B'SYS GmbH

CHO K_VLQT1/minK Cell Line

Application Note

TABLE OF CONTENTS

1 BACKGROUND

1.1 The cardiac IKs current is encoded by K_VLQT1/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_VLQT1 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_VLQT1 and minK

The paired expression of the K_VLQT1 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 K_VLQT1 currents is an about tenfold decrease of the activation kinetics as compared to currents from K_VLQT1 channels alone.

1.3 B'SYS's CHO K_VLQT1/minK Cells

B'SYS has designed a new CHO K_VLQT1/minK cell line with constitutive co-expression of human K_VLQT1/minK channels. The human K_VLQT1/minK cDNA was cloned and transfected into CHO cells and then the functional properties of the K_VLQT1/minK channels validated by means of the patch-clamp technique. Cells were validated for manual and automated patch-clamping (Sophion Q-Patch™). Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human K_VLQT1/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 K_VLQT1/MINK CELLS

3.1 Electrophysiology

K_VLQT1/minK currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 145, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40 ~305 mOsm. The pipette solution consisted of (in mM) KF 120, KCl 20, HEPES 10, EGTA 10, EDTA 10, pH (KOH) 7.20 ~290 mOsm. 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). K_VLQT1/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.

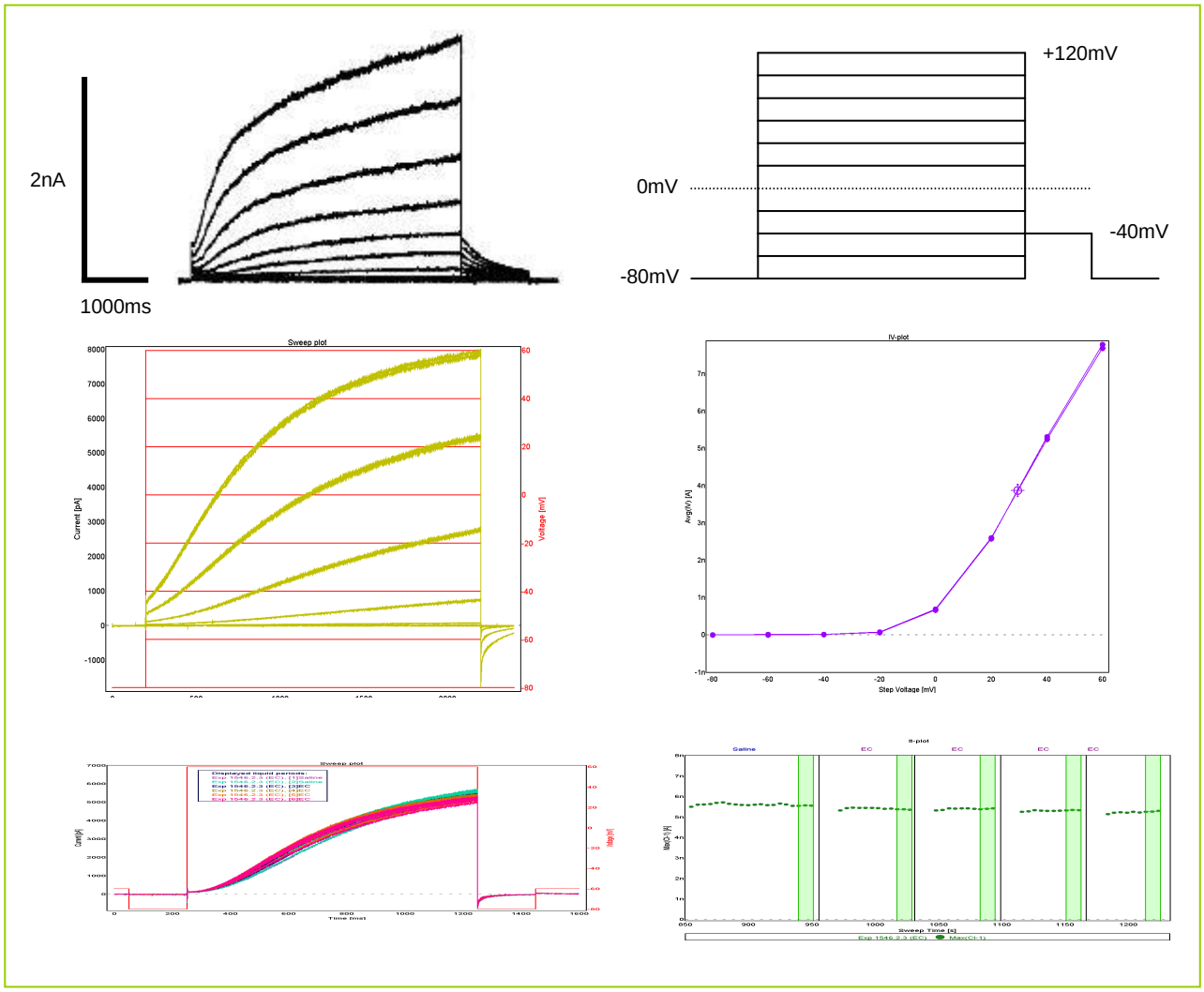

Fig.1: Activation of K_VLQT1/mink. Outward currents were observed upon depolarization of the cell membrane from holding potential (-80 mV) up to +120 mV in 20 mV increments. Top left: manual patch-clamping, top right voltage protocol, middle left: automated patch-clamping, Q-Patch, middle right: IV curve. Bottom left: repetitive stimulation of K_VLQT1 / mink, bottom right: IT plot shows only minimal decrease of the current amplitude.

3.2 Pharmacological Validtion

In order to evaluate the pharmacological properties of the KvLQT1/minK channel. The response of K_VLQT1/minK to a known blocker XE-991 was tested. Four concentrations were tested 0.01, 0.1, 1, 10 µM. An IC_{50} for XE-991 of 0.96±0.4 µM, n=7 was determined (literature value 1-6 µM).

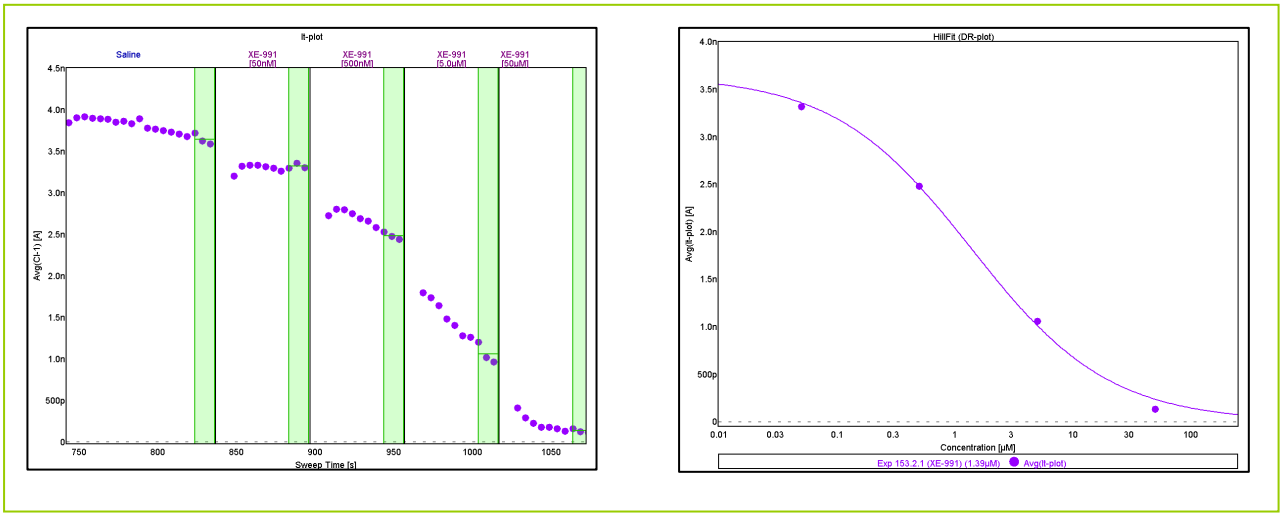

Fig.2: Block of KvLQT1/mink current with XE-991. Left: IT-plot showing the current amplitude in response to four increasing concentrations of XE-991. Right: Corresponding Hill fit.

Next, the effect on the blocker Bepridil was tested on the K_VLQT1/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 µM). Figure 3 (right) shows the corresponding Hill fit. The resulting IC_{50} = 8.96±1.0 µM, n=8 (literature value 5.3-10.5 µM).

