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CHO K_V7.2/3 Cell Line

Specification Sheet

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1. BACKGROUND

1.1. The neuronal M-current

$K_v7.2$ and $K_v7.3$ are voltage-gated potassium (K+) channels, belonging to the subfamily of $K_v7.x$. They are encoded by *KCNQ2* and *KCNQ3* genes respectively; and co-assemble together to form an M-type homomeric or heteromeric K_v channel. These channels, having the properties of the native M-current, is a slowly activating and deactivating (delayed rectifier) K+ channel that regulates synaptic plasticity and neuronal excitability by causing delayed membrane hyperpolarization.

$K_v7.2$ and 7.3 are found in hippocampus, cortex, thalamus, cerebellum and brain stem. Further they are found in nodes of Ranvier, sympathetic and dorsal root ganglia. Loss of function mutations in *KCNQ* genes can cause repeated neuronal discharges and is associated with rare hereditary human epilepsy called benign familial neonatal convulsions (BFNC). Furthermore, *KCNQ* genes are expressed in sensory neurons and are therefore also involved in nociceptive signaling.

$K_v7.2/7.3$ inhibitors are developed for the treatment of learning and memory disorders; whereas $K_v7.2/7.3$ activators for the treatment of epilepsy and pain.

1.2. B'SYS CHO $K_V7.2$ and CHO $K_v7.2/7.3$ 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.

The $K_v7.2$ and $K_v7.3$ cDNAs were cloned in the same bidirectional expression vector and were transfected on CHO cells. Their functional properties are also validated by the means of the manual patch-clamp technique.

2. PRODUCT SHIPMENT

2.1. Product Format

CHO cells stably transfected with recombinant human $K_V7.2$ or $K_v7.2/7.3$ ion 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

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 K_V7.2 AND K_V7.2/7.3

3.1. Electrophysiology

K_V7.2 and K_V7.2/7.3 currents were measured by means of the patch-clamp technique in the perforated-patch 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 130, Magnesium Chloride 1, Mg-ATP 5, HEPES 10, EGTA 5, Amphotericin B (0.5 mg/mL), pH (KOH) 7.2. After formation of a GΩ seal between the patch electrodes and individual K_V7.2 or K_V7.2/7.3 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.

