# Validation of $K_V7.X$ channel assays using the Qube 384 automated patch-clamp platform

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## 1 ABSTRACT

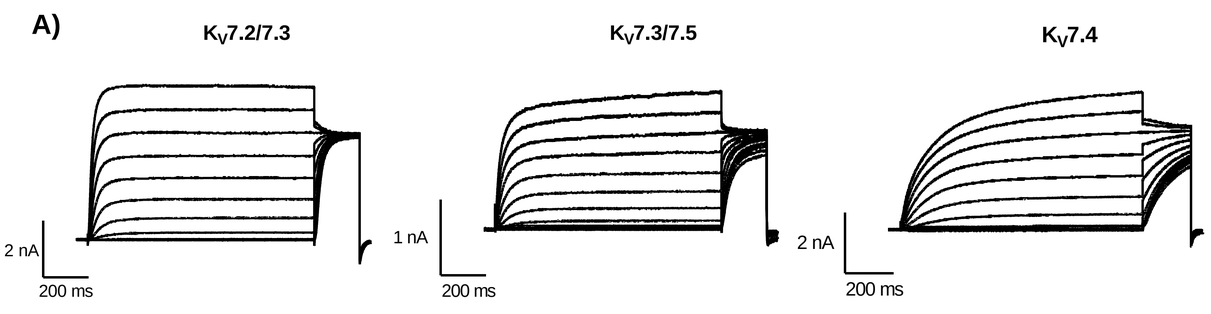
$K_V7$ (KCNQ) channels are voltage-gated $K^+$ channels with major roles in neurons, muscle cells and epithelia. The biophysical properties of $K_V7$ channels make them essential in controlling the activity of excitable cells[1]. They produce currents that are voltage-activated, slowly activating and non-inactivating, such as neuronal M current and cardiac I*Ks*[2]. These channels often work as 'excitability brakes'[3] and are targeted by various hormones and modulators to regulate cellular activity outputs. Genetic deficiencies in KCNQ genes result in human excitability disorders, including epilepsy, arrhythmias and deafness[4], making $K_V7$ channels an attractive target for the pharmaceutical research.
The aim of the present work was to demonstrate using the Sophion Qube 384-well APC platform for robust and reproducible $K_V7$ currents recordings, suitable for fast and reliable drug testing.

