

B'SYS GmbH

CHO K_vLQT1/minK Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The cardiac I_{Ks} current is encoded by KvLQT1/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 KvLQT1 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 KvLQT1 and minK

The paired expression of the KvLQT1 α subunit along with the minK β subunit in a suitable expression system results in potassium currents resembling the native I_{Ks} current. The most apparent effect of the minK β subunit on KvLQT1 currents is an about tenfold decrease of the activation kinetics as compared to currents from KvLQT1 channels alone.

1.3 B'SYS's CHO KvLQT1/minK Cells

B'SYS has designed a new CHO KvLQT1/minK cell line with constitutive co-expression of human KvLQT1/minK channels. The human KvLQT1/minK cDNA was cloned and transfected into CHO cells and then the functional properties of the KvLQT1/minK channels validated by means of the patch-clamp technique. Cells were validated for manual and automated patch-clamping (Sophion Q-Patch™ and Sophion Qube™). Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human KvLQT1/minK channel:

- 1 x 0.5 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 KvLQT1/MINK CELLS

3.1 Electrophysiology

KvLQT1/minK currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 150, KCl 5, CaCl₂ 2, MgCl₂ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 150, MgCl₂ 0.5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual KvLQT1/minK stably transfected CHO 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 +120 mV in 20 mV increments of 4 seconds duration (Fig. 1). KvLQT1/minK deactivating tail currents were elicited upon partial repolarization to -40 mV for 1 s. The voltage pulses were run at intervals of 10 s.

