B'SYS GmbH

CHO K_V2.1 Cell Line

Specification Sheet

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1. PRODUCT SHIPMENT

1.1. Product Format

CHO cells stably transfected with recombinant K_V2.1 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.

1.3. Assays

CHO K_V2.1 cells were validated for manual patch-clamping and automated patch-clamping (Q-Patch, Sophion)

2. VALIDATION OF CHO K_V2.1 CELLS

2.1. Electrophysiology

K_V2.1 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, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a Gigaohm seal between the patch electrodes and individual K_V2.1 stably transfected CHO cells, the cell membrane 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_V2.1 currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to voltages between -90 mV and +130 mV in 20 mV increments of 1.75 s duration (Fig. 1).

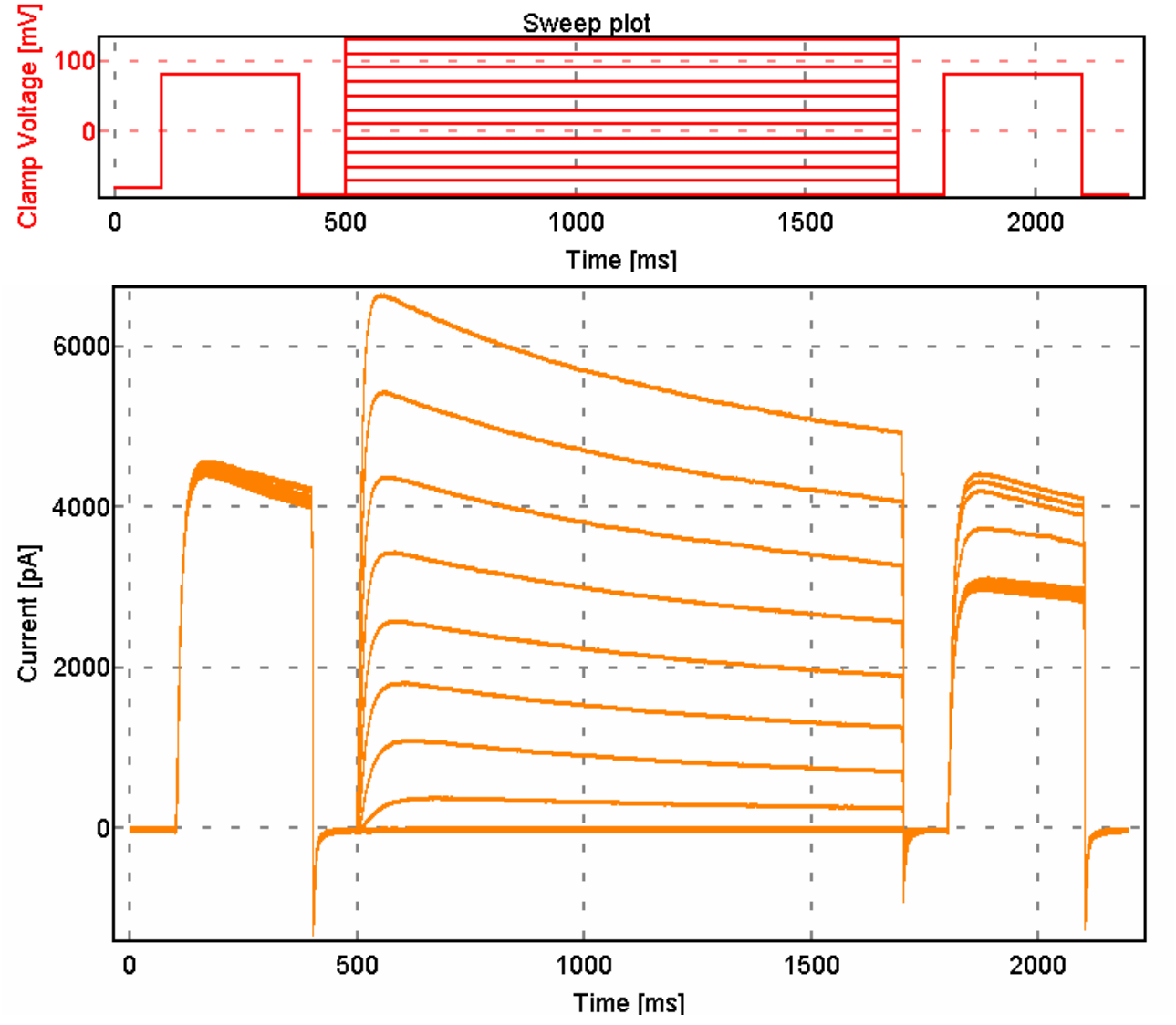

Fig. 1: Representative K_V2.1 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -90 mV and 130 mV in 20 mV increments (1750 ms). Voltage pulses to +80 mV (300 ms) elicited K_V2.1 tail currents.

2.2. K_V2.1 channel blocker Celecoxib and Nifedipine

Celecoxib and Nifedipine, which are known as K_V2.1 blockers, were used as positive control to validate the pharmacological profile of the CHO K_V2.1 cells. The cells were exposed to 0.3, 3.0, 30, 100 and 300 µM Nifedipine or 1.0, 3.0, 10, 30 and 100 µM Celecoxib and the current amplitude was analyzed. The inhibition curves (Fig. 5) were best fitted with IC_{50} values of 136.09 µM (Hill coefficient: 0.79) for Nifedipine and and 5.42 µM (Hill coefficient: 1.15) for Celecoxib.

