B'SYS GmbH

HEK 293 K_V7.1/minK Cell Line

Specification Sheet

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1. BACKGROUND

1.1. The cardiac IKs current is encoded by K_V7.1/minK

Like the native cardiac I_{Kr} current (encoded by HERG channel), also the I_{Ks} current to a large extent is responsible for the termination of the cardiac action potential. Inherited mutations in K_V7.1 potassium channel (KCNQ1) and the associated minK subunit (KCNE1) can cause the Long QT Syndrome 1 and Long QT Syndrome 5, respectively.

1.2. Coexpression of K_V7.1 and minK

The paired expression of the K_V7.1 α subunit along with the minK β subunit in suitable expression system results in potassium currents resembling the native I_{Ks} current. The most apparent effect of the minK β subunit on K_V7.1 currents is an about tenfold decrease of the activation kinetics as compared to currents from K_V7.1 channels alone.

1.3. B'SYS' HEK 293 K_V7.1/minK Cells

B'SYS has designed a new HEK 293 K_V7.1/minK cell line with constitutive coexpression of human K_V7.1/minK channels. The human K_V7.1/minK cDNA was cloned and transfected into HEK 293 cells and then the functional properties of the K_V7.1/minK channels validated by means of the patch-clamp technique. Results are outlined in section 3.

2. PRODUCT SHIPMENT

2.1. Product Format

HEK 293 cells stably transfected with recombinant human K_V7.1/minK channel:

- 1x 0.5 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 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 K_V7.1/MINK CELLS

3.1. Electrophysiology

K_V7.1/minK 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) K-Aspartate 135, Na_2ATP 2, HEPES 10, EGTA 5, Na_2-phosphocreatine 14, creatinephosphokinase 50 U/mL, PMA 0.01, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual K_V7.1/minK 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. As soon as a stable seal could be established potassium currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to +110 mV in 10 mV increments of 4 s duration (Fig. 1). K_V7.1/minK deactivating tail currents were elicited upon partial repolarization to -40 mV for 500 ms. The voltage pulses were run at intervals of 30 s. The IC_{50} of mefloquine , a quinidine-related antimalarial compound which is known to specifically inhibit K_V7.1 (Kang et al. 2001) was estimated at 3.6 µM. Other IK_S blockers were tested and the observed IC_{50} values are given table I and compared to those published in literature.

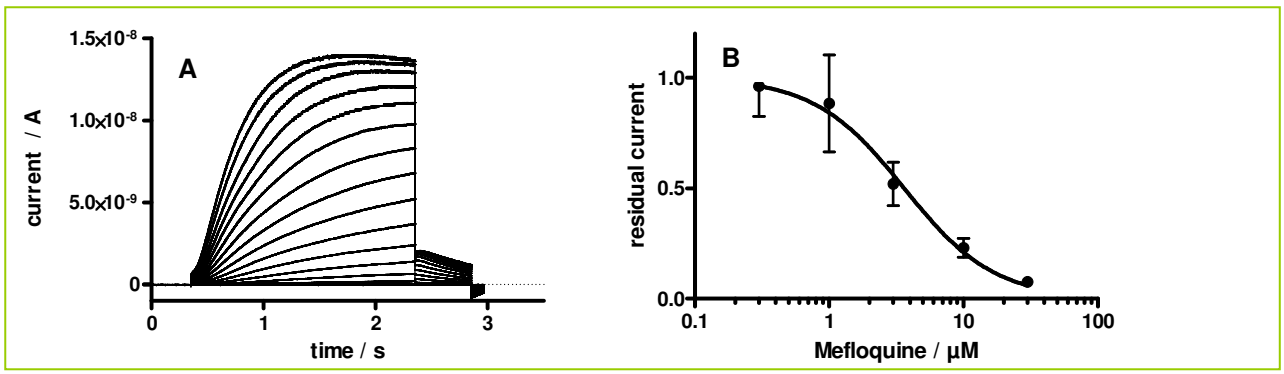

Fig.1: A) Examples of the original traces recorded to demonstrate the voltage dependence of steady state current and peak tail current. B) Concentration-response curve for mefloquine determined in three individual cells.

Table I: Comparison of observed IC_{50} values with literature reference values ranked according to potency.

