SOPHION BIOSCIENCE

Application Report

High throughput assay for K_V1.5 on Qube 384

A stable assay well-suited for a high throughput screening

Summary

- Biophysical characteristics are in line with literature values
- Constantly high success rates (>95%)
- Giga-ohm seal resistances in almost all wells
- Very low data spread
  - All CV values of DMSO control ≤12%
  - No false positives with cutoff criteria 3 X SD
  - Z' and Z > 0.7

Introduction

K_V1.5 channels conduct the ultra-rapid delayed rectifier current (I_{Kur}) that contributes to action potential repolarization of the heart. Mutations in the encoding gene (KCNA5) have been related to both atrial fibrillation and sudden cardiac death. K_V1.5 is also found in other tissues, such as vascular smooth muscle, CNS, microglia, and Schwann cells (Clarkson *et Al.*, 1998).

The pharmacological blockade of K_V1.5 has been suggested as a prevention and treatment of various arrhythmia (Karczewski *et Al.*, 2009) In the present study, we present an assay suitable for a high throughput screening campaign.

Results

A 384-well based, single concentration HTS assay was developed using the Qube 384 platform that allows for reproducible testing of many thousands of compounds per day. In the first step, the stability of the assay was determined. Figure 1 shows data from a representative multi-hole well (10 holes). Current stability was assessed for the steady-state current recorded at the end of the 1 s long stimulation pulse. Two additions of vehicle control were followed by a test period and the experiment finished with the application of 33 µM loratadine that served as a positive control (Figure 2).

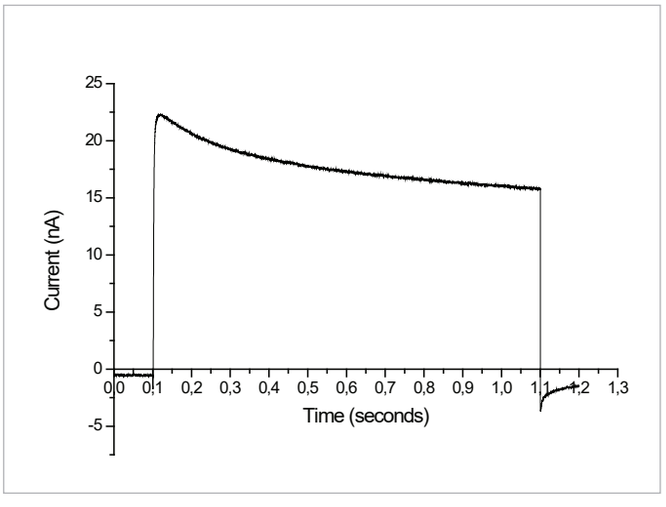

Fig. 1: Representative current trace from a multi-hole QChip (10 cells per well) that was elicited using a voltage step.

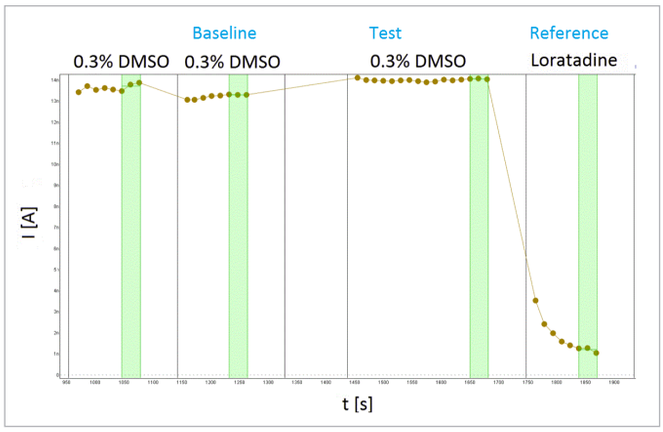

Fig. 2: Example current over time (It) plot of the steady-state current recorded during the last 50 ms of the voltage step to +20 mV. The second vehicle period was used as the baseline period. Compound effects were monitored using 16 sweeps with an intersweep interval of 15 s. 33 µM loratadine was applied at the end of the experiment as a positive control.