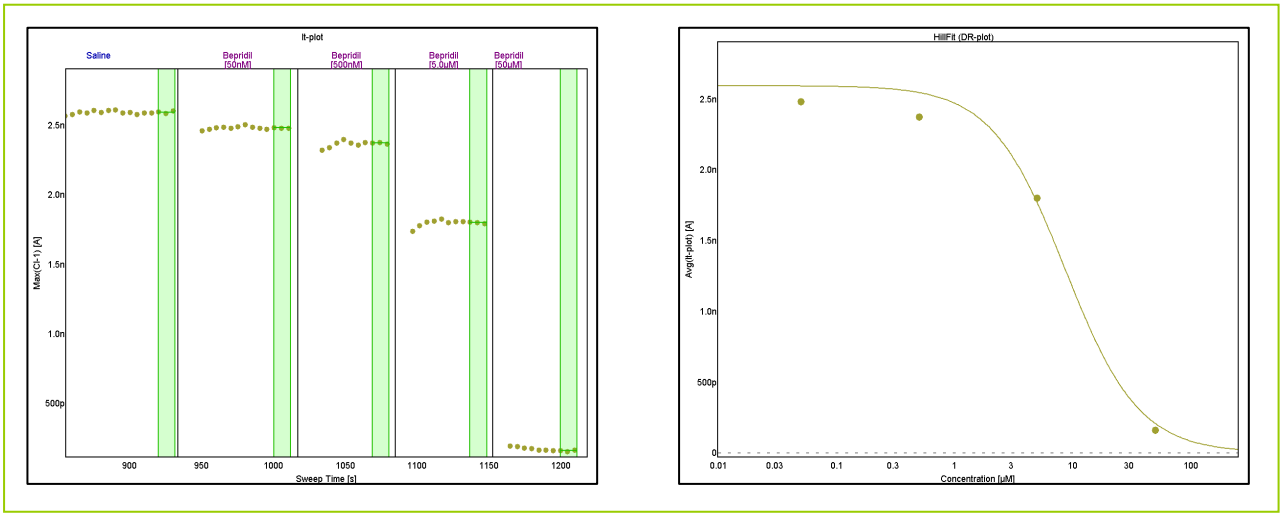

Fig.3: Block of KvLQT1/minK current with Bepridil. Left: IT-plot showing the current amplitude in response to four increasing concentrations of Bepridil. Right: Corresponding Hill fit.

Furthermore, experiments were performed in order to evaluate Chromanol 293B on K_VLQT1/minK currents. Figure 5 (left) shows the current–time plot of the peak amplitude to in relation to four concentrations of Chromanol 293B (0.05, 0.5, 5, 50 µM). Figure 4 (right) shows the corresponding Hill fit. The resulting IC_{50} for Chromanol 293B= 10.6±1.1 µM, n=13 (literature value 10-12.4 µM).

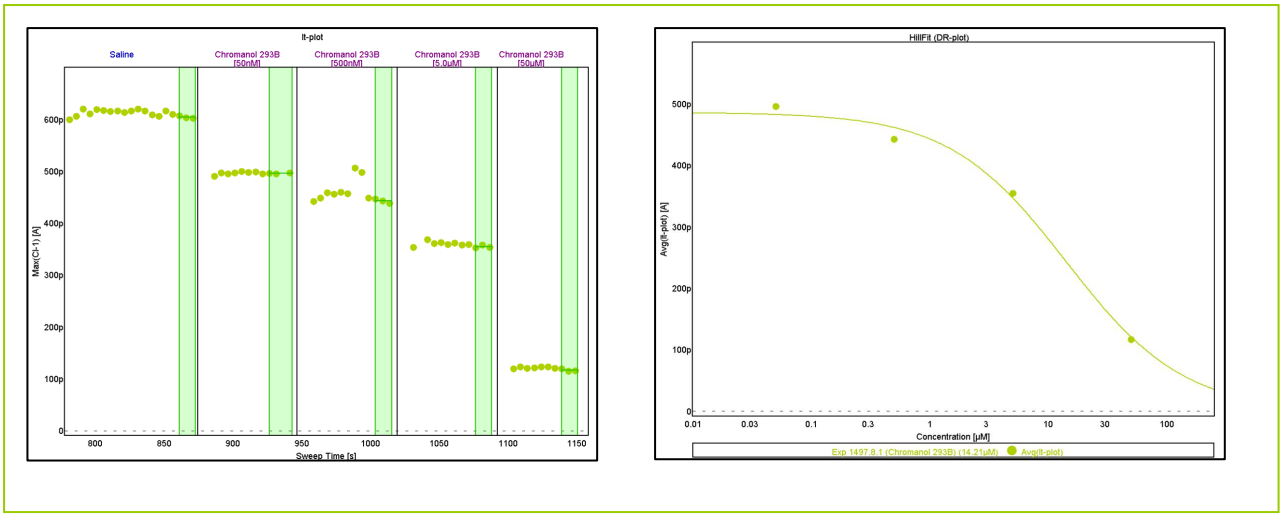

Fig.4: Block of KvLQT1/minK current with Chromanol 293B. Left: IT-plot showing the current amplitude in response to four increasing concentrations of Chromanol 293B. Right: Corresponding Hill fit.

