B’SYS GmbH

HEK 293 K2P18.1 (TRESK) Cell Line

Specification Sheet

TABLE OF CONTENTS

1. BACKGROUND

1.1. Two pore domain potassium channels (K2P)

The two-pore domain potassium channels (K2P) have been the subject of several research projects. This enable to considerably enrich the knowledge about the structure and the functions of these channels which are found in animals as well as in plants. At the present time, fifteen genes coding for one of the K2P subunits are known.
Unlike Kv and Kir channels which are composed of subunits containing only one P domain, K2P subunits have two. Thus, K2P subunits constitute dimers, unlike all the other potassium channels which are constituted of tetramers. Each P domain of a subunit is framed by two transmembrane helices (M1-M2 and M3-M4) and the N and C-terminus are both in the cytoplasmic compartment. Another specific structural properties of the K2P channels is an extracellular loop between the M1 helice and the P1 domain. This M1P1 loop constitute a coiled-coil domain which allows to interact with the M1P1 loop of another subunit to form an active dimer. This loop contains a cysteine residue (Cys69) involved in the formation of a disulfide bridge between the cysteines of the subunits ensuring the dimer formation as well as the stability of the structure.

1.2. TRESK (K2P18.1)

TRESK (K2P18) is the product of the KCKNK18 gene, the last discovered leak potassium K2P channel gene. The general properties of this channel are similar to the other K2P channels, but the protein shares only a 19% of homology with its K2P relatives. TRESK is unique in both its function and regulation. Indeed, it is the only channel of the K2P family whose mechanism of action is dependent on calcium activation. TRESK is primarily expressed in primary sensory neurons of dorsal root ganglia (DRG) and trigeminal ganglia where it controls the potassium conductance and stabilisation of the negative resting membrane potential, and thus, enables excitability adjustment, depolarisation counteraction and potassium transport through the plasma membrane. Due to its role and location, TRESK channel play an important part in the nociception. Moreover, several mutations in the KCNK18 gene leading to TRESK dysfunction are linked to diseases like pain disorders or migraine have been identified. In 2012, Andres-Enguix and al proposed a model where a dysfunction in TRESK protein would disrupts the potassium equilibrium potential by inhibiting potassium efflux, ultimately enhancing the transmission of nociceptive information and the release of neurotransmitters.

2. PRODUCT SHIPMENT

2.1. Product Format

HEK 293 cells stably transfected with recombinant human K2P18.1 channel:

- 1 x 0.5ml 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 13

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 HEK 293 K2P18.1 CELLS

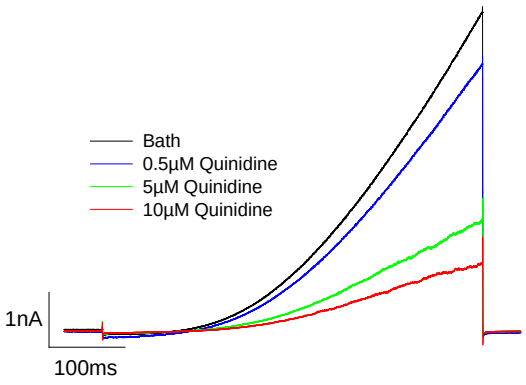
3.1. Electrophysiology

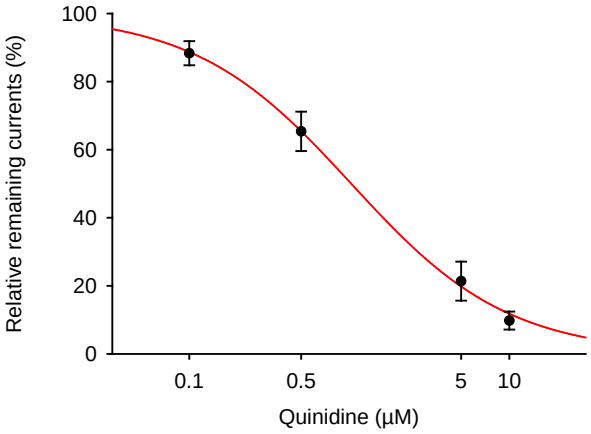
K2P18.1 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 135, MgATP 5, $MgCl_2$ 1, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a Gigaohm seal between the patch electrodes and individual K2P18.1 stably transfected HEK 293 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.