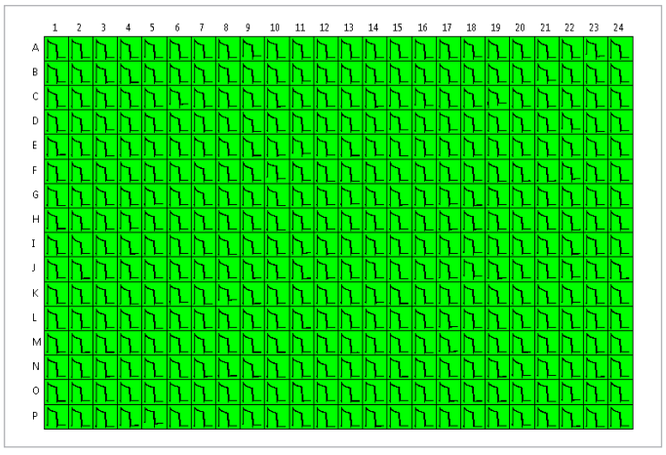

Fig. 3: Plate view of all 384 wells in the baseline period.

Assay stability was evaluated by calculating the current change between the baseline and the test period corrected for the reference period using the following equation.

$$\text{Inhibition} = \frac{I(\text{Test}) - I(\text{Baseline})}{I(\text{Reference}) - I(\text{Baseline})}$$

Equation 1

A plate view with respective inhibition values is shown in Figure 4. All green-shaded wells fall within the mean ± 3 x standard deviation (SD) range that is often used as a threshold value for identifying hits. No wells fell outside of this range, thus the assay did not show any false positives under this threshold. This is further illustrated in a histogram plot in Figure 5.

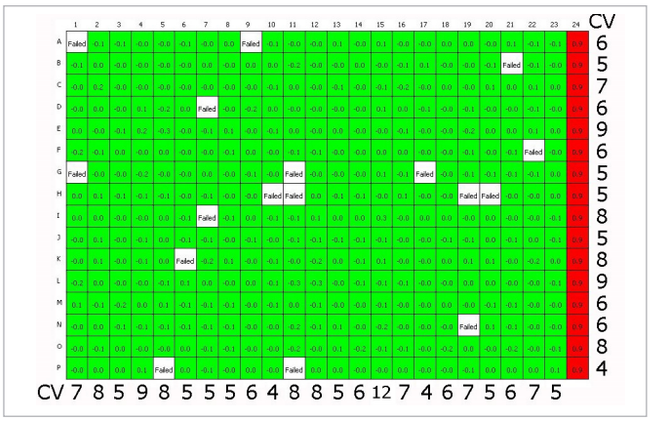

Fig. 4: Plate uniformity experiment. Steady-state current inhibition, calculated using Equation 1, are shown for each well. 0.3% DMSO was applied to column 1-23 and 33 µM loratadine to column 24. All white wells were failed by the filter criteria (R_{total} > 0.1 GΩ), all green wells lie within the mean ± 3 x SD and all red wells show an inhibition ≥ 89%. Coefficient of Variation (CV) for the individual columns are shown below the figure and values for the rows (loratadine excluded) on the right side of the figure. All CV values, except Col.15, lie below 10%.

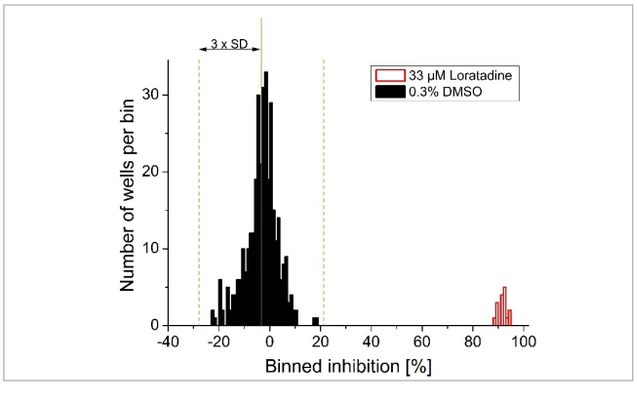

Fig. 5: Histogram plot of DMSO control % inhibition data (black) and 33 µM loratadine (red). The mean % inhibition of DMSO control is shown as a bold line and 3 x SD (25%) are indicated with dashed lines. All vehicle control data lie within 3 x SD.

In the next step, the channel was investigated biophysically using a step voltage protocol (Figure 10, right panel). Figure 6 shows a typical current response to the step protocol. The tail current at -40 mV (green cursor) was used to generate a steady-state activation curve (Figure 6, right panel). The mean $V_{½}$ of activation was calculated to $V_{½}$ =-16 ± 2 mV (n=369). This value is in good agreement with a value reported in the literature (Filipe *et Al.*, 2008).