Rank	compound	IC_{50}/µM (observed)	IC_{50}/µM (reference)	Reference
1	HMR1556	0.13	0.0838	Dong et al. 2006
2	Mefloquine	3.6	1 .43	Kang et al. 2001
3	Chromanol293B	6.2	9.2	Dong et al. 2006
4	Quinidine	>100	44	Kang et al.2001

3.2. Patch-clamp Success Rates

The patch-clamp properties of the HEK 293 K_V7.1/minK cell line were elucidated at typical working passage numbers (passage 17-28). A total of 18 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. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **96%** (n=18)
- Whole-cell successful: **80%** (n=18)
- Recording (20-30 min) successful: **72%** (n=18)

3.3. Automated patch-clamp

K_V7.1/minK currents were measured on the QPatch automate. The bath solution was the same as for the maual patch-clamp experiments. The pipette solution consisted of (in mM): KCl 130, NaCl 10, $MgCl_2$ 1, EGTA 10, HEPES 10, Mg-ATP 5, Na-GTP 2.6, cAMP 0.2, pH (KOH) 7.20. Currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to +40 mV for 5 s. The blocker mefloquine was tested at concentrations of 1, 10, 30 and 100 µM. The IC_{50} of mefloquine was determined to 12.27 µM.

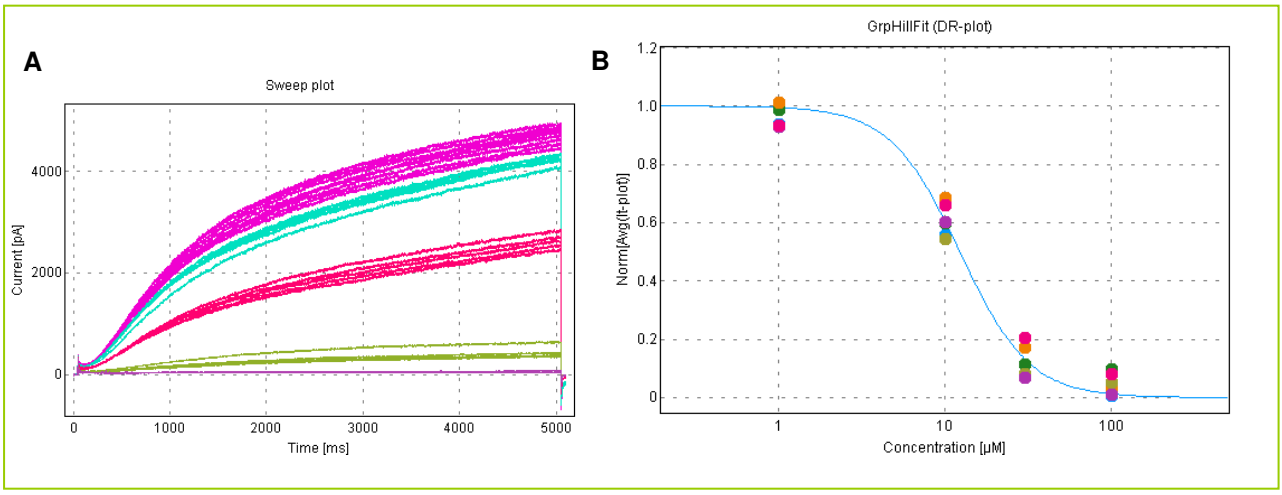

Fig.2: A) Examples of the original current traces (0, 1, 10, 30 and 100 µM mefloquine) B) Concentration-response curve for mefloquine determined in six individual cells.

4. CELL CULTURE CONDITIONS

4.1. General

HEK 293 K_V7.1/minK 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 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 800 µg/mL Geneticin. The HEK 293 K_V7.1/minK 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.

4.2. Recommended Complete Medium

- DMEM with GlutaMAX I
- 9% FBS
- 0.9% Penicillin/Streptomyci

4.3. Antibiotics

- HEK 293 K_V7.1/minK clones were selected under 800 µg/ml Geneticin, and 800 µg/mL Hygromycin antibiotic pressure
- To cultivate HEK 293 K_V7.1/minK cells, also a reduced antibiotic pressure (100 µg/mL) can be used.
- To separate HEK 293 K_V7.1/minK cells from untransfected cells, use 100 µg/mL Geneticin and 100 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: 100 µg/mL Geneticin, 100 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 0.5 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.

4.8. Stability of HEK 293 K_V7.1/minK cells

HEK K_V7.1/minK cells stably express functionally active K_V7.1/minK potassium channels over 41 passages. Under recommended cell culture conditions no variation in current density was observed over 41 cell splitting cycles.