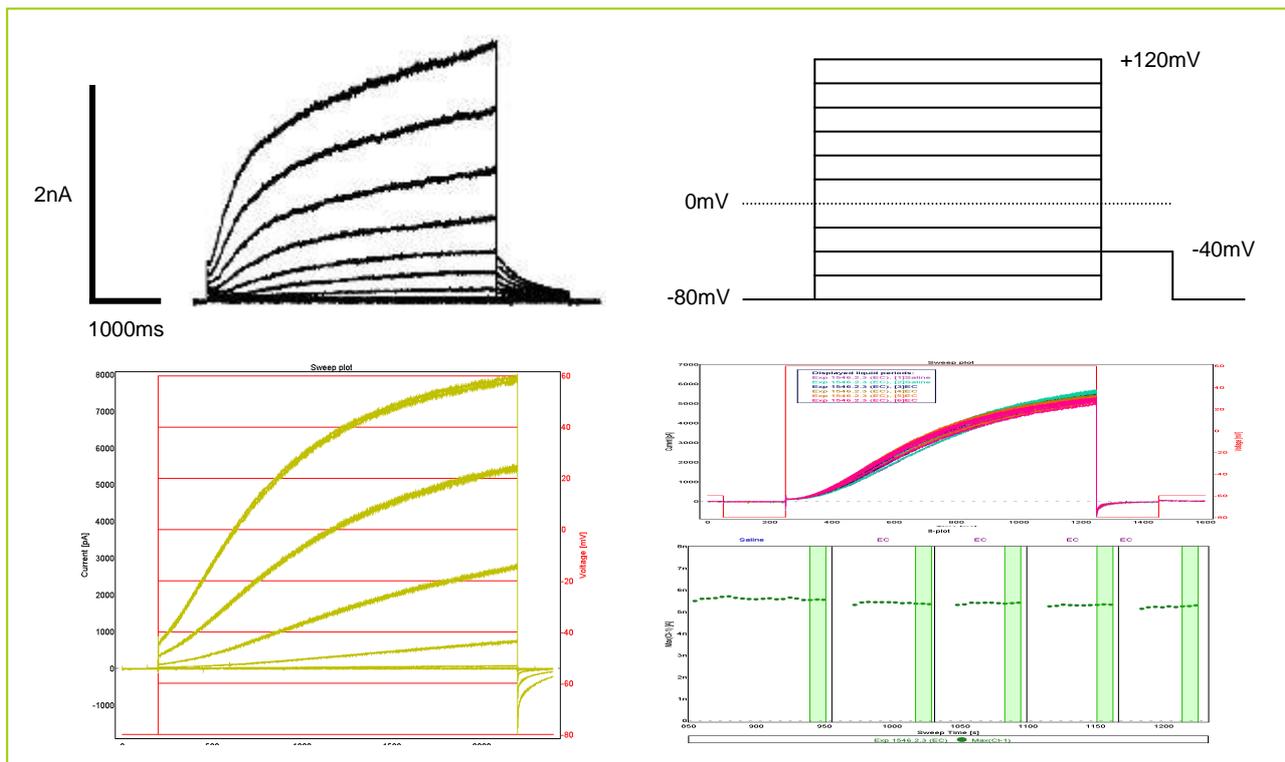


Fig.1: Activation of KvLQT1/minK. Outward currents were observed upon depolarization of the cell membrane from holding potential (-80 mV) up to +120 mV in 20 mV increments (Top: manual patch-clamping, Bottom: automated patch-clamping, Q-Patch™). Bottom right: repetitive stimulation of KvLQT1 / minK caused only minimal decrease of the current amplitude.

3.2 Pharmacological Validation

In order to evaluate the pharmacological properties of the KvLQT1/minK channel. The response of KvLQT1/minK to a known blocker XE-991 was tested. Four concentrations were tested 0.01, 0.1, 1, 10 μM. An IC₅₀ for XE-991 of 0.96±0.4 μM, n=7 was determined (literature value 1-6 μM).

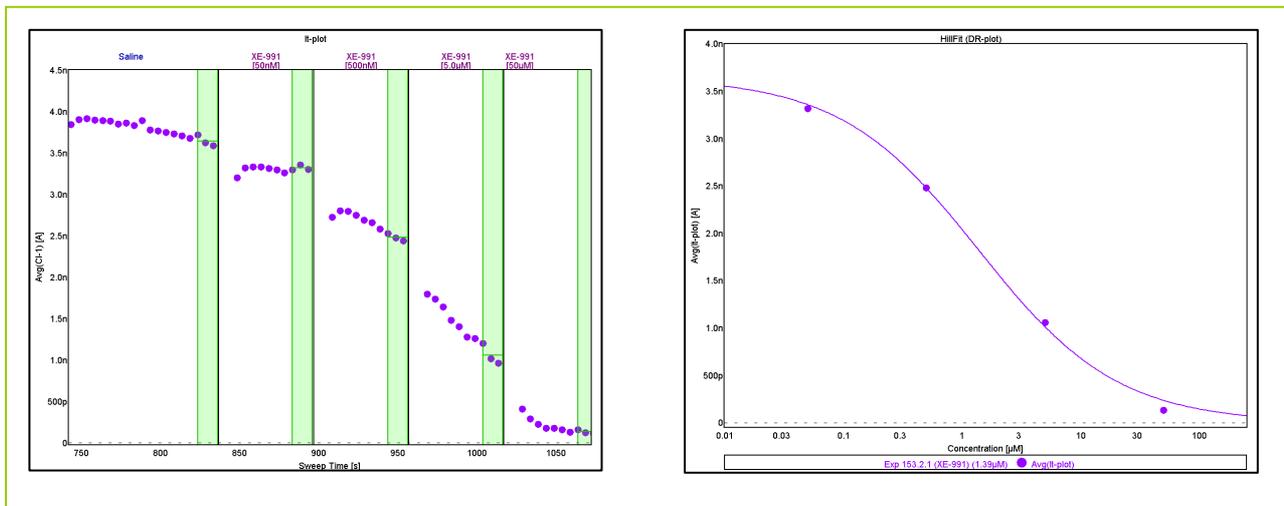


Fig.2: Block of KvLQT1/minK current with XE-991. Left: IT-plot showing the current amplitude in response to four increasing concentrations of XE-991. Right: Corresponding Hill fit.

Next, the effect on the blocker Bepridil was tested on the K_V LQT1/minK currents. Figure 3 (left) shows the current–time plot of the peak amplitude in response to four increasing concentrations of Bepridil (0.05, 0.5, 5, 50 μ M). Figure 3 (right) shows the corresponding Hill fit. The resulting $IC_{50} = 8.96 \pm 1.0 \mu$ M, $n=8$ (literature value 5.3-10.5 μ M).

