Application Report

CHO-hERG DUO optimized for QPatch

Functional validation and performance data for the CHO hERG DUO cell line available from Sophion. This cell line is made in collaboration with B'SYS GmbH (Switzerland).

Introduction

CHO hERG cells originating from B'SYS GmbH, Switzerland, were optimized at Sophion Bioscience A/S and subsequently assessed for their behaviour on QPatch.

This report describes the characterization of the optimized cell line in terms of:

- Seal rate
- Success rate for completed experiments (25 min.)
- Passage stability
- Run-down
- Biophysical properties
- Pharmacological properties

CHO (Chinese Hamster Ovary) cell line expressing the hERG (human ether-a-go-go related gene) potassium channel also known as $K_v11.1$ was obtained from B'SYS GmbH, Switzerland, and used in this study.

Results and discussion

The following electrophysiological experiments were carried out by using the intracellular solution IC0.0.0 and extracellular solution EC0.0.0. The IC0.0.0 contains (mM): $CaCl_2$, 5.37; $MgCl_2$, 1.75; HEPES, 10; EGTA, 10; Na_2ATP, 4; KCl, 120; pH was adjusted to 7.2 with KOH and osmolarity was adjusted to 285 ~ 296 with sucrose. The EC0.0.0 contains (mM): $CaCl_2$, 2; $MgCl_2$, 1; HEPES, 10; KCl, 4; NaCl, 145; glucose, 10; pH was adjusted to 7.4 with NaOH and osmolarity was adjusted to approximately 305 with sucrose.

Functional expression of hERG was monitored over time using QPatch. The numbers of wells of success seal and successful completed experiment on a 16-well trial are shown in Figure 1. With increasing number of passage, the success rates of seal and completion remain relatively unchanged up to the highest tested passage #35.
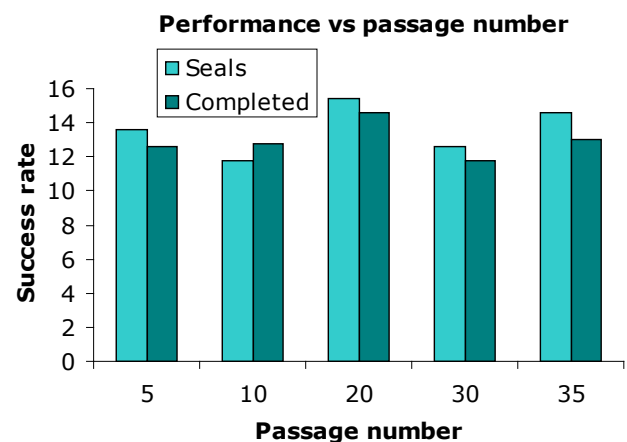

Fig. 1: The average number of seals and completed experiments (25 minutes, four points dose response experiments) were determined averaging the results from five QPlate16s at each passage number.

The average tail current amplitude, obtained at – 50 mV at the end of the saline period (see Figure 10), was 495 ± 80 pA (mean ± S.E.M n=50). Importantly, as can be observed in Figure 2, the tail current amplitude remained relatively constant over the course of 35 passages (see Figure 2). These experiments suggest that the expression of hERG in the cell line remains robust and the cell line is fit for the QPatch study of hERG potassium channels with consistent success rate.
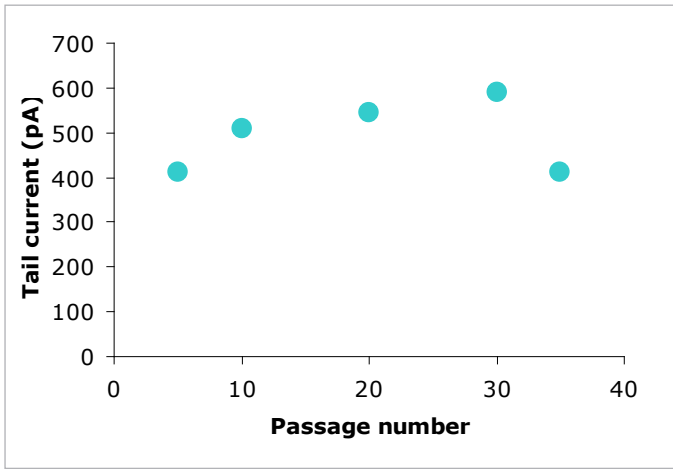
Fig. 2: The mean tail current amplitude measured at -50 mV (n=10 for each passage number).