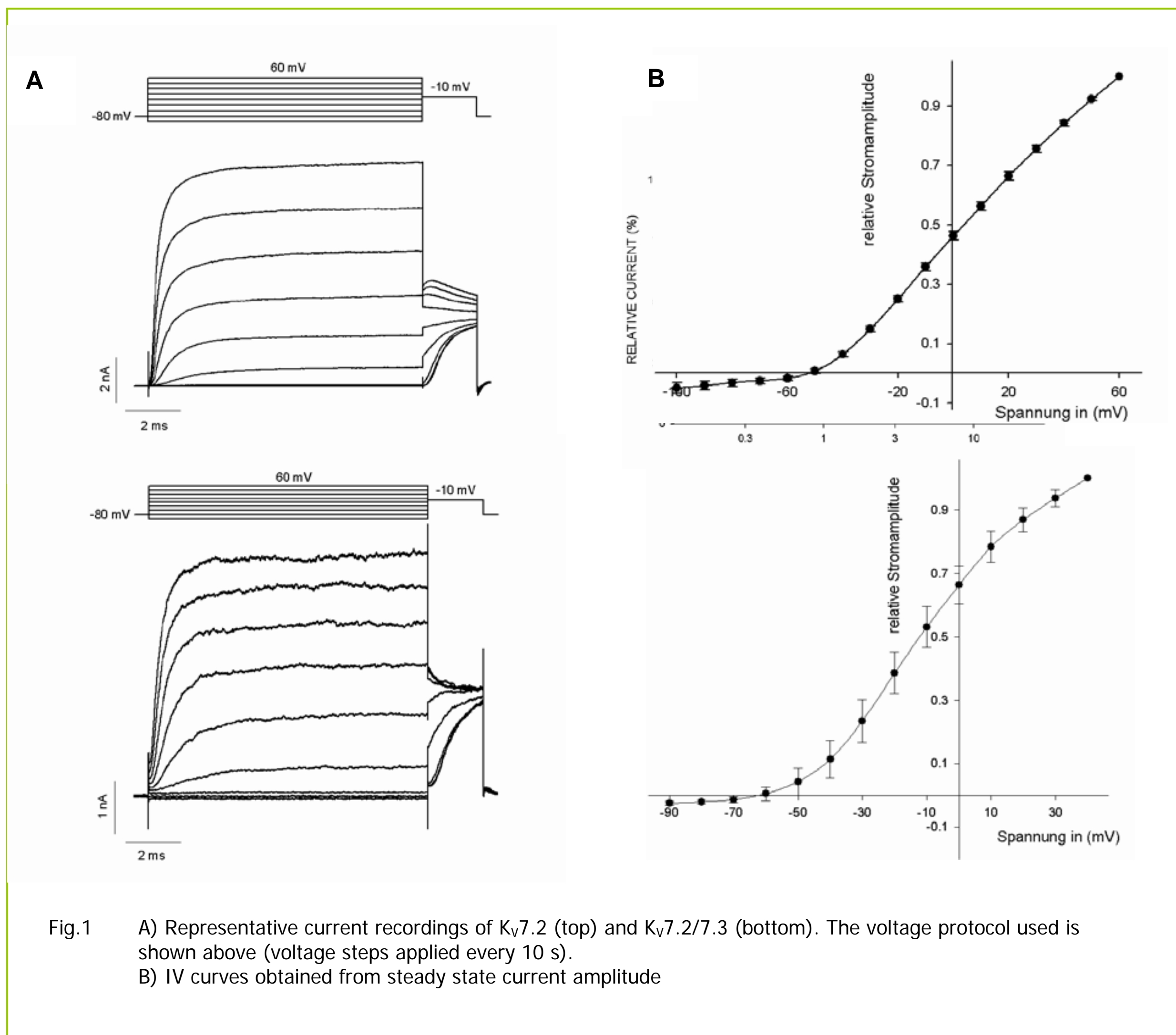

Fig.1 A) Representative current recordings of K_V7.2 (top) and K_V7.2/7.3 (bottom). The voltage protocol used is shown above (voltage steps applied every 10 s).
B) IV curves obtained from steady state current amplitude

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_V7.2 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. 2B) was best fitted with an IC_{50} value of **1.27 µM**. Similarly, for the heteromeric K_V7.2/7.3 currents were blocked by XE-991. When the cells were exposed to 0.1, 1.0, 3.0 and 10 µM XE-991, the steady-state level relative current amplitudes revealed values of 83.64 ± 6.43%, 61.74 ± 4.21%, 28.38 ± 4.87 and 15.62 ± 2.41%, respectively (mean ± SEM of 5 cells). The inhibition curve (Fig. 2D) was best fitted with an IC_{50} value of **1.29 µM**.

