# ENHANCING THROUGHPUT WITH MULTIPLE CELL LINES PER WELL WITH THE QPATCH HTX


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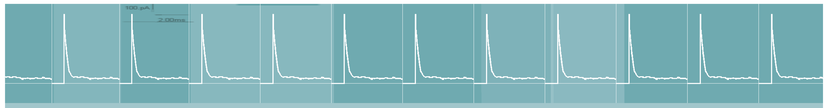


## INTRODUCTION

The QPatch HTX automated patch clamp technology was developed to 1) increase throughput in ion channel drug screening by parallel operation of 48 multi-hole patch clamp sites, each comprising 10 individual patch clamp holes, in a single measurements site on a QPlate X, and 2) diminish problems with low-expressing cell lines. Thus, parallel recording from 10 cells represents a 10-fold signal amplification, and it increases the success rate at each site substantially.

To further increase throughput we explored the possibility of simultaneous recording of a number of ion channel currents.

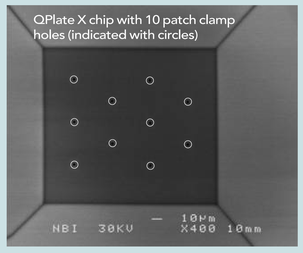
Two or three cell lines, each expressing a specific ion channel, were applied at each site simultaneously. The ion channel currents were separated temporally or pharmacologically by proper choices of voltage protocols or ion channel inhibitors. Using this strategy we were successful in recording currents from specific ion channel populations. This strategy, which ensured the exact same conditions for the cell lines with respect to Ringer solutions, temperature, pH and osmolarity, allowed a doubling or even tripling of the throughput.

We conducted a series of QPatch HTX experiments with a combination of ion channels involved in cardiac risk assessment: hERG, Kv1.5, KvLQT1/minK and Nav1.5. Specifically, tests were set up for recordings of 1) IV-relationships and concentration-responses for Kv1.5 and Nav1.5 in parallel by using multiple voltage protocols, and 2) pharmacological properties of specific blockers of hERG, KvLQT1/mink and Nav1.5 in parallel.

We present biophysical and pharmacological data obtained with QPatch HTX using multiple voltage protocols and cell lines in combination, and compare them to traditional single-hole data obtained with QPatch HT.

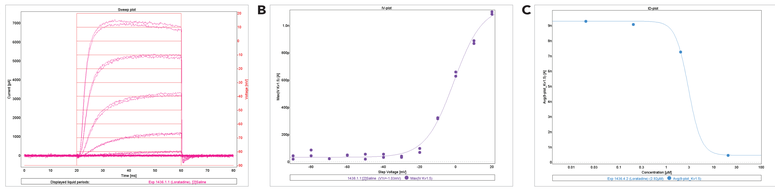
## MATERIALS AND METHODS

**Cells:** Cultured CHO cells expressing hERG, KvLQT1/minK, Nav1.5 (from B-Sys, Witterswil, Switzerland) and Kv1.5 (from STZ, Mannheim, Germany), respectively.
**Ringers:** Extracellular (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH=7.4 (NaOH), ~305 mOsm. Intracellular (In mM): 120 KF, 20 KCl, 10 HEPES, 10 EGTA, pH=7.2 (KOH), ~300 mOsm. In some cases we used an alternative intracellular Ringer: 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 120 KCl, 4 $Na_2$-ATP, pH=7.2 (w. KOH), 295 mOsm (w. sucrose). When this Ringer was used we corrected results for liquid junction potential (LJP).
**Compounds:** XE991 (Tocris, Bristol, UK), E-4031 (Sigma, Buchs, Switzerland) and Tetrodotoxin (Alomone Labs, Jerusalem, Israel) were applied in four concentrations from 0.1 – 100 µM in ten-fold increments. Loratadine (Sigma) was applied in four concentrations from 0.02 – 20 µM in ten-fold increments.
**Data analysis:** Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). IV-relationship for activation and concentration-dependent drug effects (Hill fit and $IC_{50}$) were accomplished with the QPatch Assay Software.




## QPATCH HTX MULTI-HOLE: TEMPORAL CURRENT SEPARATION BASED ON DISCRETE RECORDING TIME WINDOWS

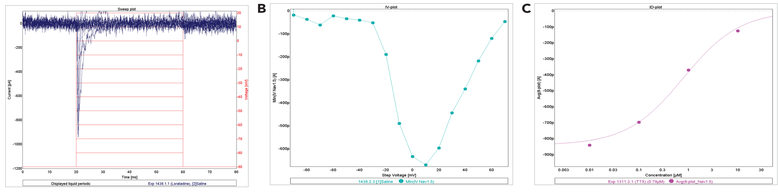
### Kv1.5 (*KCNA5*)




| | $V_{½}$ (mV) | | $IC_{50}$ (µM) loratadine | |
|---|---|---|---|---|
| | Measured | Literature | Measured | Literature |
| Kv1.5 | -11.4±3.1 (n=9) | -14 | 2.7±3.0 (n=4) | 1.2 (Ref. 1,4) |

Raw traces (A), I-V (B) and Hill plot (C) for loratadine. Currents recorded at t=100 msec after depolarization