During the test of various CHO-hERG cell lines, it became apparent that several tested cell lines exhibited more pronounced rundown of the tail current amplitude than others. For the optimized CHO-hERG DUO cell line, we found the minimal run-down. Figure 3 shows the hERG tail current recorded with time and multiple saline solution exchanges. After initial settling, the current remained relatively stable for several solution exchanges up to 15 min of recording. On average, the run-down was 0.51 ± 0.43%/min (n =184) for the optimized CHO-hERG DUO cell line.
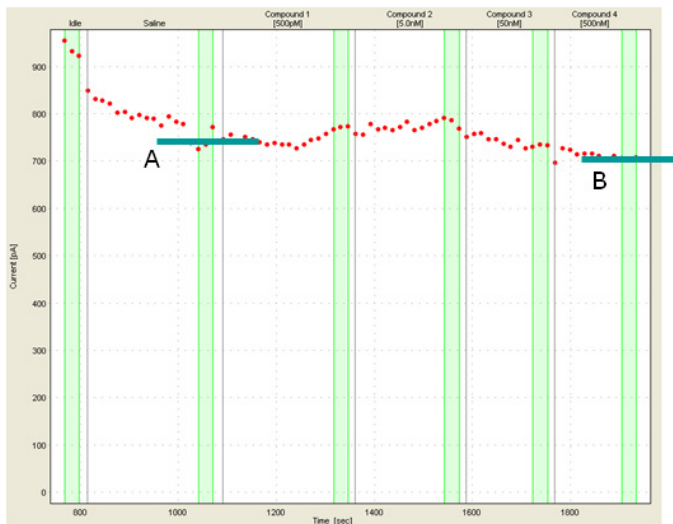

Fig. 3: A simulated dose response experiment with saline containing 0.1% DMSO was used to evaluate run-down. Run-down is calculated as the percentage/minute of initial tail current amplitude from A to B.

Biophysical characterization

Figure 4 shows the voltage protocol used for evoking hERG currents. In this protocol, the cell was held at -80 mV. For every 15 seconds, a five second depolarizing voltage step (from -80 mV to +50 mV) was followed by a five second tail step to -50 mV. A typical raw data example is shown in Figure 5.
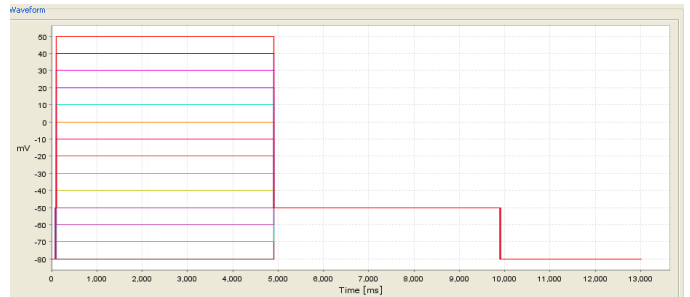

