B'SYS GmbH

CHO hERG-Duo Cell Line

Specification Sheet

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1. BACKGROUND

1.1. Drug-induced QT Prolongation

The QT interval as measured in the electrocardiogram (ECG) is determined by the duration of the cardiac action potential. The rapid delayed rectifier current (I_{Kr}) is important for cardiac action potential repolarization. Suppression of I_{Kr} function by adverse drug effects (e.g. terfenadine, cisapride, astemizole etc.) can induce a prolongation of the QT interval carrying elevated risk of life-threatening cardiac arrhythmias.

1.2. Regulatory Issues

I_{Kr} is mediated by the potassium channel hERG (human ether-à-go-go related gene). This ion channel is involved in both, congenital and acquired long QT syndrome. According to the ICH S7A and S7B guidelines the analysis of molecules for inhibition of hERG currents allows the assessment of the potential for QT prolongation.

1.3. B'SYS's CHO hERG-Duo Cells

As a new test system B'SYS has designed a CHO hERG-Duo cell line with constitutive expression of hERG channels. The hERG cDNA was cloned and transfected twice (two different vectors) into CHO cells and then the functional properties of the hERG channels validated by means of the patch-clamp technique.

B'SYS CHO hERG-Duo cells were validated found suitable for manual and automated patch-clamping (Sophion Q-Patch ™ and Qube ™).

2. PRODUCT SHIPMENT

2.1. Product Format

CHO cells stably transfected with recombinant hERG potassium channel:

- 1x 0.5 mL aliquots of frozen cells at 2.9 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO
- Cells are frozen at passage number 11

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 HERG CELLS

3.1. Electrophysiology

hERG 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 hERG 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 hERG currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to +60 mV in 10 mV increments of 2 s duration (Fig. 1). hERG tail currents were elicited upon partial repolarization to -40 mV for 3 s The voltage pulses were run at intervals of 10 s.

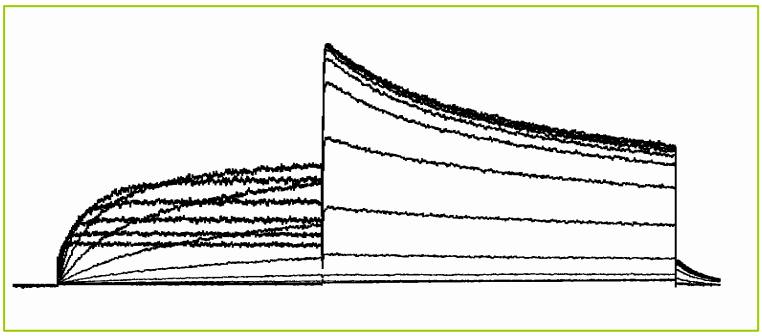


Fig. 1: Representative hERG outward currents recorded upon depolarization of the CHO membrane from -80 mV to +60 mV in 10 mV increments (2 s). Subsequent partial repolarization to -40 mV (3 s) elicited hERG tail currents.

3.2. Tail Current Activation

The hERG channel is known as an inwardly rectifying potassium channel displaying very slow activation/deactivation kinetics, but is inactivated very rapidly. To verify the functional properties of B'SYS's cloned CHO hERG-Duo cells, both the activation plot and IV curve were constructed. The activation plot of hERG tail currents was best fitted with a Boltzmann function with half-maximal activation at **-10.41 mV** (manual patch-clamping) / **-14.2 mV** (automated patch-clamping) and a slope of **9.0** (manual patch-clamping) or **8.8** (automated patch-clamping) (Fig. 2, n=5 cells).

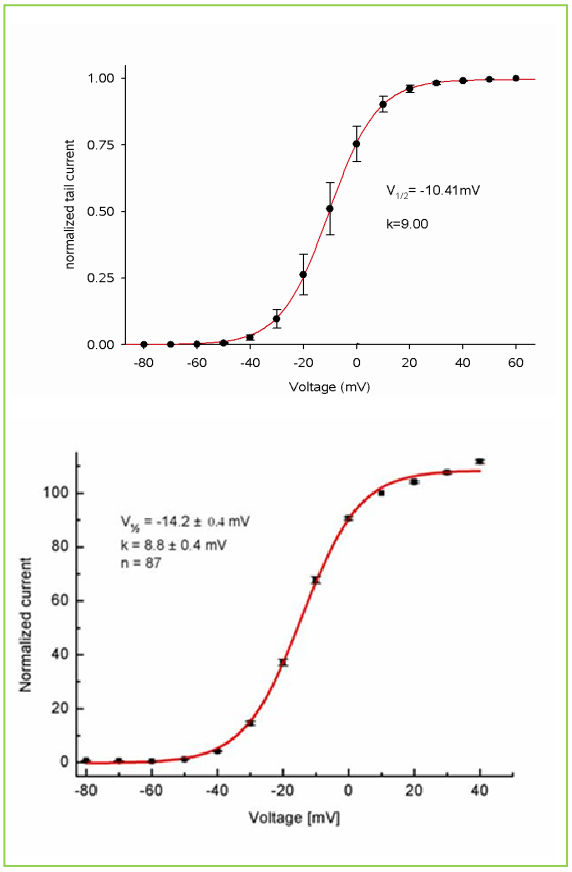


Fig. 2: Activation plot of hERG tail currents fitted to Boltzmann function with V_{50} -10.41 mV (manual patch-clamping, top) / -14.2 mV (automated patch-clamping, bottom)