3.3 Patch-clamp Success Rates

During validation cells showed stable expression levels up to passage 28 (higher passages were not tested). The following success rates were achieved using Q-Patch:

	Single hole	Multi hole
No of Q-Plates tested	5	6
Cell attachment (%)	99	100
100 MΩ<Rmem<1 GΩ (%)	28	-
Rmem > 1 GΩ (%)	58	-
Whole cells (%)	96	100
Completed experiments (%)	73	100
Whole cell life time (min)	20	35

4 K_VLQT1/MINK SEQUENCE

4.1 Human K_VLQT1 Accession Number AF000571

Cloned cDNA sequence of K_VLQT1 subunit was error-free and identical with AF000571 sequence:

ATGGCCGCGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCGCTGGGGTTGGGGCCGCCTGCCAGGCGCCCGGCGGGGCAG
CGCGGGCCTGGCCAAGAAGTGCCCCTTCTCGCTGGAGCTGGCGGAGGGCGGCCCGGCGGGCGGCGCGCTCTACGCGCCCA
TCGCGCCCGGCGCCCCAGGTCCCGCGCCCCCTGCGTCCCCGGCCGCGCCCGCCGCGCCCCCAGTTGCCTCCGACCTTGGCC
CGCGGCCGCCGGTGAGCCTAGACCCGCGCGTCTCCATCTACAGCACGCGCCGCCCGGTGTTGGCGCGCACCCACGTCCAGG
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GCCTCATCTTCAGCGTGCTGTCCACCATCGAGCAGTATGCCGCCCTGGCCACGGGGACTCTCTTCTGGATGGAGATCGTGCT
GGTGGTGTTCTTCGGGACGGAGTACGTGGTCCGCCTCTGGTCCGCCGGCTGCCGCAGCAAGTACGTGGGCCTCTGGGGGCG
GCTGCGCTTTGCCCGGAAGCCCATTTCCATCATCGACCTCATCGTGGTCGTGGCCTCCATGGTGGTCCTCTGCGTGGGCTCCA
AGGGGCAGGTGTTTGCCACGTCGGCCATCAGGGGCATCCGCTTCCTGCAGATCCTGAGGATGCTACACGTCGACCGCCAGG
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CCTCATCTTCTCCTCGTACTTTGTGTACCTGGCTGAGAAGGACGCGGTGAACGAGTCAGGCCGCGTGGAGTTCGGCAGCTAC
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GACCATCGCCTCCTGCTTCTCTGTCTTTGCCATCTCCTTCTTTGCGCTCCCAGCGGGGATTCTTGGCTCGGGGTTTGCCCTGAA
GGTGCAGCAGAAGCAGAGGCAGAAGCACTTCAACCGGCAGATCCCGGCGGCAGCCTCACTCATTCAGACCGCATGGAGGT
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AAGACAAGGTGACGCAGCTGGACCAGAGGCTGGCACTCATCACCGACATGCTTCACCAGCTGCTCTCCTTGCACGGTGGCA
GCACCCCCGGCAGCGGCGGCCCCCCCAGAGAGGGCGGGGCCCACATCACCCAGCCCTGCGGCAGTGGCGGCTCCGTCGA
CCCTGAGCTCTTCCTGCCCAGCAACACCCTGCCCACCTACGAGCAGCTGACCGTGCCCAGGAGGGGCCCCGATGAGGGGTC
CTGA

4.2 Human minK Subunit Accession Number AF135188

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

ATGATCCTGTCTAACACCACAGCGGTGACGCCCTTTCTGACCAAGCTGTGGCAGGAGACAGTTCAGCAGGGTGGCAACATG
TCGGGCCTGGCCCGCAGGTCCCCCCGCAGCAGTGACGGCAAGCTGGAGGCCCTCTACGTCCTCATGGTACTGGGATTCTTCG
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TGAAAACCATCTGGCCATAGAACAACCCAACACACACCTTCCTGAGACGAAGCCTTCCCCATGA

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