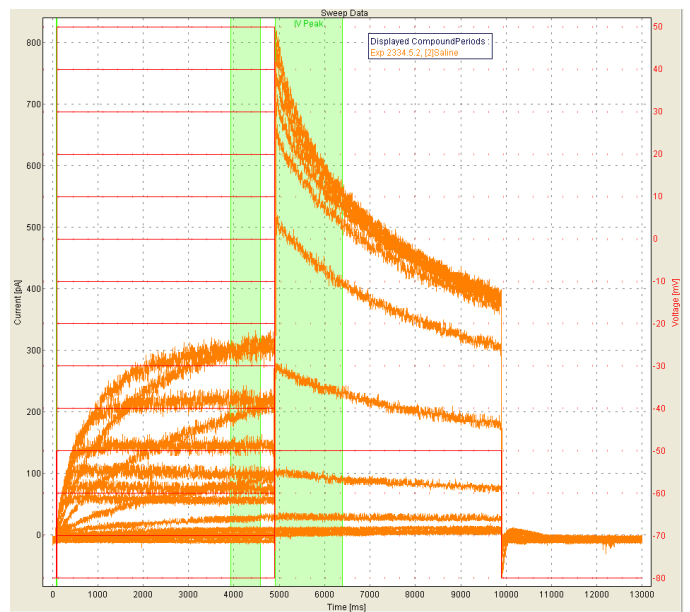Fig. 4: Voltage protocol for hERG IV characterization.

Fig. 5: Raw data IV: The "A" cursor to the left is used to measure the steady-state current for the current-voltage relationship and the "B" cursor to the right is used to measure the tail current.

The steady state current amplitude increased progressively with increase of activating voltage and peaked at approximately 0 mV. At more positive voltages than 0 mV, the current amplitudes decreased due to the onset of fast inactivation. Figure 6 displays the steady-state current-voltage (I/V) relationship of hERG. As expected, the I/V relationship is bell-shaped (Zhou et al 1998).
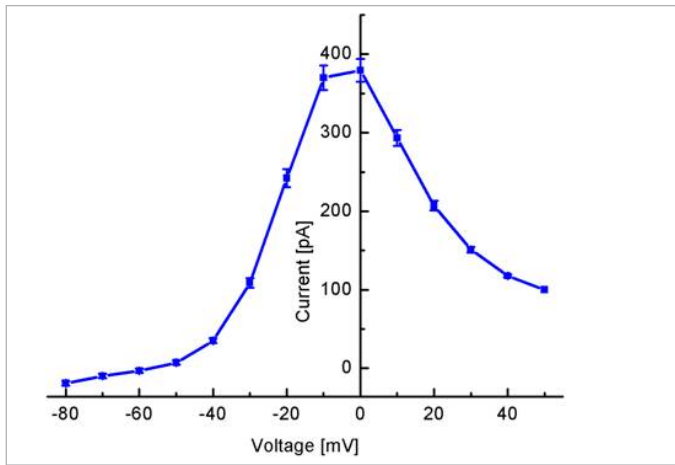
Fig. 6: The isochronal I/V relationship of hERG (mean ± S.E.M., n=79)

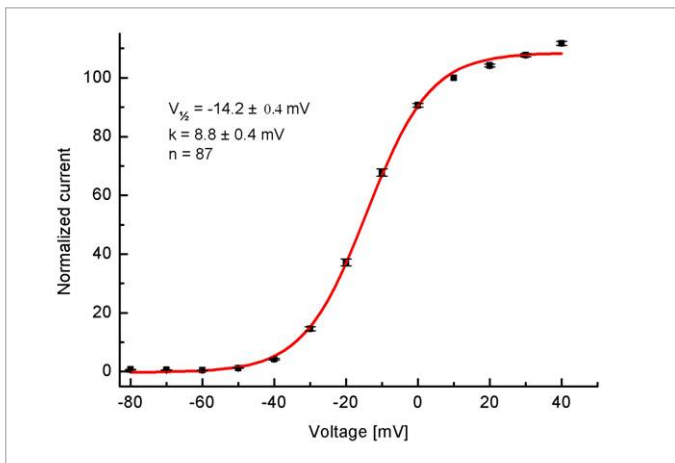$V_{1/2} = -14.2 \pm 0.4$ mV
$k = 8.8 \pm 0.4$ mV
$n = 87$

Fig. 7: Using the "B" cursor (Figure 5), hERG tail currents at potentials ranging from -80 mV to +40 mV was plotted and fitted to the Boltzmann equation. (mean current ± S.E.M., n=87).