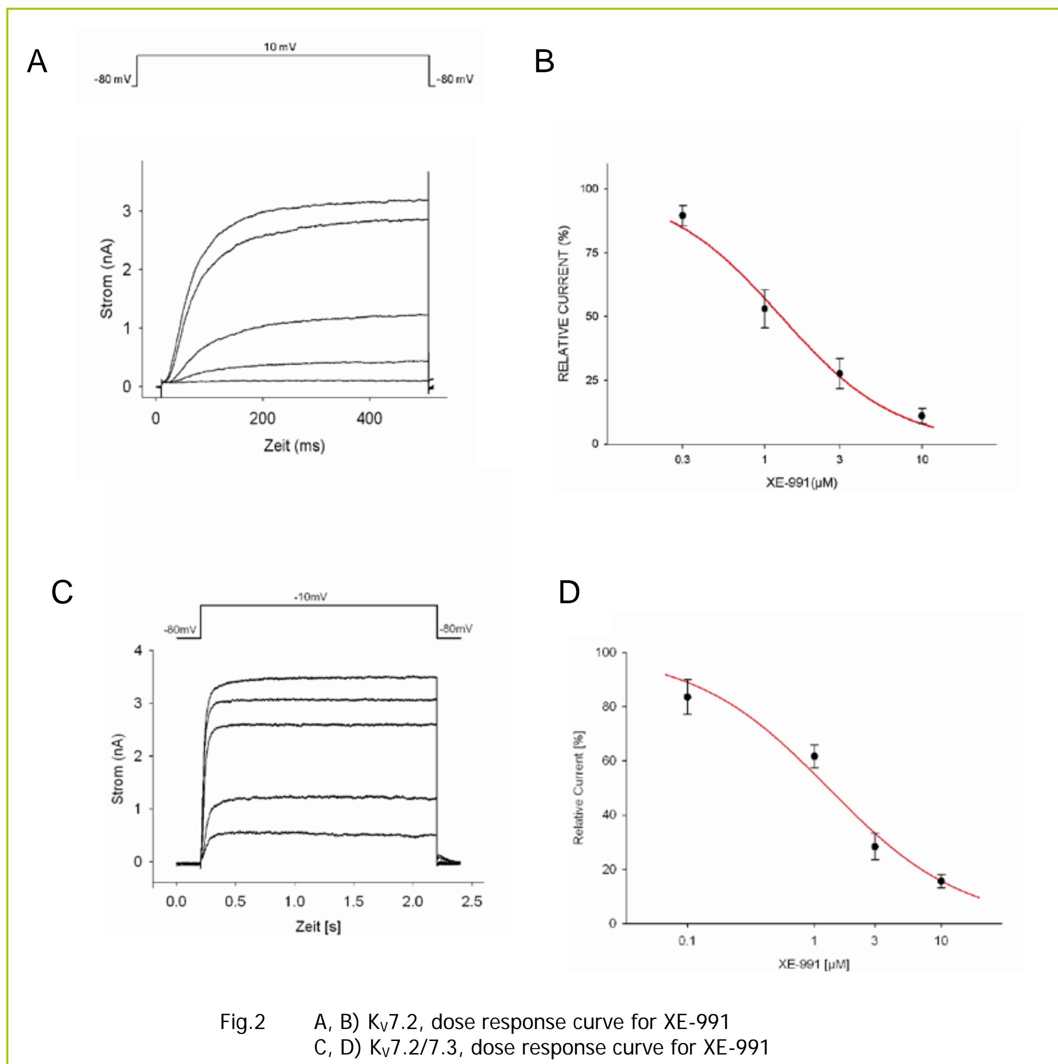

Fig.2 A, B) K_V7.2, dose response curve for XE-991
C, D) K_V7.2/7.3, dose response curve for XE-991

4. HETEROMERIC $K_V7.2/7.3$

4.1. Current amplitudes of Homo- and Heteromers

For $K_V7.2/7.3$ heteromeric channels, the current amplitude was strongly reduced in comparison to $K_V7.2$ channels, but still larger than the current amplitude that was observed in cells that were expressing with $K_V7.3$ only.

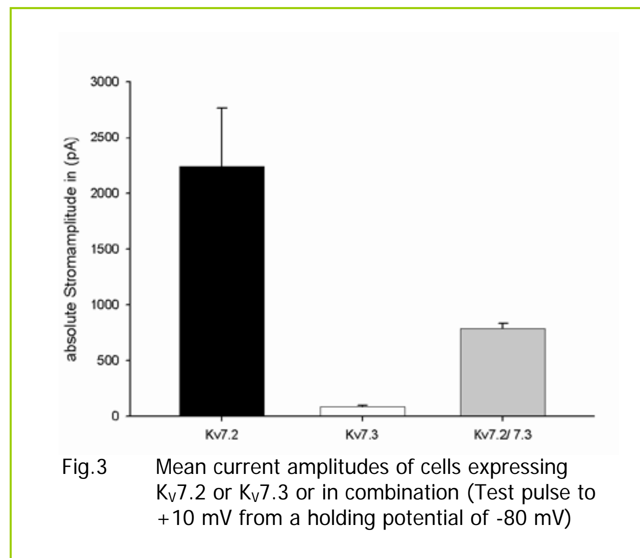

Fig.3 Mean current amplitudes of cells expressing $K_V7.2$ or $K_V7.3$ or in combination (Test pulse to +10 mV from a holding potential of -80 mV)

4.2. Positive control: TEA

$K_V7.2$ currents could be blocked by 10 µM TEA. The remaining current amplitude was 8%. Cells transfected with $K_V7.2$ / 7.3 were less sensitive, only 65% of the current amplitude was blocked.

