Application Report:

CHO-hERG DUO

Characterization and functional validation

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Sophion Bioscience A/S, Baltorpvej 154, DK-2750 Ballerup, Danmark
Phone: + 45 44 60 88 00 Fax: +45 44 60 88 99 E-mail: info@sophion.dk www.sophion.com

Introduction

CHO hERG cells originating from bSys 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 minutes)
- Passage stability
- Run-down
- Biophysical properties
- Pharmacological properties

Product Information

CHO (Chinese Hamster Ovary) cell line expressing the human ERG (ether-a-go-go related gene) potassium channel also known as Kv11.1.

Functional Validation and Performance

Functional expression of hERG was followed over time using QPatch and stable expression was found to be robust over at least 30 - 35 passages (see Figure 1).

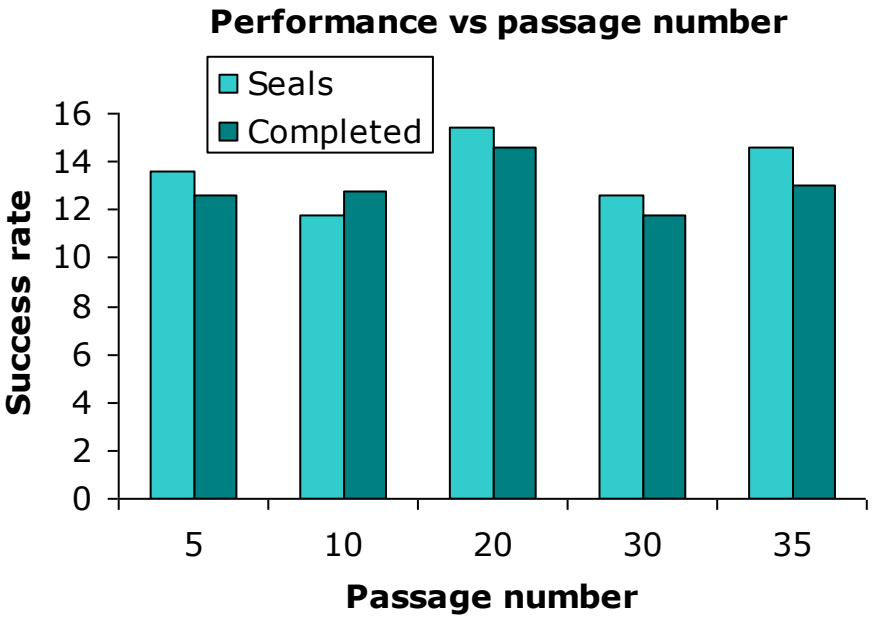


Figure 1. The average number of seals and completed experiments (25 minutes, 4 points dose response experiments) were determined averaging the results from 5 QPlate16s at each passage number.

The average tail current amplitude estimated 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, and the steady-state/tail amplitude ratio was always very low (see Figure 2). Furthermore, the optimized cell line retains close to 100% expressors even at passage 35.

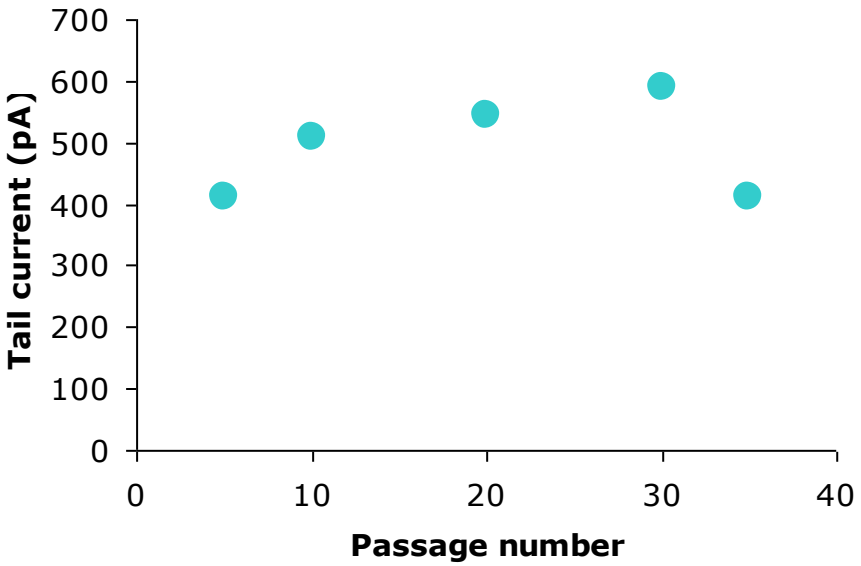


Figure 2. The mean tail current amplitude measured at -50 mV (n=10 for each passage number).