Conductance voltage (GV) relationship of hERG is shown in Figure 7. Data obtained from the hERG tail currents were fit with the Boltzman equation (y = A2 + (A1-A2)/(1 + exp((x-x0)/dx))), which yielded a half activation voltage of -14 mV (-14.2 ± 0.4 mV) with a slope of 8.8 mV.

To assess the open channel characteristics, hERG was activated by a step voltage of +10 mV from the holding potential of -80 mV. The tail current interval was then obtained between -120 mV and -10 mV at a 10 mV increment (see Figure 8). At potentials negative to the reversal potential (~-80mV), large inward tail currents were recorded (see Figure 9). The current reversed upon stepping to more depolarized potentials leading to progressively larger outward tail currents with the expected inward rectification as described by Zhou et al 1998.
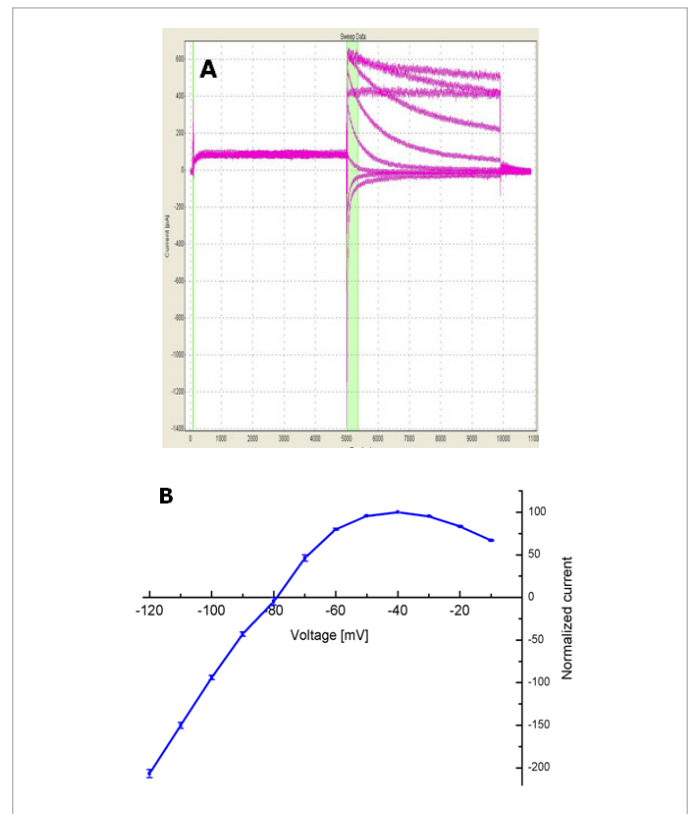

Fig. 9: hERG open channel characteristics. A) Typical raw data. B) Peak tail current plotted against membrane voltage. $E_{rev}= -79$mV (mean ± S.E.M., n=47).
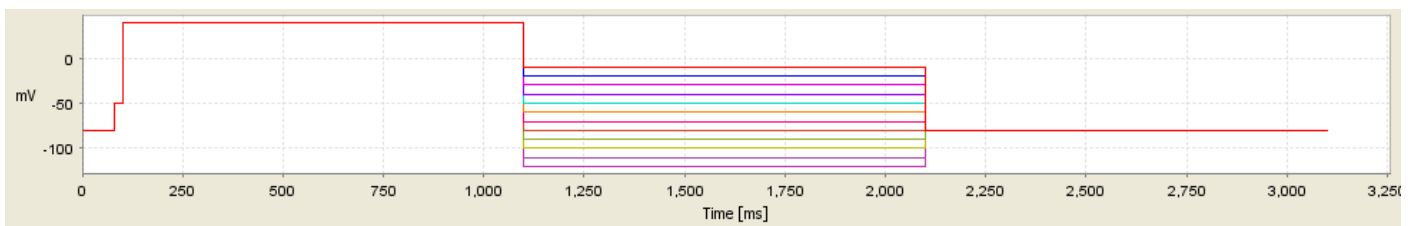

Fig. 8: Voltage protocol with repolarising steps from -120 mV to -10 mV.