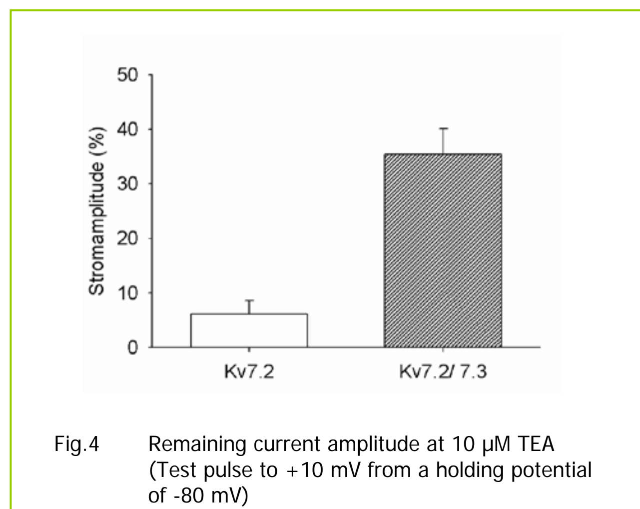

Fig.4 Remaining current amplitude at 10 µM TEA (Test pulse to +10 mV from a holding potential of -80 mV)

4.3. Potassium channel activator: Retigabine

Retigabine is a novel anti-convulsant known to act as a positive modulator of the heteromer K_V7.2/7.3. At a concentration of 10 µM the current for the K_V7.2/7.3 was stimulated by 63%, while the two homomers were only stimulated by 30%. For K_V7.2/7.3, the dose response curve was generated and the EC_{50} value was calculated to be 8.34 µM.