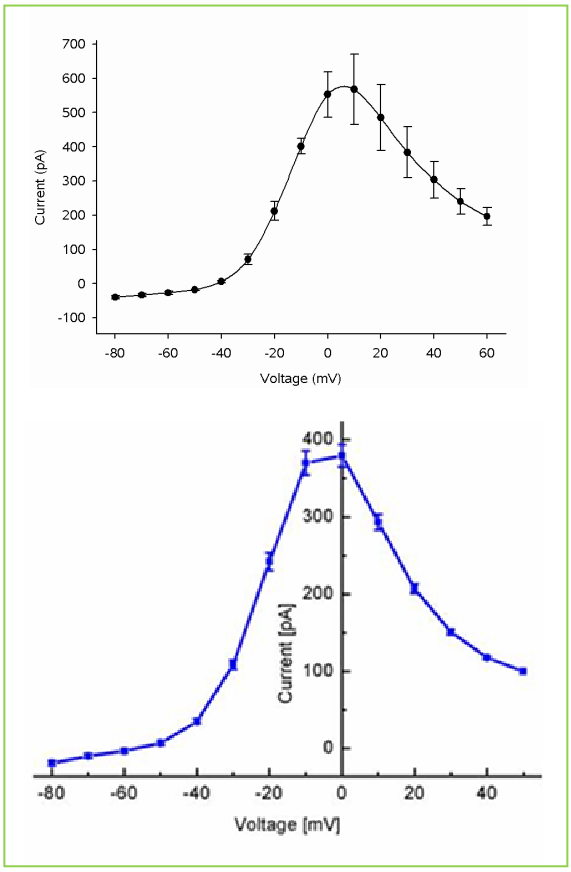


Fig. 3: IV curve of hERG activation currents. Threshold for activation was between -40 mV and -30 mV and maximal current amplitude was measured at 0 mV for manual (top) and automated (bottom) patch-clamping

3.3. Inward Rectifying

Smallest hERG activation currents could be elicited upon depolarization of the cell membrane to around -30 mV (Fig. 3). Further depolarization gradually increased the outward currents until a maximum was reached at 0 mV. Depolarization of the membrane to positive potentials resulted in a decrease of current amplitudes due to the inward rectifying properties of the hERG channel.

3.4. E-4031

E-4031, which is known as a selective I_{Kr} blocker, was used as positive control to validate the pharmacological profile of the CHO hERG-Duo cells. The effect of E-4031 was first measured at 100 nM, which blocked hERG tail currents almost completely (6.4 ± 4.1 % relative tail current amplitude, mean ± SEM of 3 cells). When the cells were exposed to 30, 10, 3 and 1 nM E-4031 (mean ± SEM of 3 cells). The inhibition curve (Fig. 4) was best fitted with an IC_{50} value of **5.26 nM**.

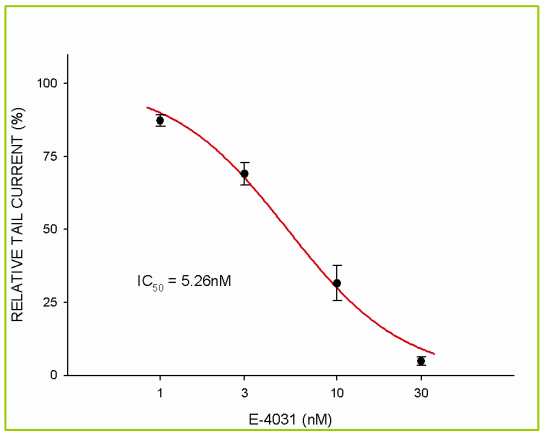
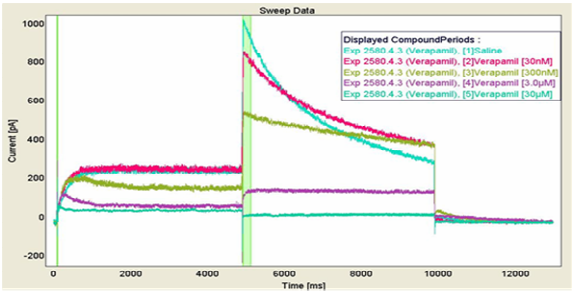
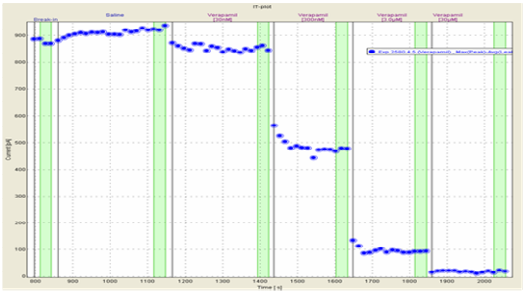


Fig. 4: hERG tail current inhibition curve of E-4031.