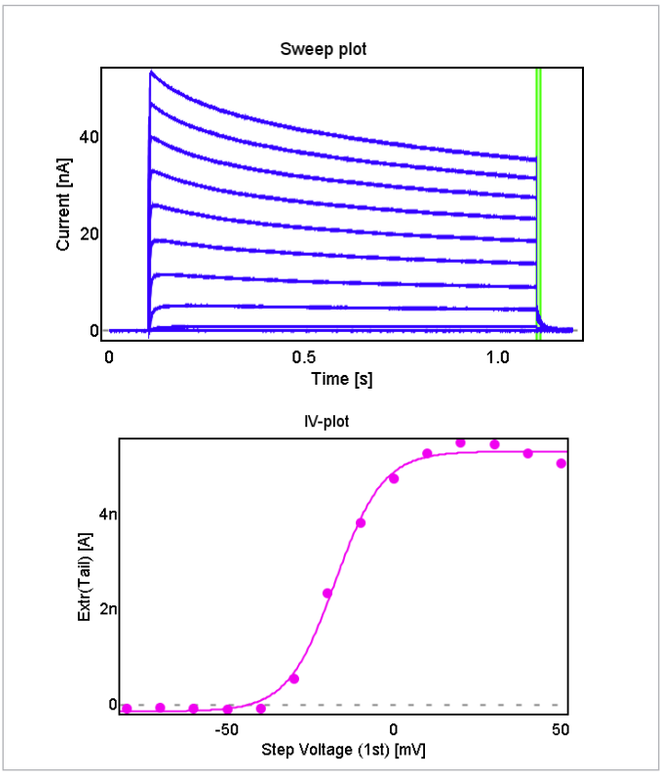

Fig. 6: Typical current traces (top) and Boltzmann fit to the tail current at -40 mV (bottom). The mean ± SD was $V_{½}$ =-16 ± 2 mV (n=369).

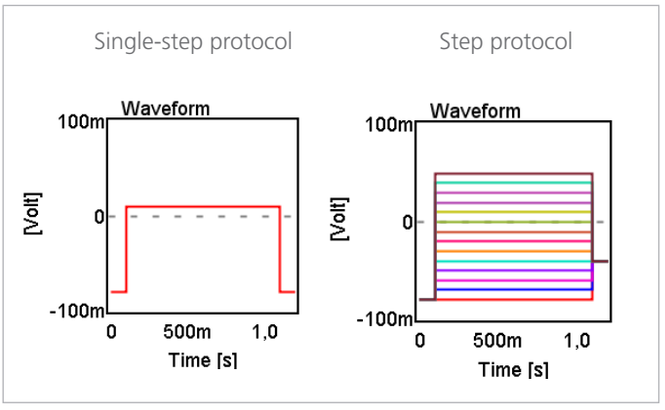

Fig. 7: Single-step voltage protocols used to assess compound effects (left). The inter sweep interval was 15 s. Right: Step protocol for biophysics characterization of K_V1.5. The inter sweep interval was 5 s.

Methods

A baseline current was recorded for 250 seconds, followed by a step protocol to characterize the cells biophysically. All data were recorded using multi-hole QChips.

- Cells
  - CHO-hK_V1.5 cells were kindly provided by B'SYS
- Electrophysiology
  - Automated patch clamp measurements were done on Qube 384 system from Sophion Bioscience.
  - The whole-cell configuration was gained using two negative pressure pulses of -250 and -350 mbar with each 1 s duration.
  - The experiment protocol consisted of a baseline period with 20 single voltage steps from -80 mV to +10 mV (Figure 10 left panel). After this, a step protocol was applied with 1 s long voltage steps from -80 mV to +50 mV in 10 mV increments (Figure 10 right panel). Next, compounds were added and 20 single step protocols were applied followed by a reference period of 8 single-step protocols.
- Data analysis
  - All data analysis was performed using Sophion`s Analyzer software, Origin7.5 (OriginLab) and Excel (Microsoft).

References:

1. Clarkson *et Al.*, 1998, Quinidine Interactions With Human Atrial Potassium Channels. Circ Res. 1998;83:1224-1231.
2. Filipe *et Al.*, 2008, K_V1.5 Association Modifies K_V1.3 Traffic and Membrane Localization. J Biol Chem. 2008 Mar 28; 283(13): 8756–8764.
3. Karczewski *et Al.*, 2009, High-throughput analysis of drug binding interactions for the human cardiac channel, Kv1.5. biochemical pharmacology 77 (2009) 177–185.

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