B'SYS GmbH

CHO Kir2.1 Cell Line

Specification Sheet

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1. PRODUCT SHIPMENT

1.1. Product Format

CHO cells stably transfected with recombinant Kir2.1 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. Validated Assays

Manual patch clamping (whole cell, voltage clamp) and automated patch-clamping (Q-Patch, Sophion)

2. VALIDATION OF CHO KIR2.1 CELLS

2.1. Electrophysiology

Kir2.1 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 GΩ seal between the patch electrodes and individual Kir2.1 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 Kir2.1 currents were measured. Voltage ramps between -120 mV and +60 mV were applied (holding potential -60 mV).

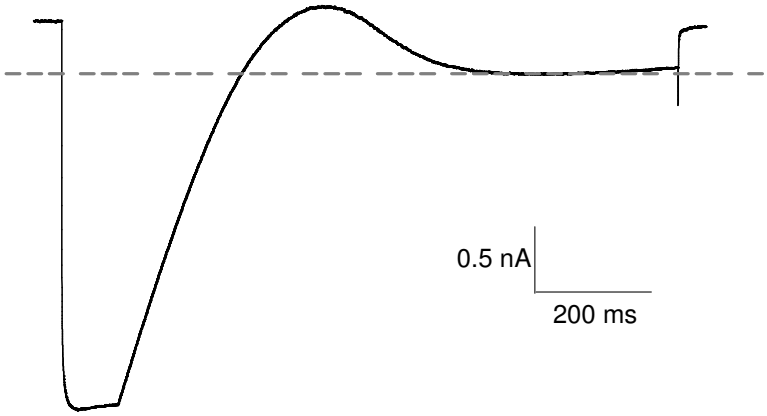

Fig. 1: Representative Kir2.1 currents recorded from a stably transfected CHO cell. The voltage was hyperpolarized to -120 mV from an holding potential of -60 mV, followed by a voltage ramp of voltages between -120 mV and +60 mV was applied during 1 s.

2.2. Effect of external potassium

The Kir2.1 channel is a selective potassium channel. To verify this characteristic, stably transfected CHO cells were perfused with external solutions containing different concentrations of potassium. The changes of the reversal potential was analyzed and compared with the theoretical reversal potentials calculated by the Nernst-e

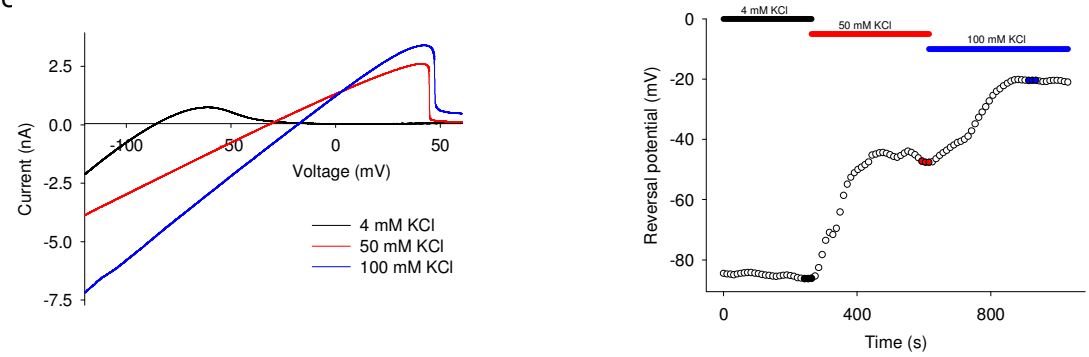

Fig. 2: Potassium dependence of Kir2.1. Left: Representative Kir2.1 currents recorded from a stably transfected CHO cell at different external potassium concentrations. Right: Long time course of reversal potential. All solutions were continuously perfused during experimentation.

2.3. Specific Kir2.1 channel Blocker ML 133 HCl

ML 133 HCl, which is known as a selective Kir2.1 blocker, was used as positive control to validate the pharmacological profile of the CHO Kir2.1 cells. The block of Kir2.1 channels by ML 133 HCl is pH sensitive. Therefore the IC_{50} was determined at pH7.4 and pH8.5. The results are summarized below:

Method (patch - clamp)		IC_{50} value (µM)		IC_{50} value (µM)
Manual	ML 133 HCl pH7.4	2.68	ML 133 HCl pH8.5	0.26
Automated (Q-Patch)	ML 133 HCl pH7.4	4.43	ML 133 HCl pH8.5	0.25