3.5. Verapamil

The tail current amplitude was measured and its time course with increasing verapamil concentration is shown in Figure 5. The resulting block at steady-state was then calculated and fitted with a logistic equation. The resulting IC_{50} for verapamil on hERG was 344 nM.





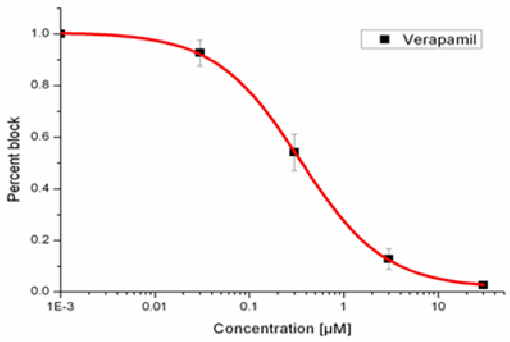


Fig. 5: hERG current trace of increasing concentrations of Verapamil (top left), The peak current amplitudes were measured and plotted versus time of experiment (top right). The IC50 was determined by fitting the normalized steady state current amplitudes to a logistic equation

3.6. Patch-clamp Success Rates

The patch-clamp properties of the CHO hERG-Duo cell line were elucidated at typical working passage numbers (passage 14-41). A total of 11 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 successfetal bovine serum, 0.9% Penicillin/Streptomycin solution, 100 µg/mL Hygromycin and 100 µg/mL G-418. The CHO hERG-Duo 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.

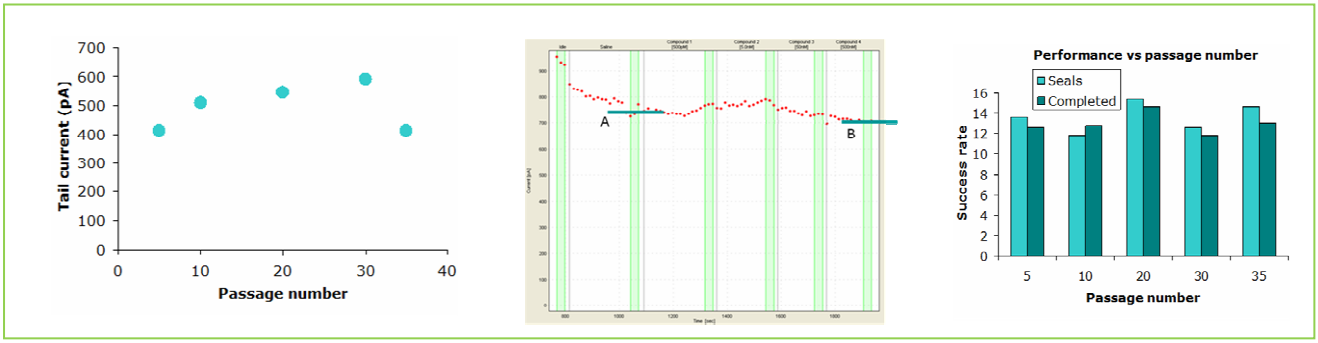


Fig. 6: Current stability of hERG DUO cells. The current amplitude was about 500 pA for more than 30 passages (left), with only minimal rundown (middle). The success rate was high and constant for also more than 30 passages (right).

4. CELL CULTURE

4.1. Recommended Complete Medium

- F12 (HAM) with GlutaMAX I
- 9% FBS
- 0.9% Penicillin/Streptomycin

4.2. Antibiotics

- CHO hERG-Duo clones were selected under 500 µg/mL Hygromycin and 500 µg/mL G-418 pressure.
- To cultivate CHO hERG-Duo cells, a reduced antibiotic pressure of 100 µg/mL must be used.
- To separate CHO hERG-Duo cells from untransfected cells, use 500 µg/mL antibiotics.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

4.3. 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 G-418 .
- Incubate cells and check them daily until 70% - 80% confluency is reached.

4.4. 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.

4.5. 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).

4.6. 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.

4.7. Stability of CHO hERG-Duo cells

CHO hERG-Duo cells stably express functionally active hERG potassium channels over 40 passages. Under recommended cell culture conditions no variation in current density was observed over 40 cell splitting cycles.

5. HERG SEQUENCE

5.1. Accession Number U04270

Cloned cDNA sequence of hERG channel was error-free and identical with U04270 sequence:

ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGCAAGTTTGAGGGCCAGAGCCGTAAGTTCAT
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