_V 7.2 currents almost completely (10.97 ± 3.06% relative current amplitude, mean ± SEM of 5 cells). When the cells were exposed to 0.3, 1.0, 3.0 and 10 µM XE-991, the steady-state level relative current amplitudes revealed values of 89.46 ± 3.88%, 52.93 ± 7.41%, 27.53 ± 5.96 and 10.97 ± 3.06%, respectively (mean ± SEM of 5 cells). The inhibition curve (Fig. 2) was best fitted with an IC_{50} value of **1.27 µM**

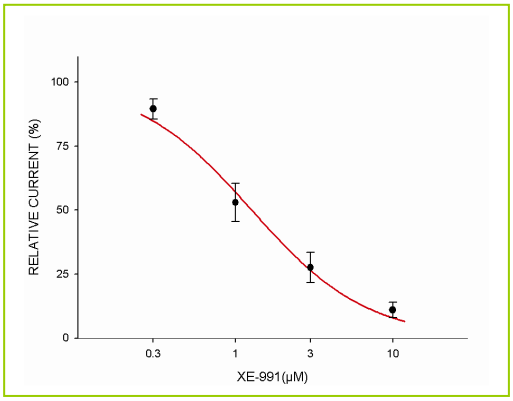

Fig. 2: Ca_V3.2 current inhibition curve of XE-991.

3.3. Patch-clamp Success Rates

The patch-clamp properties of the CHO K_V7.2 cell line were elucidated at typical working passage numbers (passage 10-20). A total of 12 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 500 MΩ and the series resistance above 30 MΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **92%** (n=11)
- Whole-cell successful: **92%** (n=11)
- Recording (20-30 min) successful: **75%** (n=9)

4. CELL CULTURE CONDITIONS

4.1. General

CHO K_V7.2 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 10 % fetal bovine serum, 1 % Penicillin/Streptomycin solution and 500 µg/mL Hygromycin. The CHO K_V7.2 cells are passaged at a confluence of about 50-80 %. 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 % confluency at 1:3 to 1:5 ratio.

4.2. Recommended Complete Medium

- F12 (HAM) with GlutaMAX I
- 10 % FBS
- 1 % Penicillin/Streptomycin

4.3. Antibiotics

- CHO K_V7.2 clones were selected under 1000 µg/ml Hygromycin antibiotic pressure.
- To cultivate CHO K_V7.2 cells, also a reduced antibiotic pressure (500 µg/mL) can be used.
- To separate CHO K_V7.2 cells from untransfected cells, use 1000 µg/mL Hygromycin.

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

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.
- Antibiotics: 500 µg/mL Hygromycin.
- Incubate cells and check them daily until 70 % - 80 % confluency is reached.

4.5. 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 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.
- 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.
- 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 0.8 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.

4.8. Stability of CHO K_V7.2 cells

CHO K_V7.2 cells stably express functionally active K_V7.2 potassium channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed over 20 cell splitting cycles.

5. K_V7.2 SEQUENCE

5.1. Human K_V7.2 Accession Number NM172107.2

Cloned cDNA sequence of K_V7.2 subunit was error-free and identical with NM_172107.2 sequence:

ATGGTGCAGAAGTCGCGCAACGGCGGCGTATACCCCGGCCCGAGCGGGGAGAAGAAGCTGAAGGTGGG
CTTCGTGGGGCTGGACCCCGGCGCGCCCGACTCCACCCGGGACGGGGCGCTGCTGATCGCCGGCTCCG
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