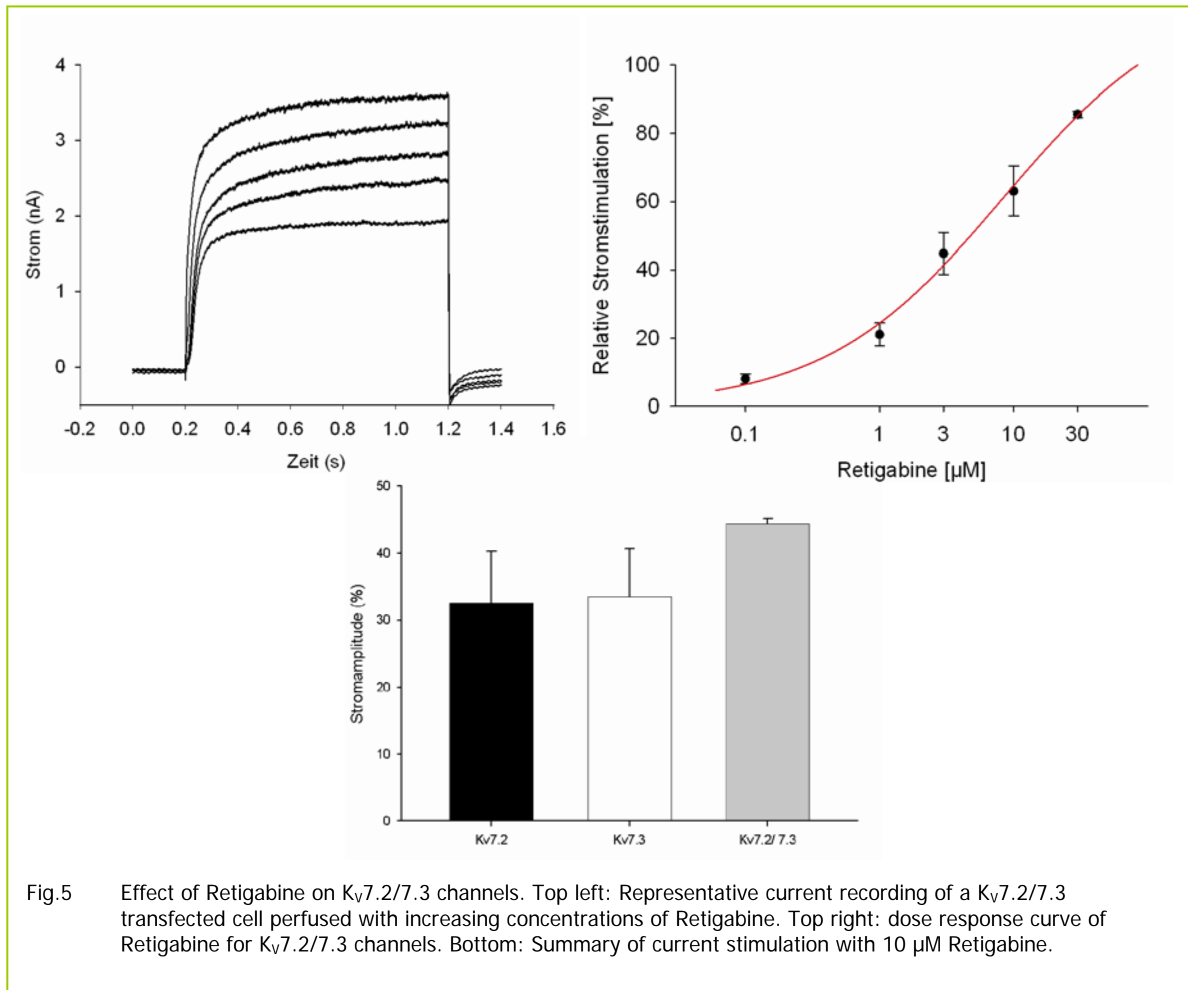

Fig.5 Effect of Retigabine on K_V7.2/7.3 channels. Top left: Representative current recording of a K_V7.2/7.3 transfected cell perfused with increasing concentrations of Retigabine. Top right: dose response curve of Retigabine for K_V7.2/7.3 channels. Bottom: Summary of current stimulation with 10 µM Retigabine.

5. CELL CULTURE CONDITIONS

5.1. General

CHO $K_V7.2$ and $K_V7.2/7.3$ 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 10% fetal bovine serum, 1% Penicillin/Streptomycin solution and 500 µg/mL Hygromycin ($K_V7.2$) or 5 µg/mL Puromycin ($K_V7.2/7.3$) and 100 µg/mL Zeocin. The CHO 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.

5.2. Recommended Complete Medium

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

5.3. 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.

- CHO $K_V7.2/7.3$ clones were selected under 10 µg/mL Puromycin and 100 µg/mL Zeocin antibiotic pressure.
- To cultivate CHO $K_V7.2/7.3$ cells, also a reduced antibiotic pressure (5 µg/mL Puromycin and 100 µg/mL Zeocin) can be used.
- To separate CHO $K_V7.2/7.3$ cells from untransfected cells, use 10 µg/mL Puromycin and 100 µg/mL Zeocine
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Remark: The permanent application of high antibiotic pressure has no effect on current density.

5.4. 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 ($K_V7.2$); 5 µg/mL Puromycin and 100 µg/mL Zeocine ($K_V7.2/7.3$).
- Incubate cells and check them daily until 70% - 80% confluency is reached.

5.5. 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.

5.6. 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).

5.7. 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.

5.8. Stability of CHO K_V7.2 and K_V7.2/7.3 cells

CHO K_V7.2 and K_V7.2/7.3 cells stably express functionally active K_V7.2 or K_V7.2/7.3 potassium channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed over 20 cell splitting cycles.

6. SEQUENCES

Human K_V7.2 Accession Number NP_742105.1
Human K_V7.3 Accession Number NP_004510.1

7. CONTACT INFORMATION

- B'SYS GmbH
  Technology Center Witterswil
  Benkenstrasse 254
  4108 Witterswil
  Switzerland

  Tel: +41 61 721 77 44
  Fax: +41 61 721 77 41
  Email: info@bsys.ch
  Web: www.bsys.ch