SOPHION BIOSCIENCE

Application Report

8 hours unattended hERG run with ≥97% success rate and consistent pharmacology results

Qube 384 offers more than 8 hours unattended hERG screening with stable current amplitude and reliable pharmacology. Verapamil was tested on 11 plates over night with results that are easy to replicate.

Summary

- 8 hours of unattended hERG experiments, using Qube with the stacker
- Use of temperature control, keeping the QChip at 25˚C
- Success rates ≥97% for all plates
- Consistent pharmacology values over time
- Z' values >0.5 for all plates

Introduction

The cardiac ion channel encoded by KCNH2, also known as human ether-à-go-go-related gene (hERG) or K_V11.1 can lead to cardiac arrhythmia when blocked and is thus a major off-target in drug discovery. Mutations in this channel can lead to congenital long QT syndrome (LQTS), associated with irregular heartbeat, fainting and sudden cardiac arrest.

Automated patch clamp (APC) allows assessing the effects of compounds on the hERG channel in the preclinical development process, enabling an early evaluation in drug development. Unattended runs – like we show here on the Qube – are a crucial advantage by saving time and resources. We here report the results of an overnight run with CHO-hERG-Duo (B'SYS) cells at 25˚C, made possible by the Qube supplemented with stacker function and temperature control.

Results and discussion

hERG currents

Cells were clamped to a holding potential of -80 mV and potassium currents were evoked by application of a depolarization step to +20 mV for 4.5 s, followed by a 1 s step to -40 mV (Fig. 1).

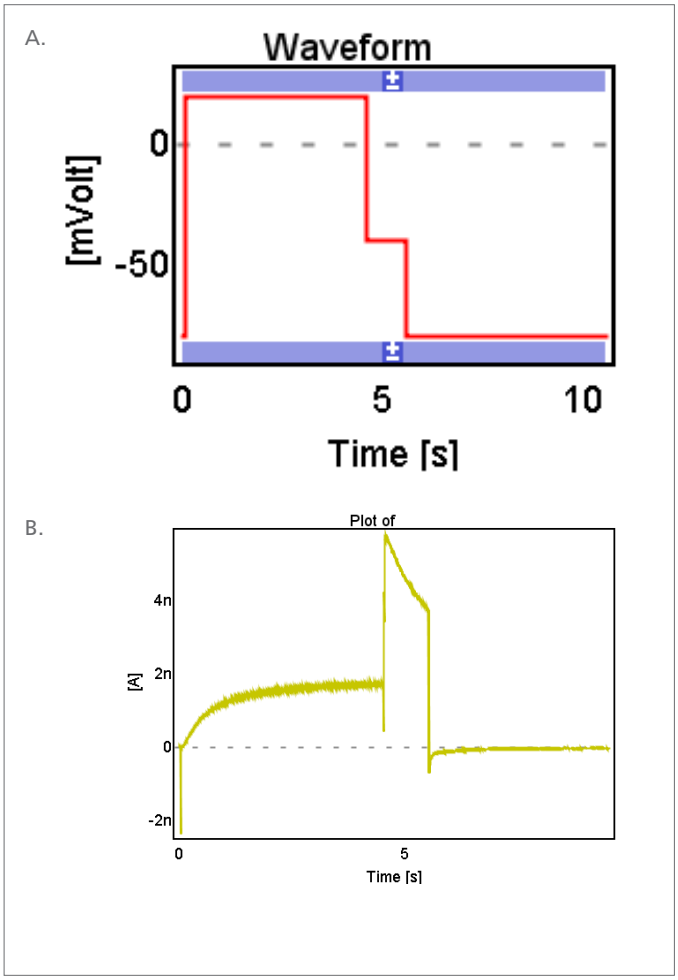


Fig. 1 A: Voltage protocol for hERG cells. After 50 ms at -80 mV, cells were depolarized for 4.5 seconds to +20 mV. The peak current is measured at the 1 second repolarization step to -40 mV, before a 5 second interval at the -80 mV holding potential. B: An example of the resulting current trace (raw data).