During the test of various CHOhERG cell lines, it became apparent that several of the tested cell lines exhibited more pronounced run-down of the tail current amplitude than others. For the optimized CHOhERG DUO we found minimal run-down 0.51%/min ± 0.43 (n =184) (see Figure 3).

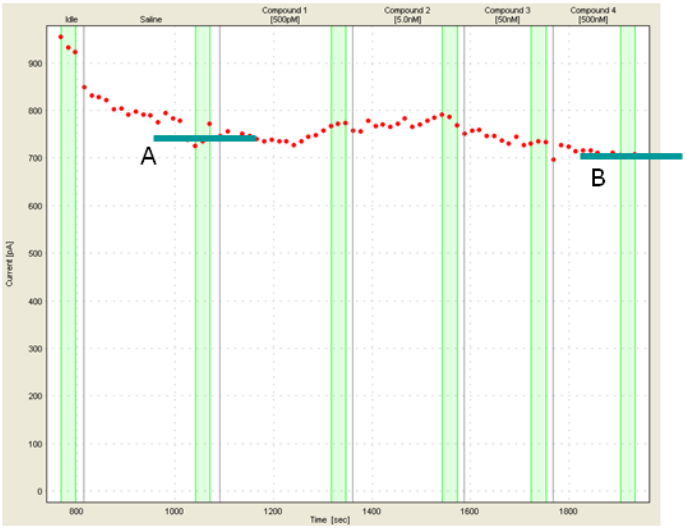


Figure 3. A simulated dose response experiment with saline containing 0.1% DMSO was used to evaluate run-down. Run-down is calculated as the percentage/minute of initial tail current amplitude from A to B.

Biophysical characterization

Figure 4 shows the voltage protocol used for evoking hERG currents: Every 15 seconds a 5 second depolarizing voltage step (from -80 mV to +50 mV) was followed by a 5 second tail step to -50 mV. A typical raw data example is plotted in Figure 5.

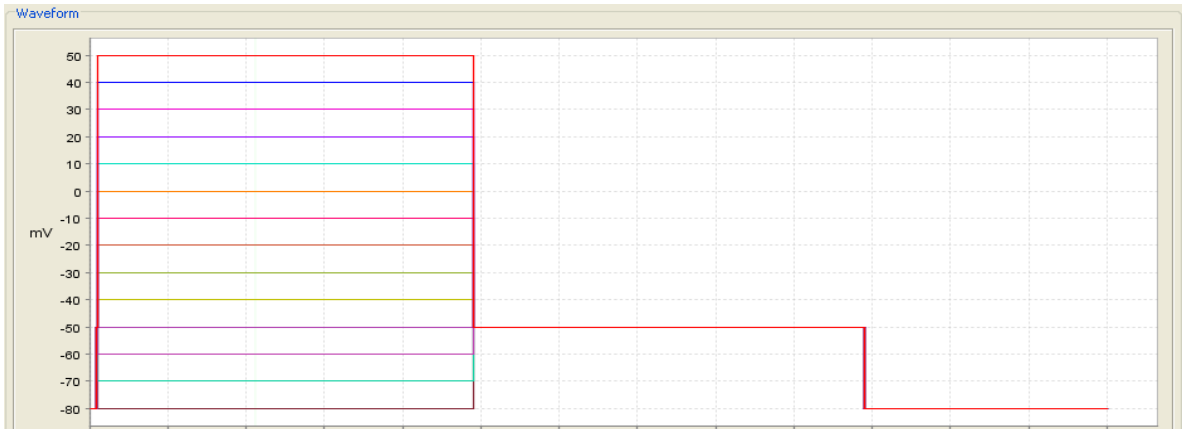


Figure 4. Voltage protocol for hERG IV characterization.