Pharmacology

We used verapamil to assess hERG pharmacology. The hERG channel was activated by a +20 mV activation potential for 5 sec. The tail current was obtained by stepping back the holding potential from +20 to -50 mV for 5 sec. This voltage protocol was repeated every 15 sec and representative verapamil data were shown in Figure 10 and 11.

After establishing a stable recording for approximately 5 min, verapamil was applied in four increasing concentrations with 10-fold dilutions starting from 30 nM. Typical raw current traces from the steady-state window are shown in Figure 10. The tail current amplitude was measured and its time course with increasing verapamil concentration is shown in Figure 11. The resulting block at steady-state was then calculated and fitted with a logistic equation as illustrated in Figure 12 and Table 1. The resulting IC_{50} for verapamil on hERG was 344 nM. Using the integrated QPatch analysis software package, the analysis sequence from raw data to IC_{50} values could be easily employed to generate files for export and/or graphic illustrations.
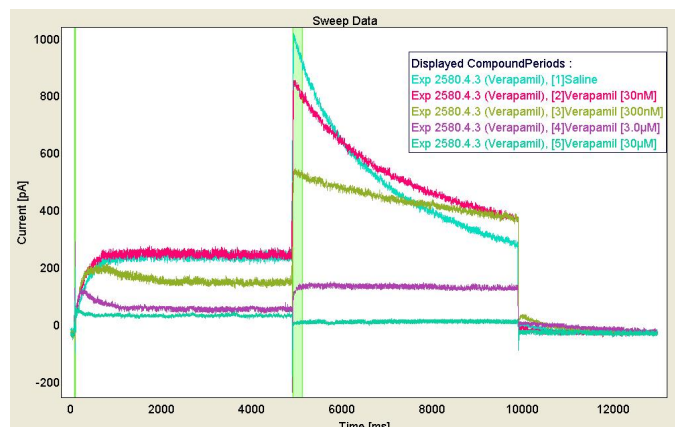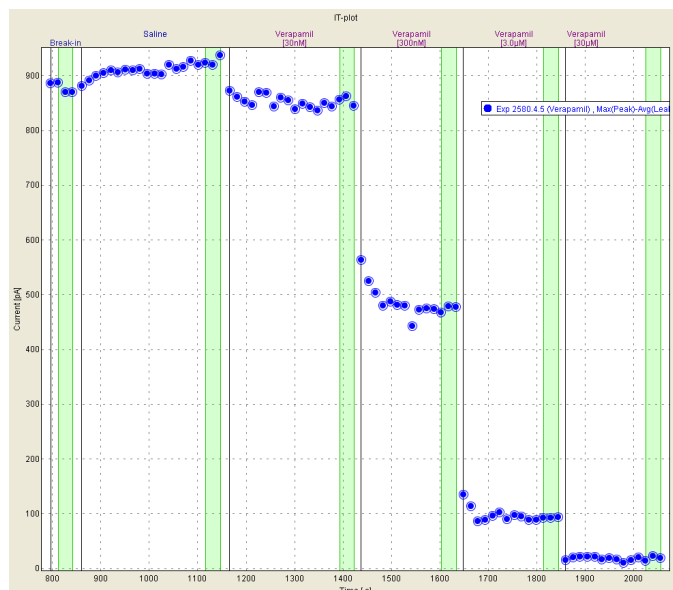
Fig. 11: IT plot showing the effect of verapamil on the hERG tail current. Green boxes indicate the steady-state window used for down-stream Hill plots.

Fig. 10: The hERG current raw trace. One trace depicted per application of verapamil.

Table 1: Percentage inhibition of hERG currents by verapamil.