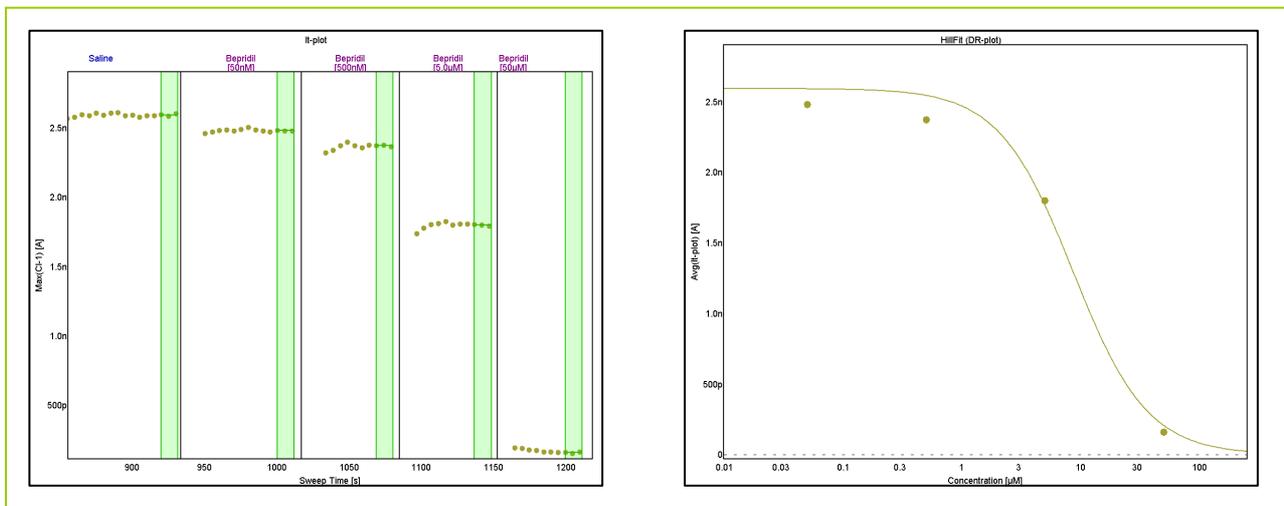


Fig.3: Block of KvLQT1/minK current with Bepridil. Left: IT-plot showing the current amplitude in response to four increasing concentrations of Bepridil. Right: Corresponding Hill fit.

Furthermore, experiments were performed in order to evaluate Chromanol 293B on K_V LQT1/minK currents. Figure 5 (left) shows the current–time plot of the peak amplitude in relation to four concentrations of Chromanol 293B (0.05, 0.5, 5, 50 μ M). Figure 4 (right) shows the corresponding Hill fit. The resulting IC_{50} for Chromanol 293B = $10.6 \pm 1.1 \mu$ M, $n=13$ (literature value 10-12.4 μ M).

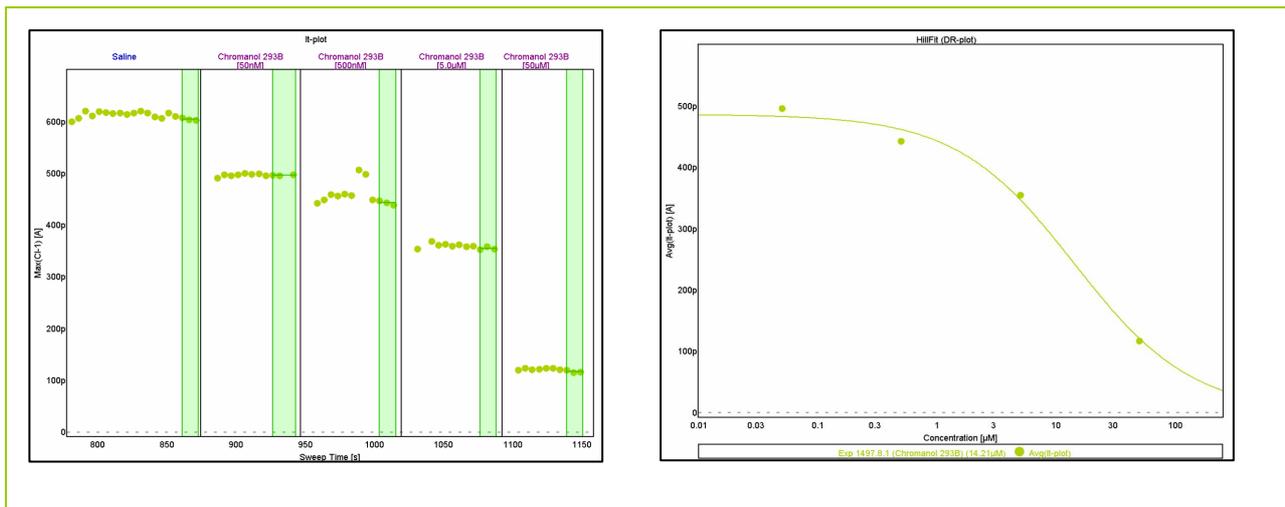


Fig.4: Block of KvLQT1/minK current with Chromanol 293B. Left: IT-plot showing the current amplitude in response to four increasing concentrations of Chromanol 293B. Right: Corresponding Hill fit.