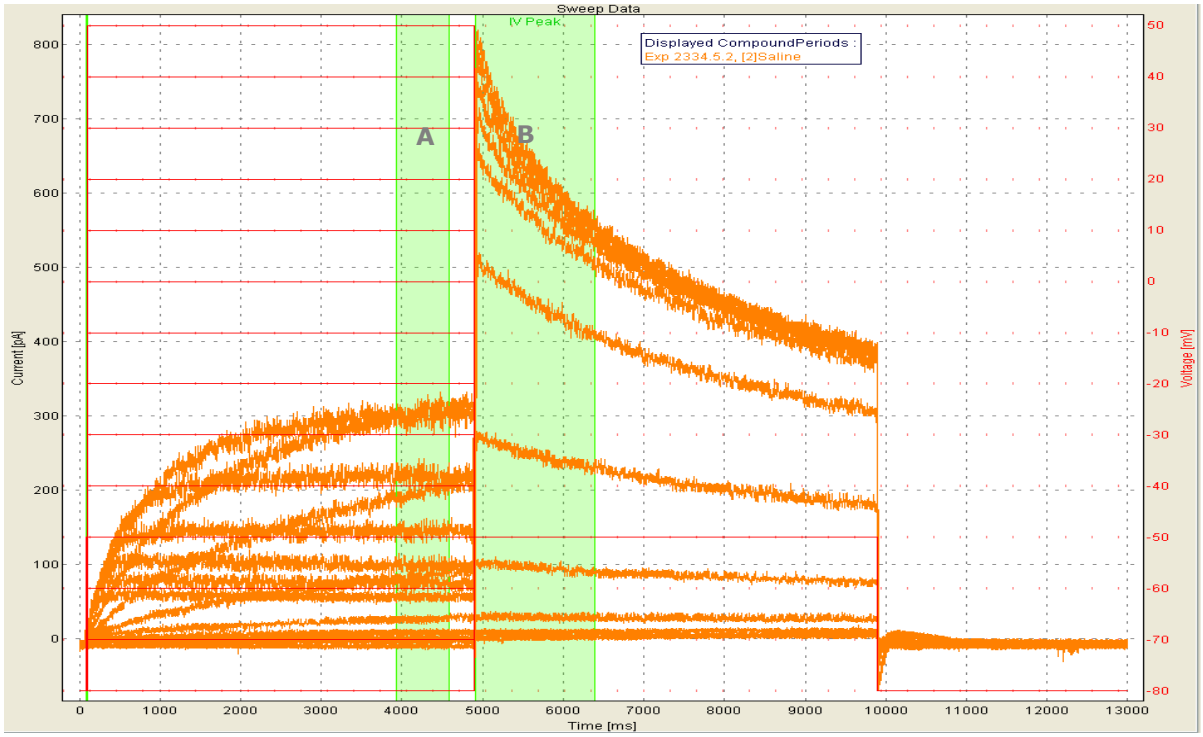


Figure 5. Raw data IV: The "A" cursor to the left is used to measure the Steady-state current for the current-voltage relationship and the "B" cursor to the right is used to measure the tail current.

The steady state current amplitude increased progressively up to -10 mV and peaked at 0 mV. At even more positive voltages the current amplitudes became smaller due to the onset of fast inactivation. Figure 6 displays the obtained I/V relationship of hERG. As expected, the I/V relationship is bell-shaped (Zhou et al 1998).

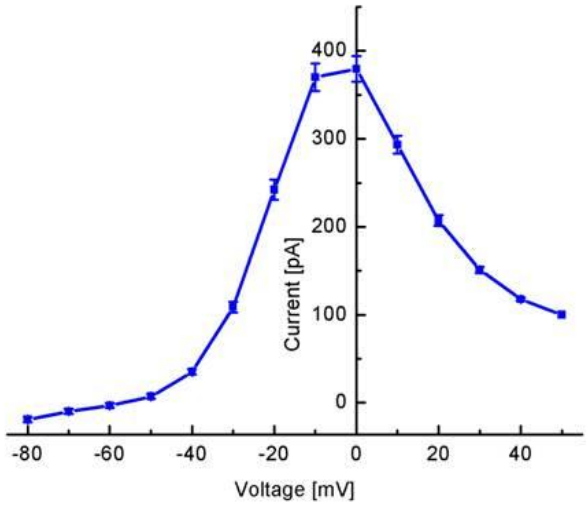


Figure 6. The isochronal I/V relationship of hERG (mean ± S.E.M, n=79)

