B'SYS GmbH

HEK hERG 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' HEK hERG Cells

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

2 PRODUCT SHIPMENT

2.1 Product Format

HEK cells stably transfected with recombinant hERG potassium channel:

- 1 x 0.5 mL aliquots of frozen cells at 2.4 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 HEK 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 HEK 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.

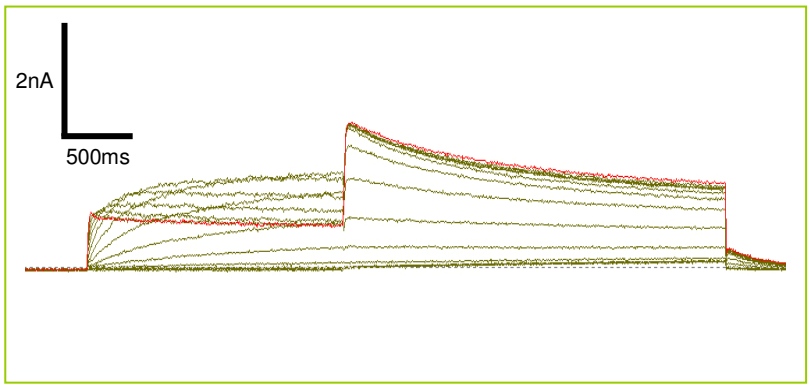

Fig. 1: Representative hERG outward currents recorded upon depolarization of the HEK membrane from -80 mV to +60 mV in 10 mV increments (2 s). Subsequent partial repolarization to -40 mV (3 s) elicited hERG tail currents.

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 HEK hERG 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 **-9.7 mV** and a slope of **9.1** (Fig. 2, n=6 cells).

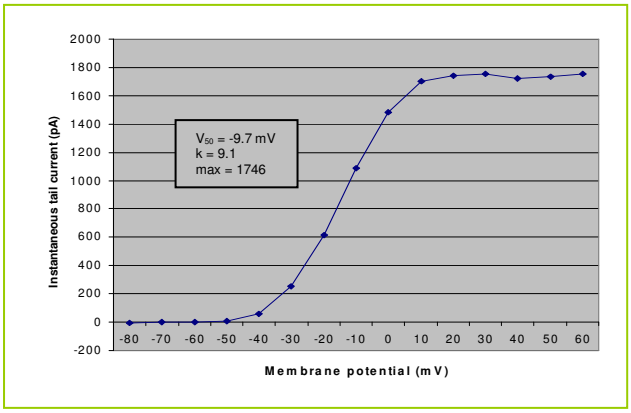

Fig. 2: Activation plot of hERG tail currents fitted to Boltzmann function with V_{50} = -9.7 mV, k = 9.1 and max = 1746 pA (mean ± SEM of 6 cells).

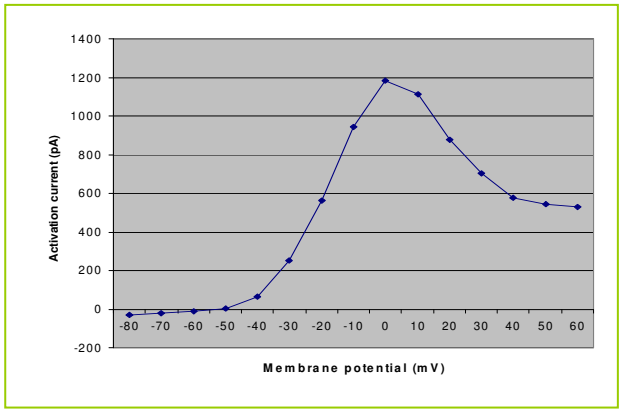

Fig. 3: IV curve of hERG activation currents. Threshold for activation was between -40 mV and -30 mV and maximal current amplitude was measured at 0 mV (mean ± SEM of 6 cells).

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 Positive Control 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 HEK hERG cells. The effect of E-4031 was first measured at 100 nM, which blocked hERG tail currents almost completely (5.3 ± 0.7 % relative tail current amplitude, mean ± SEM of 3 cells). When the cells were exposed to 30, 10, 3 and 1 nM E-4031, the steady-state level relative tail current amplitudes revealed values of 22.3 ± 2.6%, 53.1 ± 1.8%, 85.7 ± 4.2% and 94.5 ± 4.2%, respectively (mean ± SEM of 3 cells). The inhibition curve (Fig. 4) was best fitted with an IC_{50} value of **11.7 nM**.

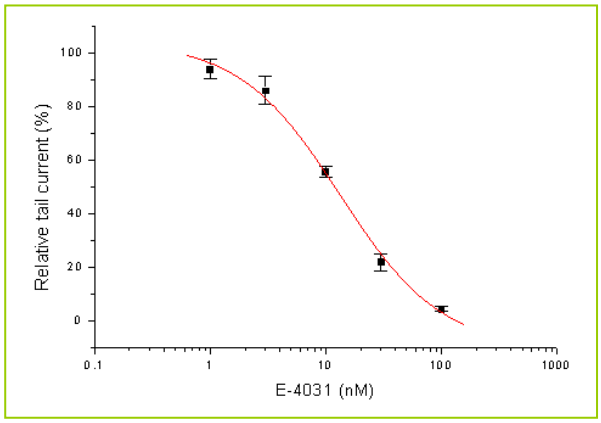

Fig. 4: hERG tail current inhibition curve of E-4031.

3.5 Patch-clamp Success Rates

The patch-clamp properties of the HEK hERG cell line were elucidated at typical working passage numbers (passage 21-43). A total of 17 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: **94%** (n=17)
- Whole-cell successful: **86%** (n=17)
- Recording (20-30 min) successful: **71%** (n=17)

4 CELL CULTURE CONDITIONS

4.1 General

HEK hERG 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/F12 medium supplemented with 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 100 µg/mL G-418 suplhate. The HEK hERG 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/F12 with GlutaMAX I
- 9% FBS
- 0.9% Penicillin/Streptomycin

4.3 Antibiotics

- HEK hERG clones were selected under 500 µg/mL G-418 suplhate antibiotic pressure.
- To cultivate HEK hERG cells, also a reduced antibiotic pressure (100 µg/mL) can be used.
- To separate HEK hERG cells from untransfected cells, use 500 µg/mL G-418 suplhate.

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-25 culture flask containing 5 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 5 mL complete medium & antibiotics.
- Antibiotics: 100 µg/mL G-418 suplhate.
- 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).
- 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 HEK hERG cells

HEK hERG 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:

ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGCAAGTTTGAGGGCCAG
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6 CONTACT INFORMATION

6.1 Contact Address for Technical Support & Ordering Information

- B'SYS GmbH
  Technology Center Witterswil
  Benkenstrasse 254
  4108 Witterswil
  Switzerland

  Tel: +41 61 721 77 44
  Fax: +41 61 721 77 41
  Email: info@bsys.ch
  Web: www.bsys.ch