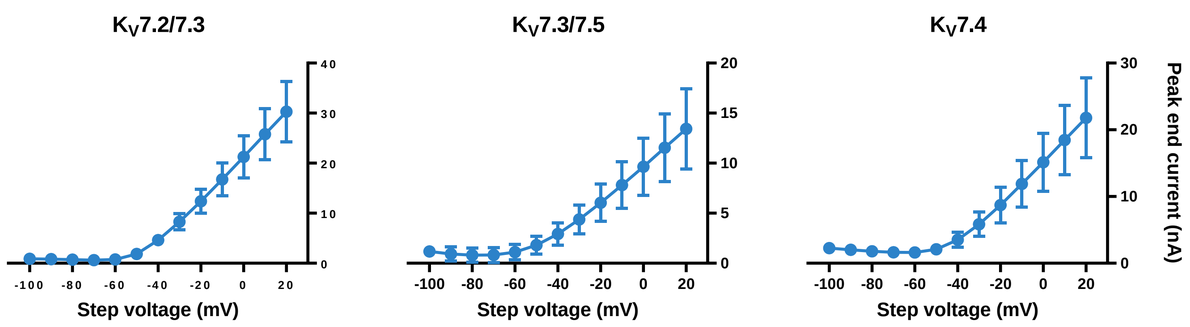
## 2 MATERIAL AND METHODS

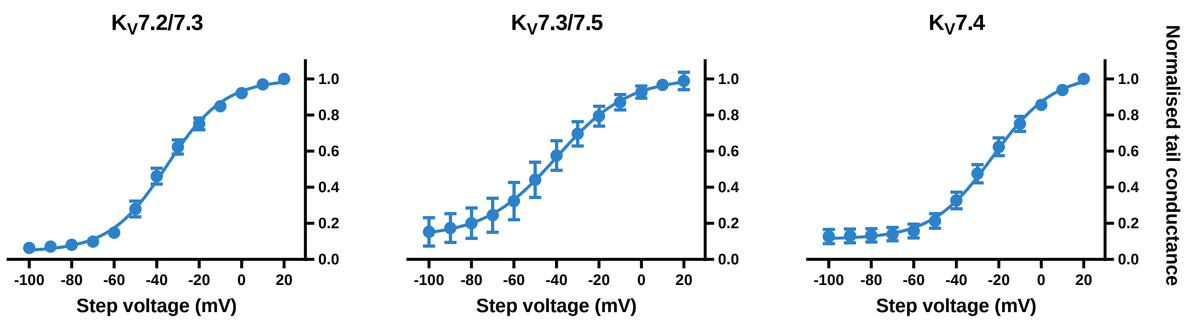
**Cell Culture:** $K_V7.2/7.3$, $K_V7.3/7.5$ and $K_V7.4$ cells were produced at Charles River Laboratories and are commercially available.
**Qube experiments:** Experiments were conducted using Multi-hole QChips on the Sophion Qube 384 platform, using EC000 and modified IC000 as prescribed by Sophion. Cells were held at -80 mV throughout the experiment. $K_V7.x$ currents were recording with 1 s voltage steps from -100 to +20 mV. Currents were sampled at 25 kHz, with a cut off at 5 kHz and Bessel filtering. QC filters used: RSeal > 3 or 4 MΩ, Capacitance > 20 pF, resting peak current > 1.5 or 3 nA.
**Analysis:** Data analysis was performed using Qube Analyzer and GraphPad Prism (9.0). Compound effects on the $V_{Half}$ of activation and current size at -40 and +20 mV voltage steps were normalised to vehicle application.

## 3 RESULTS

### 3.1 Biophysical properties of $K_V7$ cell lines on the Sophion Qube



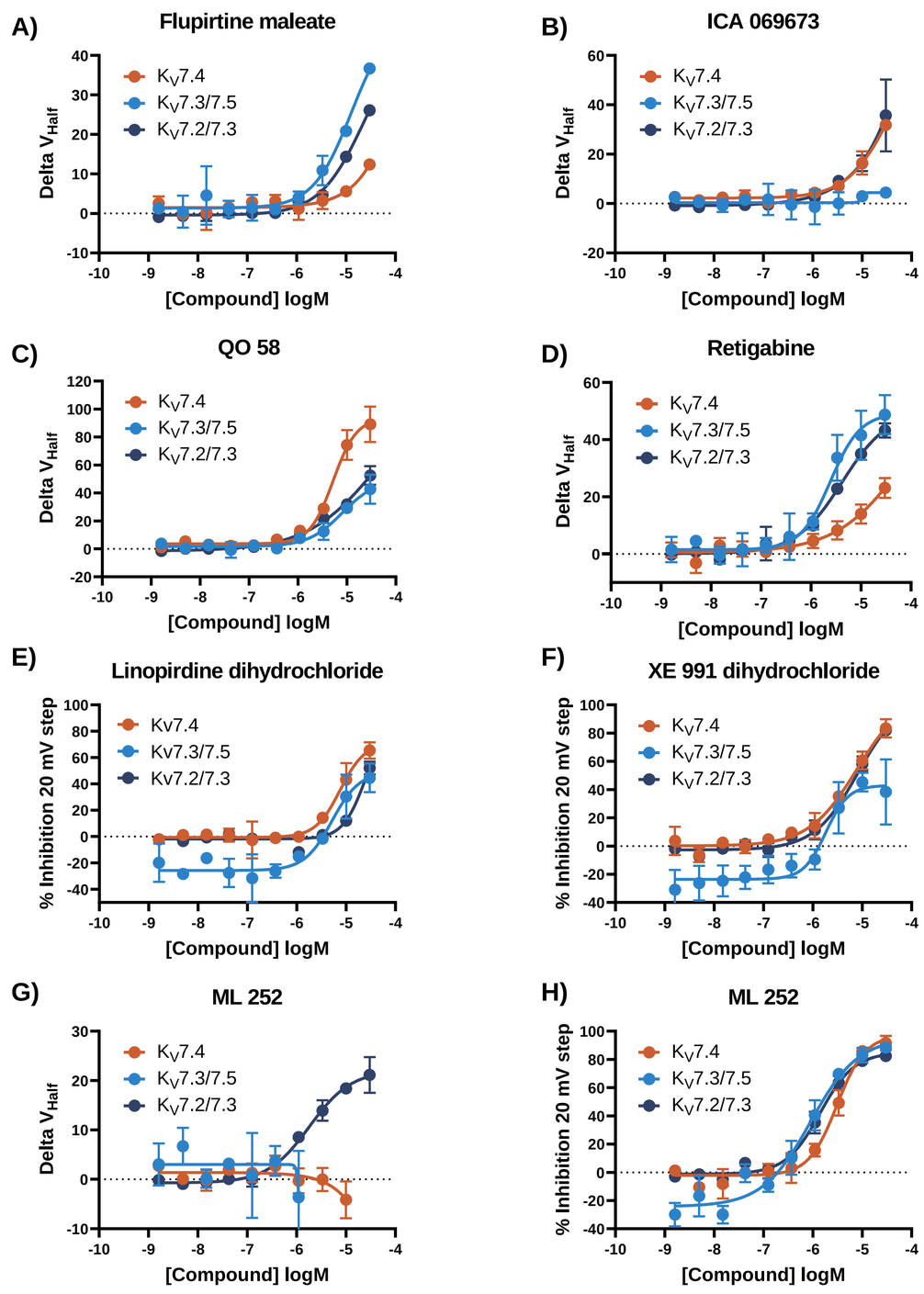




D)