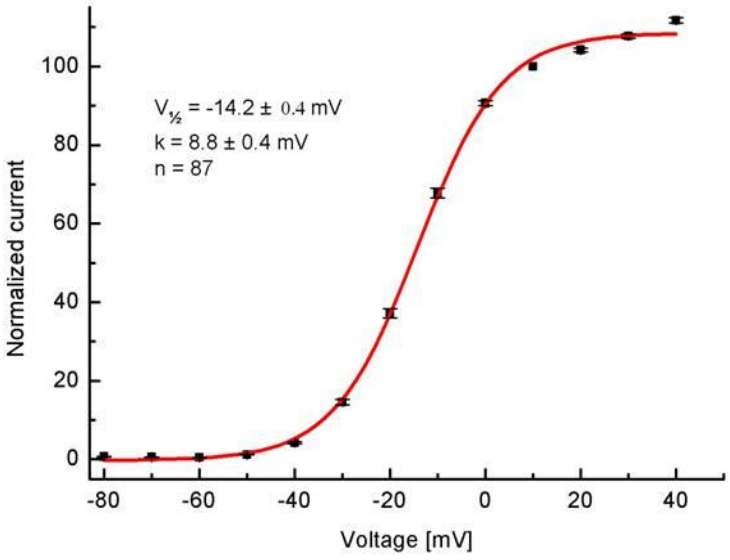


Figure 7. Using the "B" cursor (Fig. 5), hERG tail currents at potentials ranging from -80 mV to +40 mV was plotted and fitted to the Boltzmann equation. (mean current ± S.E.M., n=87)

Data from the hERG tail currents were fit with the Boltzman equation (y = A2 + (A1-A2)/(1 + exp((x-x0)/dx))). The data and the fit are shown in Figure 7.

To assess the open channel characteristics, the tail current interval was stepped at 10 mV increments between -120 mV and -10 mV from a holding potential of -80 mV (see Fig. 8). At potentials negative to the reversal potential (~-80mV), large inward tail currents were recorded (see Fig. 9). The current reversed upon stepping to more depolarised levels leading to progressively larger outward tail currents with the expected inward rectification as also described by Zhou et al 1998.

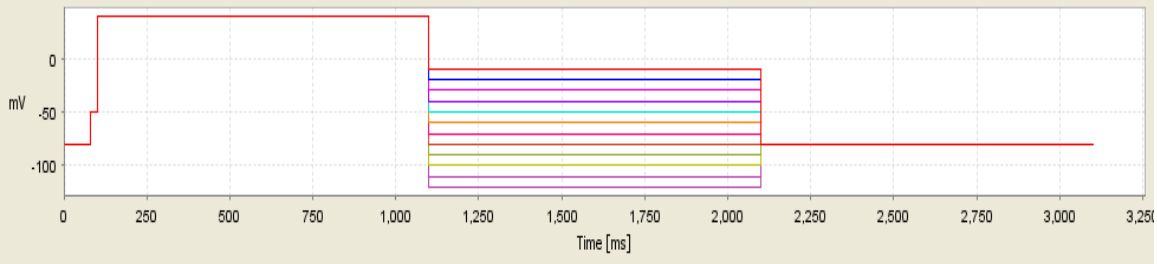


Figure 8. Voltage protocol with repolarising steps from -120 mV to -10 mV.