3.2. Pharmacology

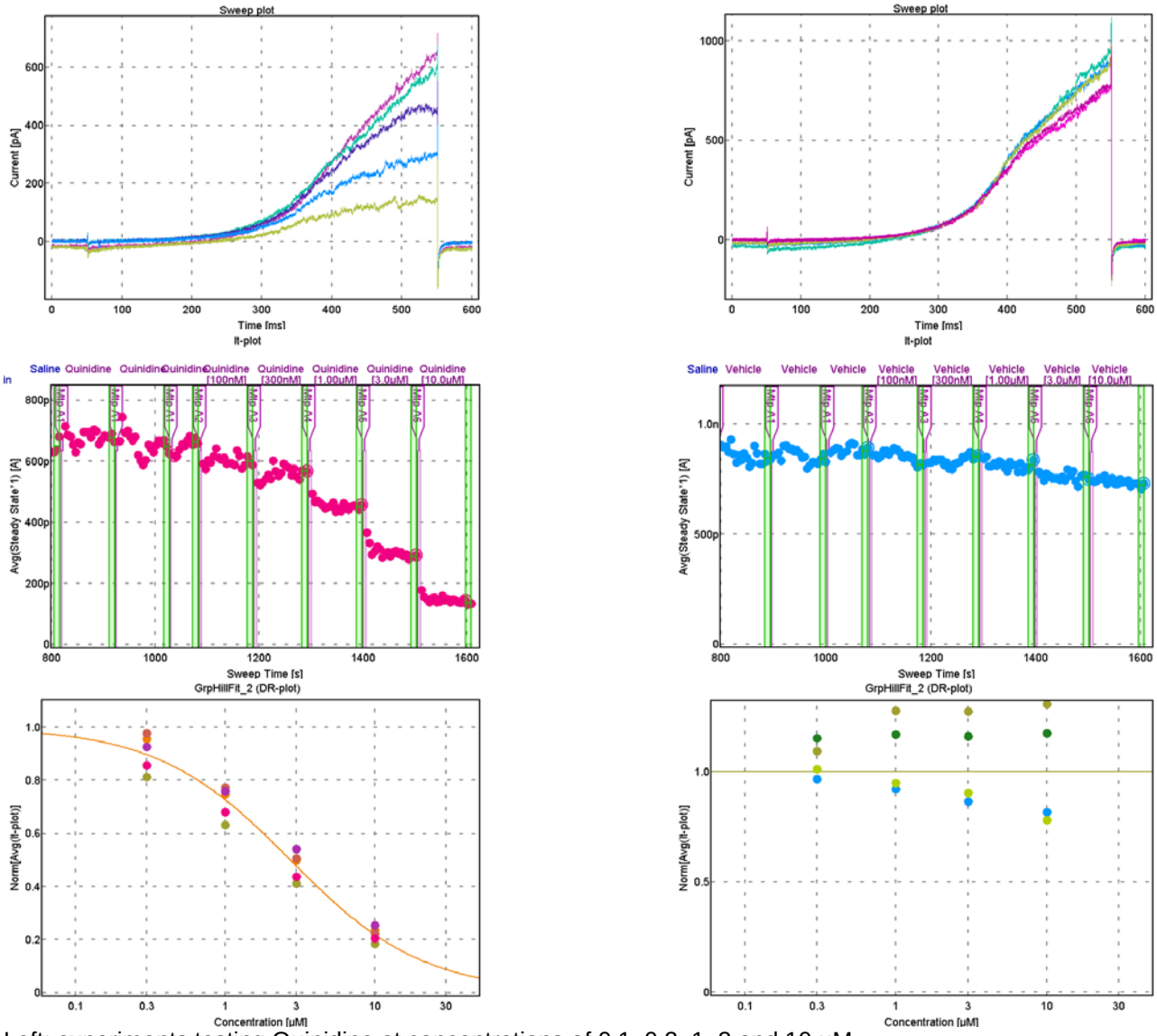
For Quinidine a significant decrease of the current at +60 mV was observed from the application of 100 nM to 10 µM. The mean remaining fraction of the initial current at +60 mV was 88.05 ± 2.05%, 66.51 ± 3.33%, 22.45 ± 3.24% and 9.73 ± 1.45% (HEK293 cells) for an application of 100 nM, 500 nM, 5 µM and 10 µM of Quinidine (n=5). The IC_{50} has been determined to be 1.01 µM.

Perfusing the cells with Lidocaine with concentrations between 1 and 10 mM also induced a significant current reduction at +60 mV for HEK293 TRESK cells lines. With this compound, the mean remaining fraction of the initial current was 81.28 ± 1.32%, 50.87 ± 1.03%, 34.03 ± 0.85% and 15.26 ± 0.4% for an application of 1 mM, 2 mM, 5 mM and 10 mM of lidocaine (n=5). The IC_{50} has been determined to be 2.56 mM.





The same experiments for Quinidine were repeated with automated patch-clamping (Q-Patch, Sophion). Concentrations of 0.1, 0.3, 1, 3 and 10 µM were tested. The IC_{50} was determined to be 2.73 µM and a Hill coefficient if 0.98.
For 14 of 16 tested cells the whole cell configuration was reached. All of these showed the TRESK currents, and 9 cells were used for data analysis.