3.3 Patch-clamp Success Rates

The patch-clamp properties of the CHO KvLQT1/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: $> 1 \text{ G}\Omega$. The whole-cell configuration was not accepted if the membrane resistance was below $500 \text{ M}\Omega$. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **97%** (n=18)
- Whole-cell successful: **81%** (n=18)
- Recording (20-30 min) successful: **72%** (n=18)

4 CELL CULTURE CONDITIONS

4.1 General

CHO KvLQT1/minK cells are incubated at 37°C in a humidified atmosphere with 5% CO₂ (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing F12 (HAM) medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 300 µg/mL Geneticin and 100 µg/mL Hygromycin. The CHO KvLQT1/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

- F12 (HAM) with Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- CHO KvLQT1/minK clones were selected under 500 µg/mL Geneticin and 300 µg/mL Hygromycin antibiotic pressure.
- To cultivate CHO KvLQT1/minK cells, also a reduced antibiotic pressure (300 µg/mL Geneticin and 100 µg/mL Hygromycin) can be used.
- To separate CHO KvLQT1/minK cells from untransfected cells, use 500 µg/mL Geneticin and 300 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: 300 µg/mL Geneticin and 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.
- Re-suspend 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 styrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4.8 Stability of CHO KvLQT1/MINK cells

CHO KvLQT1/minK cells stably express functionally active KvLQT1/minK potassium channels over 41 passages. Under recommended cell culture conditions no variation in current density was observed over 41 cell splitting cycles.

5 KvLQT1/MINK SEQUENCE

5.1 Human KvLQT1 Accession Number AF000571

Cloned cDNA sequence of KvLQT1 subunit was error-free and identical with AF000571 sequence:

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ATGCGCCGCGCCTCCTCCCGCCAGGGCCGAGAGGAAGCGCTGGGGTTGGGGCCGCTGCCAGGCGCCCGGGGGGCGAG
CGCGGGCCTGGCCAAGAAGTGCCCTTCTCGCTGGAGCTGGCGGAGGGCGCCCGGGCGGGCGCGCTCTACGCGCCCA
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AGGGGACAGGTGTTTCCACGTGCGCCATCAGGGGCATCCGCTTCTCGAGATCCTGAGGATGTACACGTGACCGCCAGG
GAGGCACCTGGAGGCTCCTGGGCTCCGTGGTCTTCCATCCAGGAGCTGATAACCACCTGTACATCGGCTTCTGGG
CCTCATCTTCTCCTCGTACTTTGTGTACCTGGCTGAGAAGGACGCGGTGAACGAGTCAAGCCGCGTGGAGTTCCGCGACTAC
GCAGATGCGCTGTGGTGGGGGGTGGTACAGTCAACCATCGGCTATGGGGACAAGGTGCCCCAGACGTGGGTGGGAA
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AAGACAAGGTGACGCAGCTGGACCAGAGGCTGGCACTCATCCGACATGCTTACCAGCTGCTCTCCTTGCACGGTGGCA
GCACCCCGGCAGCGGGCGGCCCGCCAGAGAGGGCGGGCCACATCACCAGCCCTGCGGCAGTGGCGGCTCCGTCGA
CCCTGAGCTTCTCCTGCCAGCAACACCCTGCCACCTACGAGCAGCTGACCGTGGCCAGGAGGGGCCCCGATGAGGGGTC
CTGA

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5.2 Human minK Subunit Accession Number AF135188

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

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ATGATCCTGTCTAACCCACAGCGGTGACGCCCTTCTGACCAAGCTGTGGCAGGAGACAGTTCAGCAGGGTGGCAACATG
TCGGGCTGGCCCGCAGGTCCCCCGCAGCAGTGACGGCAAGCTGGAGGCCCTCTACGTCTCATGGTACTGGGATTCCTCG
GCTTCTTACCCTGGGCATCATGCTGAGCTACATCCGCTCCAAGAAGCTGGAGCACTCGAACGACCCATTAACGTCTACAT
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TGAAAACCATCTGGCCATAGAACAACCAACACACACCTTCTGAGACGAAGCCTTCCCCATGA

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