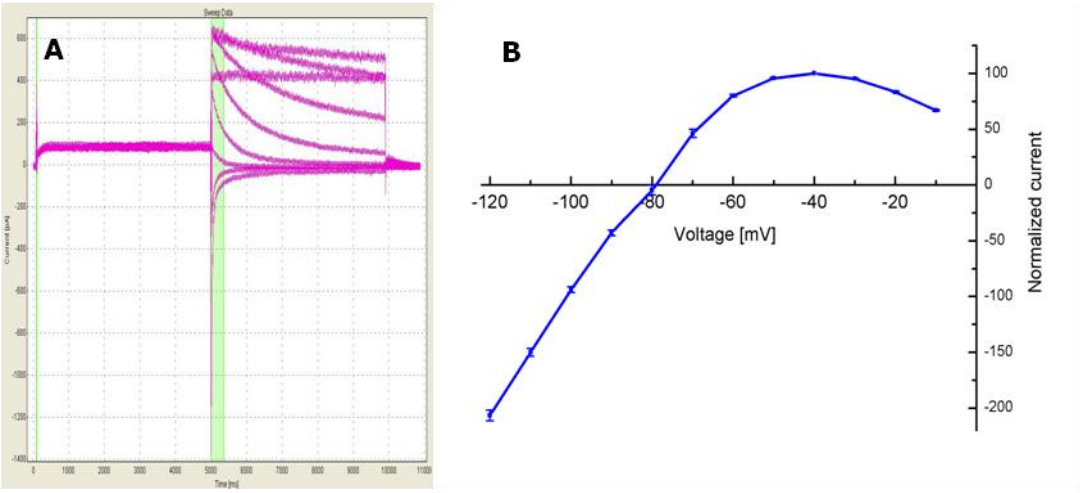


Figure 9. *hERG open channel characteristics. A) Typical raw data. B) Peak tail current plotted against membrane voltage.* E_{rev} *= -79mV (mean ± S.E.M., n=47).*

Pharmacology

We used verapamil to assess hERG pharmacology. IC_{50} values reported in the literature are highly dependent on the voltage protocol used. Our experiments are performed with the Sophion standard 5 s hERG voltage protocol described above (see Figure 4).

Verapamil was applied in four increasing concentrations with 10-fold dilutions starting from 30 µM. Typical current raw traces from the steady-state window are depicted in Figure 10 (see also Figure 11). The tail cursor amplitude was measured and the resulting block calculated at steady-state (see Figure 11 and Table 1). Using

the integrated analysis software package, the analysis sequence from raw data to IC50 values was easily employed to generate files for export and/or graphic illustrations.

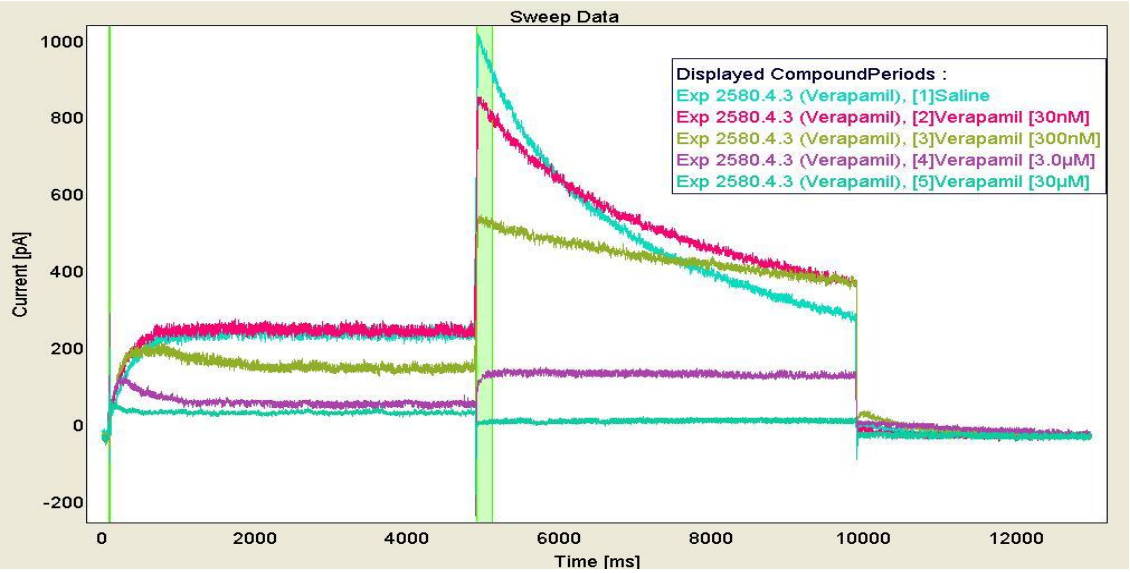


Figure 10. The hERG current raw trace. One trace depicted per application of verapamil.