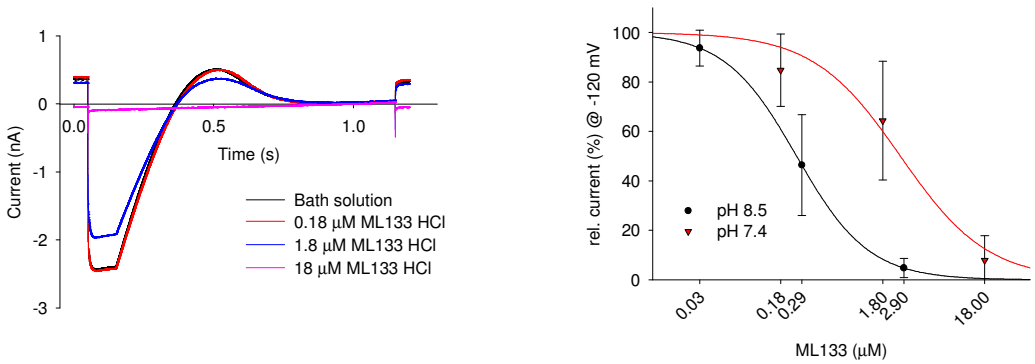

Fig. 3: pH dependent block of Kir2.1 currents by ML 133 (manual patch-clamping). Left: Representative Kir2.1 currents recorded from a stably transfected CHO cell at different ML 133 HCl concentrations (pH7.4). Right: Dose response curves of ML 133 HCl at pH7.4 and pH8.5.

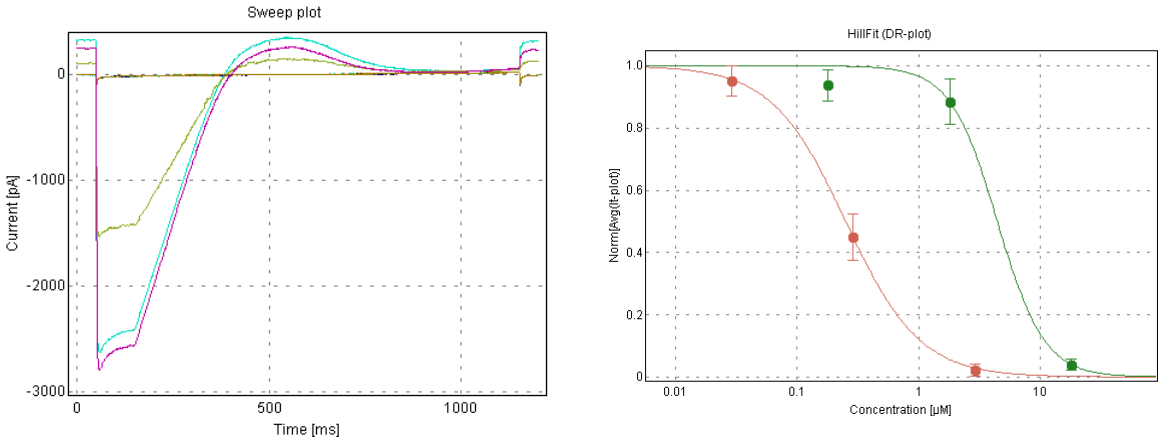

Fig. 4: pH dependent block of Kir2.1 currents by ML 133 (automated patch-clamping). Left: Representative Kir2.1 currents recorded from a stably transfected CHO cell at different ML 133 HCl concentrations (pH8.5). Concentrations of 29 nM, 290 nM and 2.9 µM were tested. Right: Dose response curves of ML 133 HCl at pH7.4 and pH8.5.

2.4. Patch-clamp Success Rates

The patch-clamp properties of the CHO Kir2.1 cell line were elucidated at typical working passage numbers (passage 5 - 12). Average current amplitude at -120 mV was more than 2 nA.

Manual patch-clamping:

A total of 14 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: **100%** (n=14)
- Whole-cell successful: **86%** (n=14)
- Recording (20-30 min) successful: **79%** (n=14)

Automated patch-clamping:

A total of 48 cells were analyzed. The whole-cell configuration was not accepted if the membrane resistance was below 0.5 GΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- Recording (20-30 min) successful: 62.5% (n=48)

3. CELL CULTURE CONDITIONS

3.1. General

CHO Kir2.1 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 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 10 µg/mL Puromycin. The CHO Kir2.1 cells are passaged at a confluence of about 80%. For manual 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
- 10% FBS
- 1.0% Penicillin/Streptomycin

3.3. Antibiotics

- CHO Kir2.1 clones were selected under 10 µg/mL Puromycine antibiotic pressure.
- To separate CHO Kir2.1 cells from untransfected cells, use 15 µg/mL Puromycin.

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-75 culture flask containing 10ml 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: 10 µg/mL Puromycin.
- 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 Kir2.1 cells

CHO Kir2.1 cells stably express functionally active Kir2.1 potassium channels over at least 20 passages. Under recommended cell culture conditions no variation in current density was observed over 42 cell splitting cycles.

4. KIR2.1 SEQUENCE

4.1. Accession Number NP_000882

Cloned cDNA sequence of Kir2.1 channel was error-free and encodes for NP_000882 sequence:

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MGSVRTNRYSIVSSEEDGMKLATMAVANGFGNGKSKVHTRQQCRSRFVKKDGHCNVQFIN
VGEKGQRYLADIFTTCVDIRWRWMLVIFCLAFVLSWLFFGCVFWLIALLHGDLDASKEGK
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LAEKKYILSNANSFCYENEVALTSKEEDDSENGVPESTSTDTPPDIDLHNQASVPLEPRP
LRRESEI
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5. CONTACT INFORMATION

5.1. Contact Address for Technical Support and Ordering Information

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