| Plate statistics | $K_V7.2/7.3$ | $K_V7.3/7.5$ | $K_V7.4$ |
|---|---|---|---|
| Well QC pass rate (%) | 80.7 | 70.8 | 65.1 |
| Max end peak current (nA, mean ± SD) | 30.3 ± 6.0 | 13.4 ± 4.0 | 21.8 ± 6.0 |
| $V_{Half}$ of activation (mV) | -35.1 | -39.9 | -22.8 |

**Figure 1. Representative $K_V7$ currents, IV and normalised tail conductance activation curves, with plate statistics used for assay QC.** Representative current traces (A), current-voltage (IV, B) and normalised tail conductance activation curves (C) for $K_V7.2/7.3$, $K_V7.3/7.5$ and $K_V7.4$. Data are plotted as mean ± SD, n ≥ 250. Plate statistics used for assay quality control represented in (D).

### 3.2 $K_V7$ pharmacology of reference compounds




I)

| Channel subtype | $EC_{50}$ (µM) | Flupirtine maleate | ICA 069673 | QO 58 | Retigabine |
|---|---|---|---|---|---|
| $K_V7.2/7.3$ | Delta $V_{Half}$ | 23.4 | >30 | 4.00 | 4.17 |
| | -40 mV | 10.64 | 12.1 | 2.77 | 2.37 |
| $K_V7.3/7.5$ | Delta $V_{Half}$ | 13.0 | >30 | 9.68 | 2.44 |
| | -40 mV | 3.14 | >30 | 8.03 | 9.68 |
| $K_V7.4$ | Delta $V_{Half}$ | >30 | 24.6 | 5.23 | >30 |
| | -40 mV | >30 | 1.21 | 8.09 | >30 |

**Figure 2. $K_V7$ pharmacology data.**
Example graphs showing reference compound profiling in $K_V7$ assays, with concentration-response curves from delta $V_{Half}$ and % activation/inhibition at two step voltages (+20 and -40 mV). Reference activators flupirtine maleate (A), ICA 069673 (B), QO 58 (C) and retigabine (D), alongside reference inhibitors Linopirdine (E), XE 991 dihydrochloride (F) and ML 252 (G and H). Summary table showing $EC_{50}$/$IC_{50}$ for all compounds tested (I and J).

J)

| Channel subtype | $IC_{50}$ (µM) | Linopirdine | ML 252 | XE 991 |
|---|---|---|---|---|
| $K_V7.2/7.3$ | Delta $V_{Half}$ | >30 | 1.81 | 8.18 |
| | 20 mV | 25.2 | 1.40 | 8.13 |
| $K_V7.3/7.5$ | Delta $V_{Half}$ | >30 | >30 | >30 |
| | 20 mV | 5.16 | 0.89 | 1.83 |
| $K_V7.4$ | Delta $V_{Half}$ | 10.5 | >30 | 8.18 |
| | 20 mV | 7.61 | 3.08 | 7.89 |

## 4 SUMMARY

$K_V7.2/7.3$, $K_V7.3/7.5$ and $K_V7.4$ can be assessed by automated patch clamp on the Sophion Qube platform. Biophysical characterization shows robust current amplitudes and $V_{Half}$ of activation, which correspond to those reported in the published literature, with good assay performance.
Pharmacological tool compounds with different mechanisms of action, both activators and inhibitors, were profiled in concentration response curves. Some compounds show subtype selectivity, for example, QO 58 shows stronger activation of $K_V7.4$ compared to $K_V7.2/7.3$ and $K_V7.3/7.5$, shown in both delta $V_{Half}$ and % activation at the -40 mV step voltage. ML 252 shows strong inhibition of all $K_V7$ subtypes at the 20 mV step voltage, whilst only alters the $V_{Half}$ of activation in the $K_V7.2/7.3$ subtype.
Overall, these results demonstrate a robust screening methodology to detect compounds which inhibit and activate $K_V7.X$ channels through both alterations in the $V_{Half}$ of activation and current amplitude, using the Sophion Qube platform.

**Acknowledgement**
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**References**
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