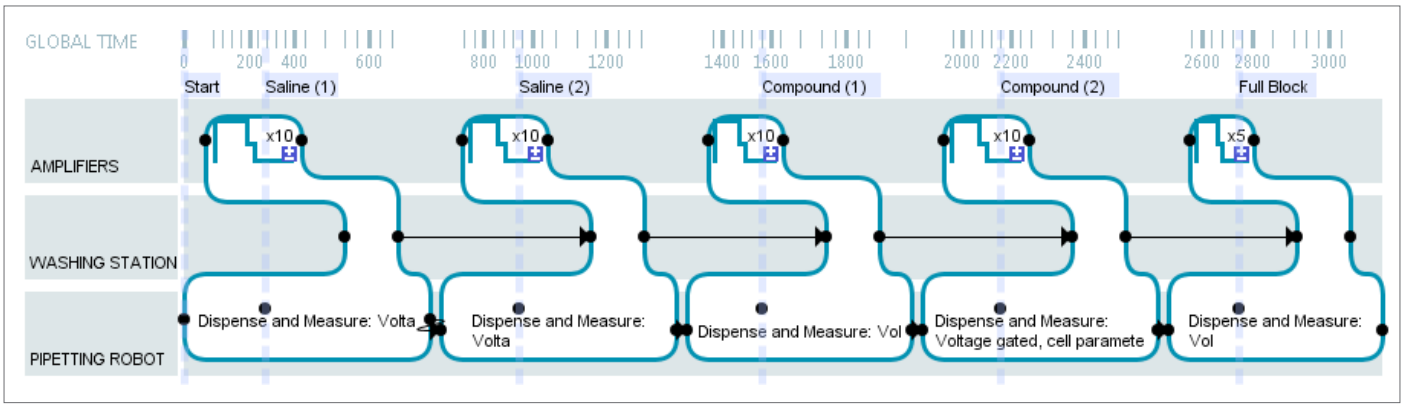


Fig. 2: Sophion ViewPoint software, depicting the liquid application protocol for the experiment: Two liquid applications of saline were followed by 2 liquid applications of a control or compound of the same concentration. The experiment was ended with a full block (1 µM cisapride) application.

The time between start of each voltage protocol execution was 25 seconds. 2 times 10 sweeps of baseline establishment in the presence of saline were followed by 2 times 10 sweeps in the presence of compound or saline, concluded by 5 sweeps at 1 µM cisapride (Fig. 2).

11 multi-hole QChips (10 patch holes/well) were run consecutively and unattended by a Qube with stacker and autofill reservoir.

Success rates and sealing

The success criteria were evaluated in the second saline period:

- >100 MΩ sealing resistance per cell
- >6.5 pF capacitance per cell
- >0.5 nA peak current amplitude per well

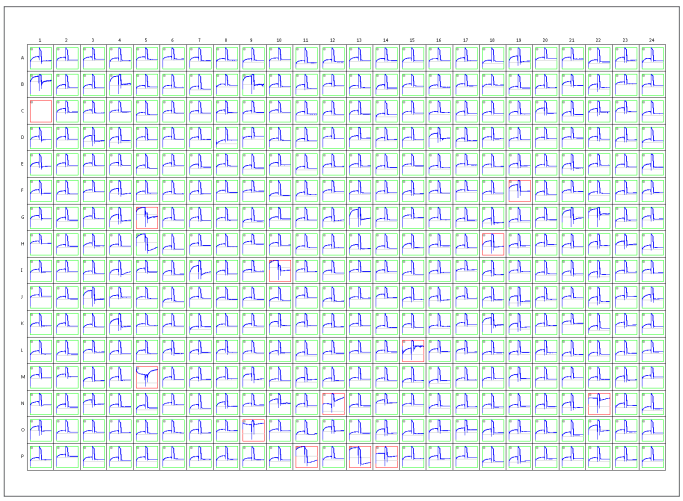


Fig. 3: Plate view of the 8th QChip (control condition).

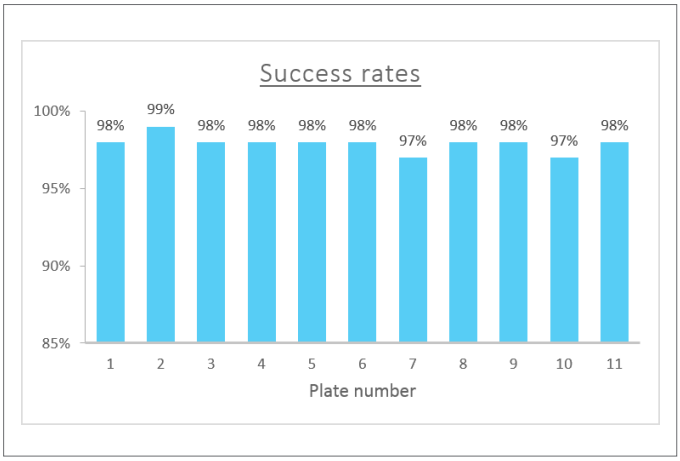


Fig. 4: Success rate over time of CHO-hERG-Duo assay.

The average resistance values for each QChip were stable over time and ranged between a minimum of 540±180 MΩ / cell and a maximum of 710± 220 MΩ / cell, with 95% of all tested wells having a sealing of >250 MΩ before compound addition. 99% of the latter maintained the sealing throughout the rest of the experiment. Overall, at least 97% of wells in each experiment fulfilled the defined success criteria (fig. 4).