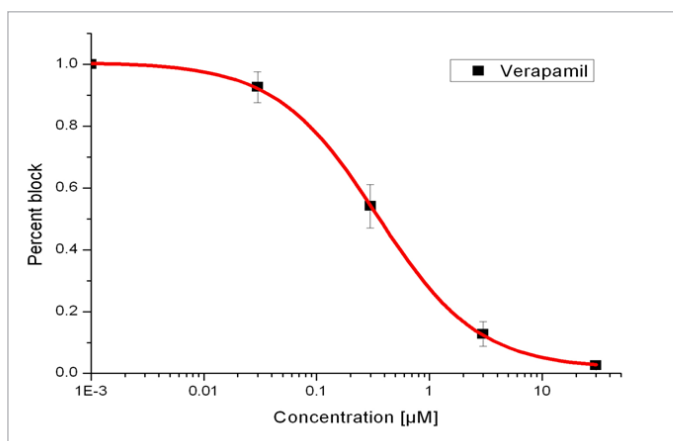| Concentration of verapamil [µM]	% block of control current average ± S.E.M (n=26)
0.03	8.65 ± 0.8
0.3	47.00 ± 1.3
3	87.53 ± 0.7
30	97.20 ± 0.6

Fig. 12: Hill fit using average block effects in a four points dose response experiment with verapamil on QPatch. IC_{50} = 344 nM (n=26).

Conclusion

When operating automated patch clamp, the results do not improve upon the biology used with the system.

This report describes our effort to identify a CHO-hERG expressing cell line that exhibits optimal performance with respect to stability, overall success rates, and run-down.

While 100% success in completed usable experiments is still not an every-day-occurence, the performance of the optimized CHO-hERG cells is getting quite close and we are very happy to be able to launch the QPatch optimized CHO-hERG cells for high quality patch clamp experiments.

For further information on culturing etc. please contact us (info@sophion.dk).

Methods

Cell Culture Protocol

Sub-culturing (T175)

1. Remove old culture media and wash with 7 ml PBS (Cat. #: D8537, Sigma).
2. Add trypsin (Cat. #: T4174, Sigma), gently swirl the flask and aspirate (leave about 1 ml).
3. Place the culture flask in 37°C incubator for ~2 min. (ensure that the cells have a round shape before tapping).
4. Gently tap on the side of the flask and add 5-7 ml culture media and resuspend the cells by working the cell suspension up and down 5-10 times.
5. Determine the cell density and viability by counting the cells in a hemocytometer using trypan blue.
6. Add the number of cells to the mother flask and the experiment flasks according to the sub-culturing plan below.
7. Grow the cells at 37°C, 5% CO_2 to maximum 70-80% confluence.

Sub-culturing plan for making mother flasks and experiment flasks
w
1. Add 3x104 cells/cm2 for sub-culturing/experiments after 24 hours.
2. Add 1.6x104 cells/cm2 for sub-culturing/experiments after 48 hours.*
3. Add 8x103 cells/cm2 for sub-culturing/experiments after 72 hours.*
4. Add 6x103 cells/cm2 for sub-culturing/experiments after 96 hours.

For passage of CHO cells we recommend to sub-culture cells every Monday, Wednesday and Friday.

*We recommend 48 or 72 hours of sub-culturing for best results.

Cells for experiments (for T175 for three QPlates)

1. Remove culture media and wash with 7 ml PBS.
2. Add 3 ml trypsin, gently swirl the flask and aspirate (leave about 1 ml).
3. Place the culture flask in a 37°C incubator for ~2 min (ensure that the cells have reached a round shape before tapping).
4. Add 5 ml serum-free media (Cat. #: C5467, Sigma) and resuspend the cells by working the cell suspension up and down 5-10 times.
5. Determine the cell density and viability by diluting an aliquot 1:2 in trypan blue (Cat. #: T8154, Sigma) and count the cells in a hemocytometer.

Make sure that there are 2-5 mio/ml cells added to the QStirrer

References

Zhou et al. 1998. Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Jan (74)1, 230-241.