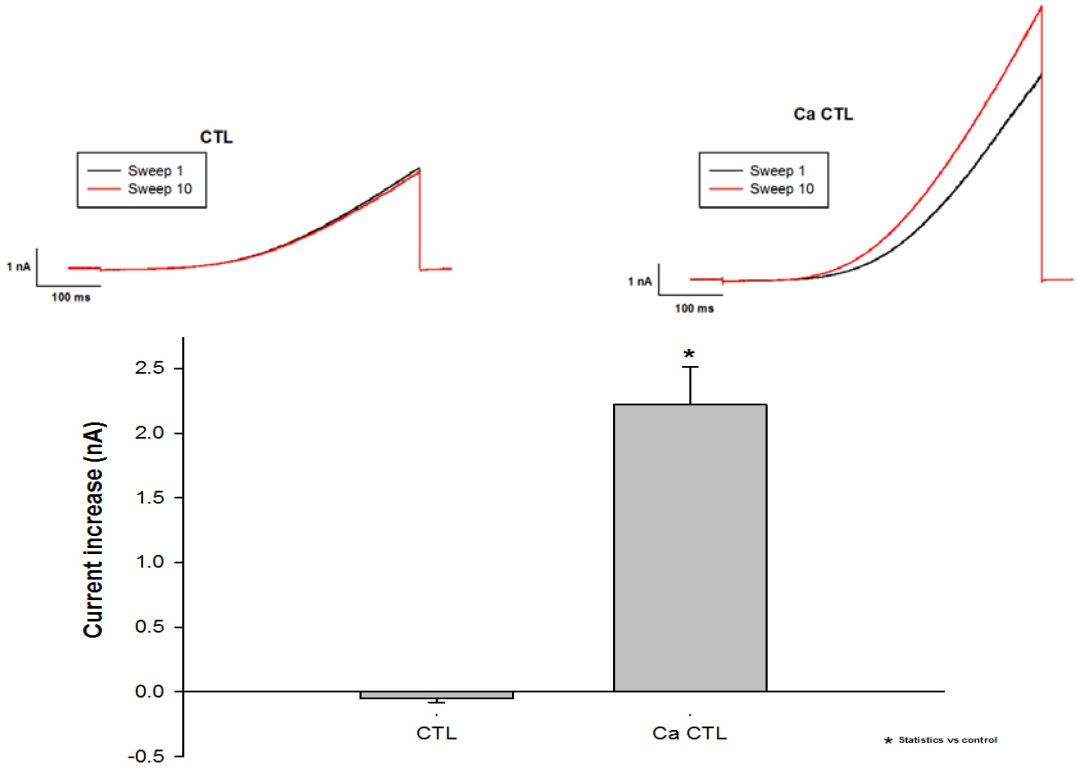


Left: experiments testing Quinidine at concentrations of 0.1, 0.3, 1, 3 and 10 µM,
Right: negative control experiments: time matched vehicle (0.1% DMSO)
Top: representative current traces at end of liquid period, middle: IT plot of one experiment testing the effect of Quinidine or 0.1% DMSO, bottom: dose-response curves

3.3. Calcium dependent activation of K2P18.1

As shown in figure below, the use of a pipette solution containing 5 mM of calcium induced a significant increase of TRESK current after a ten sweeps (50 seconds) recording (+2.22 ± 0.29 nA (n=5), while no significant increase of the current has been observed with standard pipette solution (-0.052 ± 0.029 nA (n=5)). The PKC inhibitor (Staurosporine (1µM)) did not interfere with this calcium dependent TRESK activation as shown in figure below (+1.86 ± 0.1 nA (n=5)), unlike the calcineurin inhibitor cyclosporine A (100nM), which seemed to strongly and significantly inhibit this calcium activation of the TRESK channel (+0.05 ± 0.03 nA (n=5).

As CsA, the second calcineurin inhibitor (FK506 (200nM)) also had this significant inhibitory effect on calcium dependant TRESK activation (+0.07 ± 0.02 nA (n=5)).



4. CELL CULTURE CONDITIONS

4.1. General

HEK K2P18.1 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 DMEM medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 100 µg/mL Hygromycin and 15 µg/mL Blasticidin. The HEK K2P18.1 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% confluency at 1:3 to 1:5 ratio.

IMPORTANT: To induce the expression of K2P18.1 receptors 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 DMEM/F12 with L-Glutamine or GlutaMax I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3. Antibiotics

- HEK K2P18.1 clones were selected under 500 µg/mL Hygromycin and 15 µg/mL Blasticidin antibiotic pressure.
- To cultivate HEK K2P18.1 cells, 100 µg/mL Hygromycin and 15 µg/mL Blasticidin are recommended.
- To separate HEK K2P18.1 cells from untransfected cells, use 500 µg/mL Hygromycin and 15 µg/mL Blasticidin

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: 100 µg/mL Hygromycin and 15 µg/mL Blasticidin.
- 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 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.

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 0.5 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 K2P18.1

To induce the expression of K2P18.1 receptors 2.5 µg/mL Tetracycline has to be added at least 16 h - 24 h before experimentation.

5. K2P18.1 SEQUENCE

5.1. Human K2P18.1 Accession Number Q7Z418.1

Cloned cDNA sequence encodes for K2P18.1, Q7Z418.1:

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MEVSGHPQARRCCPEALGKLFPGLCFLCFLVTYALVGAVVFSAIEDGQVLVAADDGEFEKFLEELCRILNCSETVVEDRKQDLQGHLQK
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LVQNRLIDIYKNVMLFFAKGKFYHLVKK
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