Pharmacology and Z' values

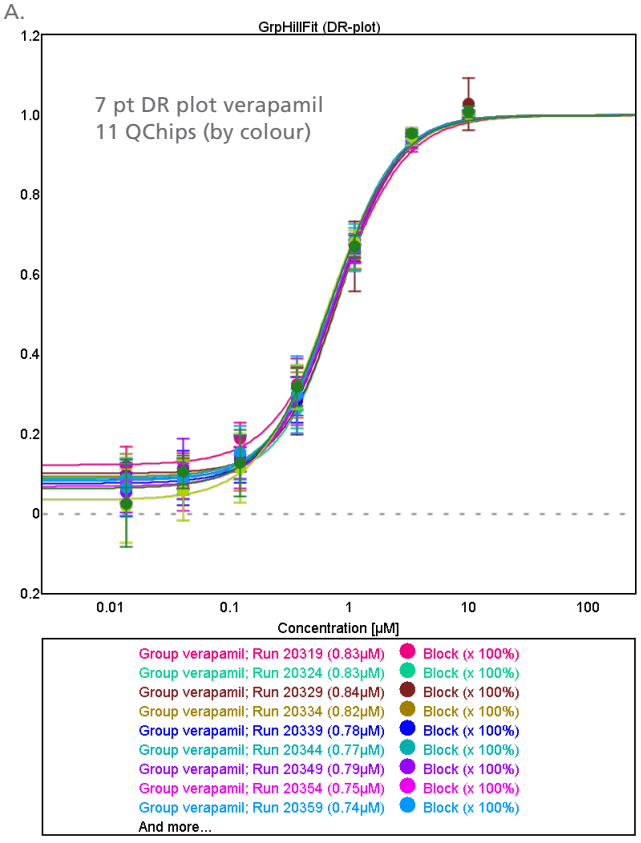
Verapamil was tested in a non-cumulative manner at 7 concentrations with a maximum of 10 µM (3-fold serial dilution). The drug effect was normalised before fitting the Hill equation.

Baseline = The average current amplitude of the last three depolarization steps of the liquid period "Saline (2)" (see Fig. 2).

Full block = The average current amplitude of the last three depolarization steps of the liquid period "Full Block" (1 µM cisapride) (see Fig. 2).

The normalised average current amplitude of the last three depolarization steps of the liquid period "Compound (2)" is shown in Fig. 5. The IC_{50} values were consistent over time and in agreement with literature values[1].

A.



B.

Experiment No.	IC_{50} verpamil	Z' value
1	0.83	0.84
2	0.83	0.86
3	0.84	0.81
4	0.82	0.75
5	0.78	0.83
6	0.77	0.84
7	0.79	0.725
8	0.75	0.67
9	0.74	0.81
10	0.67	0.57
11	0.71	0.67

Fig. 5 A: Dose-response relationship for verapamil of 11 multi-hole QChips. The data point values are mean ± SD. B: Table of verapamil IC_{50} values for each experiment, estimated by fitting the Hill equation to the data. Z' values for each experiment were calculated with 1 µM cisapride as positive control and 0.1% DMSO as negative control.

For Z' values, column 24 (16 wells) was used as a positive control (1 µM cisapride) and column one served as a negative control (0.1% DMSO). The Z' values were calculated as

$$Z' = 1 - \frac{3\chi(\sigma\rho+\sigma\eta)}{|\mu\rho-\mu\eta|}$$

With µ being the mean normalized current of the positive (µp) or the negative (µn) control and σ being the standard deviation of the positive (σρ) or the negative (ση) control.

Conclusion

Qube is an ideal platform if you want to run hERG for over 8 hours with stable data quality and results. Extremely reliable pharmacology results can be seen by the consistent Hill-curves for verapamil over the entire length of the experiment. Additionally, the stacker offers the possibility of completely unattended runs overnight and during the daytime. With Z' values consistently above 0.5, this assay overall proves to having an assay window suitable for high throughput screening on an ion channel target.

Materials and methods

Cell culture

Experiments in this study were performed on CHO-hERG Duo cells, which were kindly provided by B'SYS. CHO-hERG Duo cells were cultured and harvested according to Sophion standard procedures. The rate of cell growth in your laboratory can vary and cell numbers might therefore have to be adjusted to the individual rate of cell division. It is recommendable to thaw fresh cells when the current amplitude starts to decrease.

Cell and induction media were used according to the B'SYS CHO hERG Duo specification sheet. The cells were harvested in SFM with a density of 2 million cells/ml and transferred to the Qube where the cells were prepared for experiments using the automated cell preparation module.

Experimental setup

For worktable, cell preparation and clean-up, Qube default protocols were used.

Whole-cell protocol:
A four second suction pulse from -10 mbar to -200 mbar was applied. For more parameters, see Fig. 6.

Voltage protocol:
Cells were held at -80 mV holding potential and were depolarized for 4.8 s to +20 mV. The output of the following step for 1 s to -40 mV was used to determine the maximal current (Fig. 1 A).

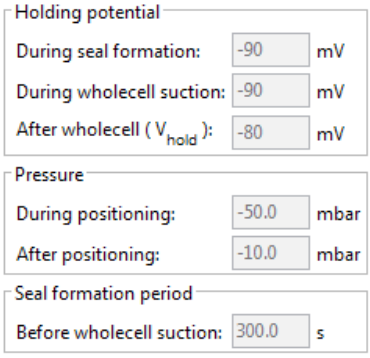


Fig. 6: Details of whole-cell formation protocol.

References:

1. Kirsch GE et al. 2004. Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J Pharmacol Toxicol Methods; 50(2):93-101.Quick, M. W., & Lester, R. A. J. (2002). Desensitization of neuronal nicotinic receptors. Journal of Neurobiology, 53(4), 457–478.

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