5. K_V7.1/MINK SEQUENCE

5.1. Human K_V7.1 Accession Number AF000571

Cloned cDNA sequence of K_V7.1 subunit was error-free and identical with AF000571 sequence:

ATGGCCGCGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCGCTGGGGTTGGGGCCGCCTGCCAGGCGC
CCGGCGGGGCAGCGCGGGCCTGGCCAAGAAGTGCCCCTTCTCGCTGGAGCTGGCGGAGGGCGGCCCGG
CGGGCGGCGCGCTCTACGCGCCCATCGCGCCCGGCGCCCCAGGTCCCGCGCCCCCTGCGTCCCCGGCC
GCGCCCGCCGCGCCCCCAGTTGCCTCCGACCTTGGCCCGCGGCCGCCGGTGAGCCTAGACCCGCGCGT
CTCCATCTACAGCACGCGCCGCCCGGTGTTGGCGCGCACCCACGTCCAGGGCCGCGTCTACAACTTCC
TCGAGCGTCCCACCGGCTGGAAATGCTTCGTTTACCACTTCGCCGTCTTCCTCATCGTCCTGGTCTGC
CTCATCTTCAGCGTGCTGTCCACCATCGAGCAGTATGCCGCCCTGGCCACGGGGACTCTCTTCTGGAT
GGAGATCGTGCTGGTGGTGTTCTTCGGGACGGAGTACGTGGTCCGCCTCTGGTCCGCCGGCTGCCGCA
GCAAGTACGTGGGCCTCTGGGGGCGGCTGCGCTTTGCCCGGAAGCCCATTTCCATCATCGACCTCATC
GTGGTCGTGGCCTCCATGGTGGTCCTCTGCGTGGGCTCCAAGGGGCAGGTGTTTGCCACGTCGGCCAT
CAGGGGCATCCGCTTCCTGCAGATCCTGAGGATGCTACACGTCGACCGCCAGGGAGGCACCTGGAGGC
TCCTGGGCTCCGTGGTCTTCATCCACCGCCAGGAGCTGATAACCACCCTGTACATCGGCTTCCTGGGC
CTCATCTTCTCCTCGTACTTTGTGTACCTGGCTGAGAAGGACGCGGTGAACGAGTCAGGCCGCGTGGA
GTTCGGCAGCTACGCAGATGCGCTGTGGTGGGGGGTGGTCACAGTCACCACCATCGGCTATGGGGACA
AGGTGCCCCAGACGTGGGTCGGGAAGACCATCGCCTCCTGCTTCTCTGTCTTTGCCATCTCCTTCTTT
GCGCTCCCAGCGGGGATTCTTGGCTCGGGGTTTGCCCTGAAGGTGCAGCAGAAGCAGAGGCAGAAGCA
CTTCAACCGGCAGATCCCGGCGGCAGCCTCACTCATTCAGACCGCATGGAGGTGCTATGCTGCCGAGA
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ACCACTTCTCTGTCGACGGCTATGACAGTTCTGTAAGGAAGAGCCCAACACTGCTGGAAGTGAGCATG
CCCCATTTCATGAGAACCAACAGCTTCGCCGAGGACCTGGACCTGGAAGGGGAGACTCTGCTGACACC
CATCACCCACATCTCACAGCTGCGGGAACACCATCGGGCCACCATTAAGGTCATTCGACGCATGCAGT
ACTTTGTGGCCAAGAAGAAATTCCAGCAAGCGCGGAAGCCTTACGATGTGCGGGACGTCATTGAGCAG
TACTCGCAGGGCCACCTCAACCTCATGGTGCGCATCAAGGAGCTGCAGAGGAGGCTGGACCAGTCCAT
TGGGAAGCCCTCACTGTTCATCTCCGTCTCAGAAAAGAGCAAGGATCGCGGCAGCAACACGATCGGCG
CCCGCCTGAACCGAGTAGAAGACAAGGTGACGCAGCTGGACCAGAGGCTGGCACTCATCACCGACATG
CTTCACCAGCTGCTCTCCTTGCACGGTGGCAGCACCCCCGGCAGCGGCGGCCCCCCCAGAGAGGGCGG
GGCCCACATCACCCAGCCCTGCGGCAGTGGCGGCTCCGTCGACCCTGAGCTCTTCCTGCCCAGCAACA
CCCTGCCCACCTACGAGCAGCTGACCGTGCCCAGGAGGGGCCCCGATGAGGGGTCCTGA

5.2. Human minK Subunit Accession Number AF135188

Cloned cDNA sequence of minK subunit was error-free and identical with AF135188 sequence:

ATGATCCTGTCTAACACCACAGCGGTGACGCCCTTTCTGACCAAGCTGTGGCAGGAGACAGTTCAGCA
GGGTGGCAACATGTCGGGCCTGGCCCGCAGGTCCCCCCGCAGCAGTGACGGCAAGCTGGAGGCCCTCT
ACGTCCTCATGGTACTGGGATTCTTCGGCTTCTTCACCCTGGGCATCATGCTGAGCTACATCCGCTCC
AAGAAGCTGGAGCACTCGAACGACCCATTCAACGTCTACATCGAGTCCGATGCCTGGCAAGAGAAGGA
CAAGGCCTATGTCCAGGCCCGGGTCCTGGAGAGCTACAGGTCGTGCTATGTCGTTGAAAACCATCTGG
CCATAGAACAACCCAACACACACCTTCCTGAGACGAAGCCTTCCCCATGA

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