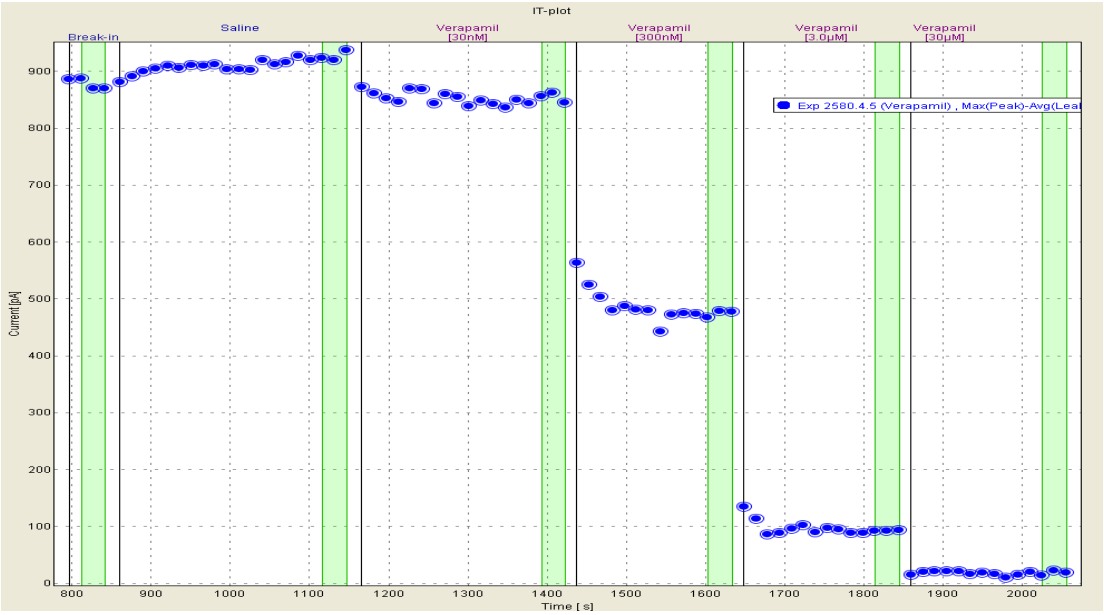


Figure 11. IT plot showing the effect of Verapamil on the hERG tail current. Green boxes indicate the steady-state window used for down-stream Hill plots.

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

Table 1. Percentage inhibition of hERG currents by verapamil.