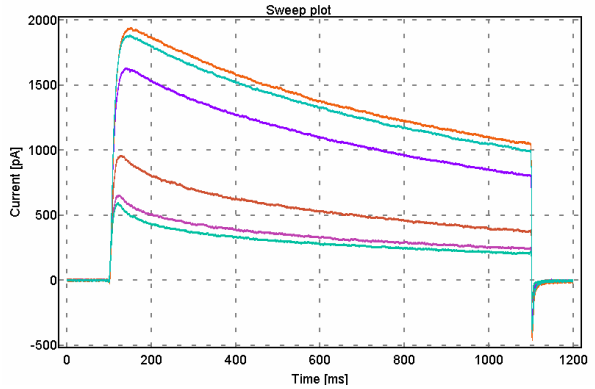

Fig. 2: Representative current recording of a K_V2.1 transfected cell treated with 0.3, 3.0, 30, 100 and 300 µM Nifedipine. The cell was depolarized from a holding potential of -80 mV to +40 mV.

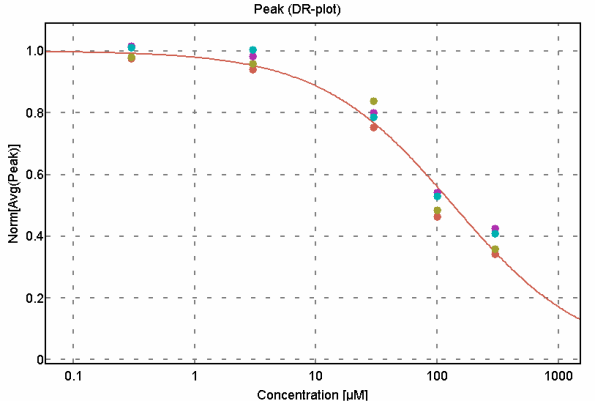

Fig. 3: K_V2.1 peak current inhibition curve of Nifedipine. The inhibition curve was best fitted with a 3 parameter logistic equation.

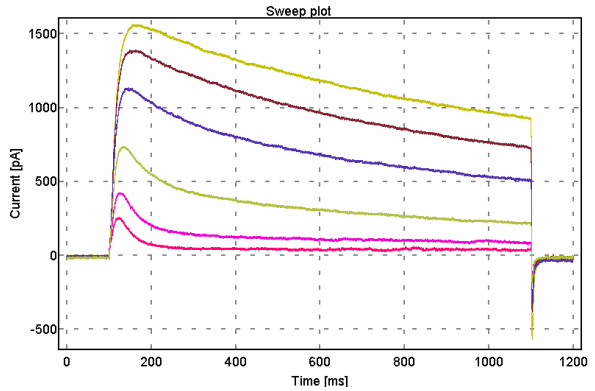

Fig. 4: Representative current recording of a K_V2.1 transfected cell treated with 1.0, 3.0, 10, 30 and 100 µM Celecoxib. The cell was depolarized from a holding potential of -80 mV to +40 mV.

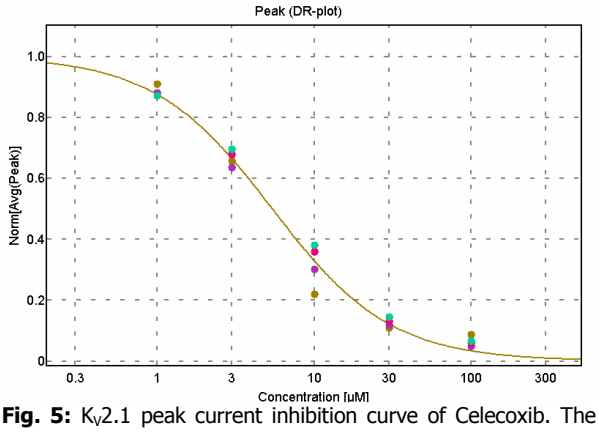

Fig. 5: K_V2.1 peak current inhibition curve of Celecoxib. The inhibition curve was best fitted with a 3 parameter logistic equation.

2.3. Patch-clamp Success Rates – automated Patch-Clamping (Q-Patch)

The patch-clamp properties of the CHO K_V2.1 cell line were elucidated at typical working passage numbers (passage 3 - 15). A total of 48 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: **96%** (n=46/48)
- Whole-cell successful: **94%** (n=45/48)
- Recording (20-30 min) successful: **81%** (n=39/48)

3. CELL CULTURE CONDITIONS

3.1. General

CHO K_V2.1 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% foetal bovine serum, 1.0% Penicillin/Streptomycin solution and 200 µg/mL Hygromycin. The CHO K_V2.1 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 L-Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

3.3. Antibiotics

- CHO K_V2.1 clones were selected under 200 µg/mL Hygromycin antibiotic pressure.
- To cultivate CHO K_V2.1 cells, a reduced antibiotic pressure (200 µg/mL) must be used.
- To separate CHO K_V2.1 cells from untransfected cells, use 500µg/mL Hygromycin.

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-25 culture flask containing 5 mL complete medium.
- Incubate cells at 37°C for 4-6 hours to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 5mL complete medium & antibiotics.
- Antibiotics: 200 µg/mL Hygromycin.
- 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 K_V2.1 cells

CHO K_V2.1 cells stably express functionally active K_V2.1 potassium channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed during this time.

4. K_V2.1 SEQUENCE

4.1. Accession Number NP_004966.1

Cloned cDNA encodes for the protein of the K_V2.1 channel (NP_004966.1):

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MPAGMTKHGSRSTSSLPPEPMEIVRSKACSRRVRLNVGGLAHEVLWRTLDRLPRTRLGKL
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VRVLPGGGAHGSTRDQSI
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5. CONTACT INFORMATION

5.1. Contact Address for Technical Support and Ordering 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