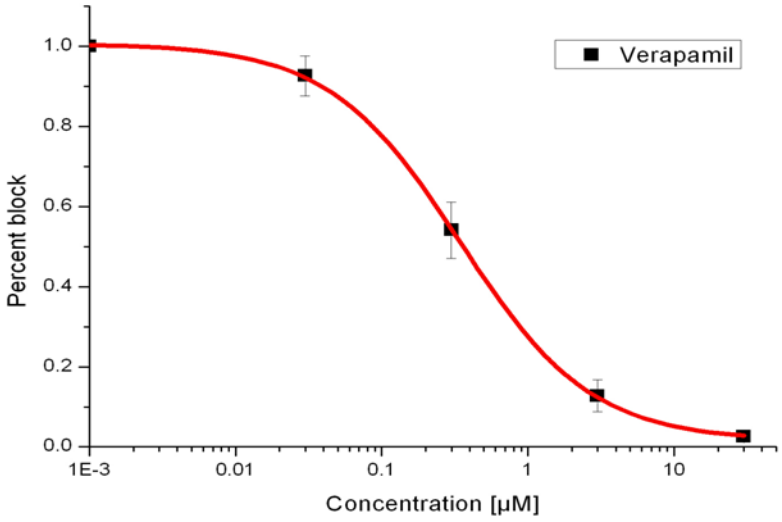
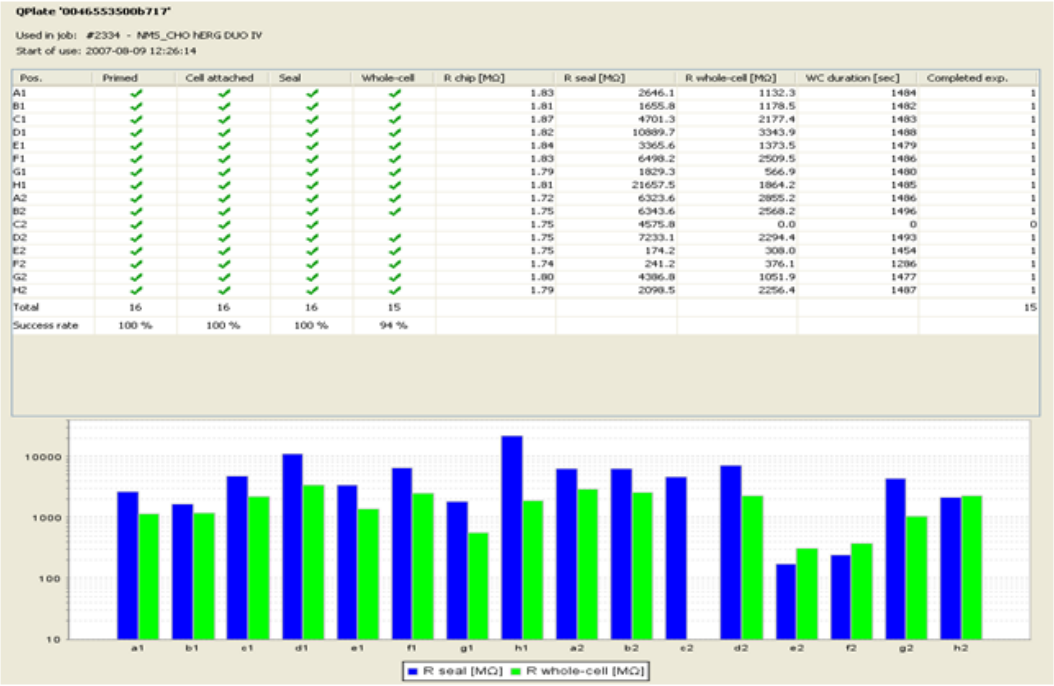


Figure 12. Hill fit using average block effects in a 4 points dose response experiment with Verapamil on QPatch. IC_{50} = 344 nM (n=26).



QPlate '0046553500b717'

Used in job: #2334 - NMS_CHO hERG DUO IV

Start of use: 2007-08-09 12:26:14

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	1.83	2646.1	1132.3	1484	1
B1	✓	✓	✓	✓	1.81	1655.8	1178.5	1482	1
C1	✓	✓	✓	✓	1.87	4701.3	2177.4	1483	1
D1	✓	✓	✓	✓	1.82	10889.7	3343.9	1488	1
E1	✓	✓	✓	✓	1.84	3365.6	1373.5	1479	1
F1	✓	✓	✓	✓	1.83	6498.2	2509.5	1486	1
G1	✓	✓	✓	✓	1.79	1829.3	566.9	1480	1
H1	✓	✓	✓	✓	1.81	21657.5	1864.2	1485	1
A2	✓	✓	✓	✓	1.72	6323.6	2855.2	1486	1
B2	✓	✓	✓	✓	1.75	6343.6	2568.2	1496	1
C2	✓	✓	✓		1.75	4575.8	0.0	0	0
D2	✓	✓	✓	✓	1.75	7233.1	2294.4	1493	1
E2	✓	✓	✓	✓	1.75	174.2	308.0	1454	1
F2	✓	✓	✓	✓	1.74	241.2	376.1	1286	1
G2	✓	✓	✓	✓	1.80	4386.8	1051.9	1477	1
H2	✓	✓	✓	✓	1.79	2098.5	2256.4	1487	1
Total	16	16	16	15					15
Success rate	100 %	100 %	100 %	94 %					

Figure 13. Overview of a typical QPlate. The Qpatch assay software provides information on giga seals, whole cells and completed experiments. The cells used in this experiment were from passage 25.

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., contact Sophion Bioscience (info@sophion.dk).