### Nav1.5 (*SCN5A*)

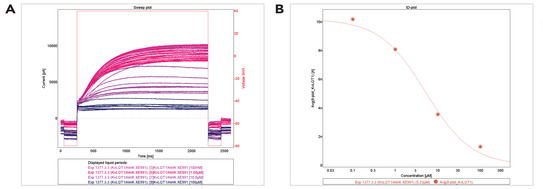



| | $V_{I_{max}}$ (mV) | $IC_{50}$ (µM) TTX | |
|---|---|---|---|
| | Measured | Measured | Literature |
| Nav1.5 | -9.8±2.2 (n=3) | 0.8±0.2 (n=3) | 1-2 (Ref. 2) |

Raw traces (A), I-V (B) and Hill plot (C) for TTX. Currents recorded at t=0.5 msec after depolarization

## QPATCH HTX MULTI-HOLE: PHARMACOLOGICAL SEPARATION BASED ON ION CHANNEL INHIBITORS

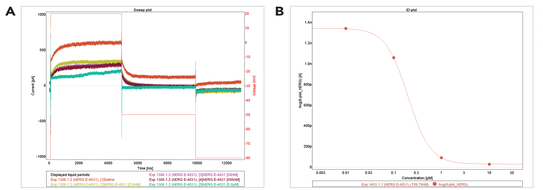
### KvLQT1/minK (*KCNQ1/KCNE1*)




Raw traces (A) and Hill plot (B) for XE991. hERG and Nav1.5 blocked by E-4031 and TTX, respectively.

| | $IC_{50}$ (µM) XE991 | |
|---|---|---|
| | Measured | Literature |
| KvLQT1 | 3.5±1.0 (n=12) | 1-6 (Ref. 5,6) |

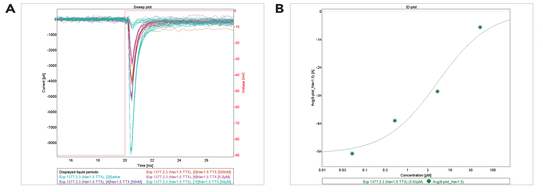
### hERG (*KCNH2*)




Raw traces (A) and Hill plot (B) for E-4031. KvLQT1 and Nav1.5 blocked by XE991 and TTX, respectively.

| | $IC_{50}$ (nM) E-4031 | |
|---|---|---|
| | Measured | Literature |
| hERG | 258±111 (n=4) | 18-200 (Ref. 1,3) |

### Nav1.5 (*SCN5A*)

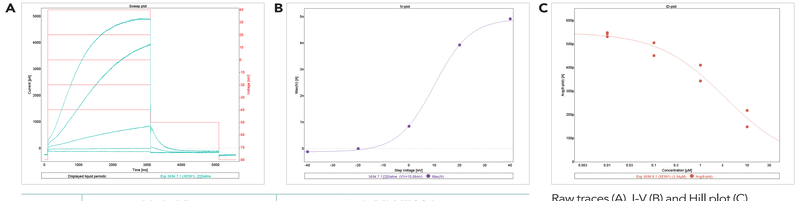



Raw traces (A) and Hill plot (B) for TTX. KvLQT1 and hERG blocked by XE991 and E-4031, respectively.

| | $IC_{50}$ (µM) TTX | |
|---|---|---|
| | Measured | Literature |
| Nav1.5 | 2.6±0.7 (n=9) | 1-2 (Ref. 2) |

## QPATCH HT SINGLE-HOLE: CONTROL

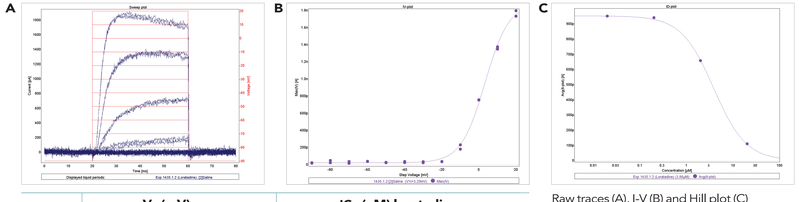
### KvLQT1/minK (*KCNQ1/KCNE1*)




| | $V_{½}$ (mV) | | $IC_{50}$ (µM) XE991 | |
|---|---|---|---|---|
| | Measured | Literature | Measured | Literature |
| KvLQT1 | 9.2±1.2 (n=5) | 8 | 5.1±2.6 (n=7) | 1-6 (Ref. 5,6) |

Raw traces (A), I-V (B) and Hill plot (C) for XE991.

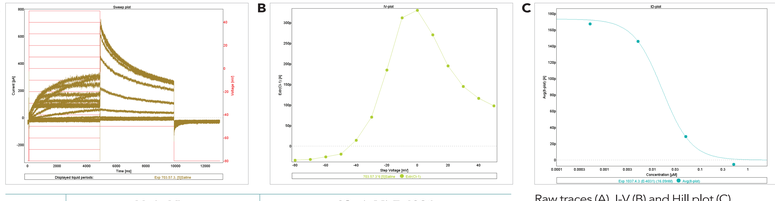
### Kv1.5 (*KCNA5*)




| | $V_{½}$ (mV) | | $IC_{50}$ (µM) loratadine | |
|---|---|---|---|---|
| | Measured | Literature | Measured | Literature |
| Kv1.5 | -11.1±0.7 (n=9) | -14 | 3.9±0.5 (n=6) | 1.2 (Ref. 1,4) |

Raw traces (A), I-V (B) and Hill plot (C) for loratadine.

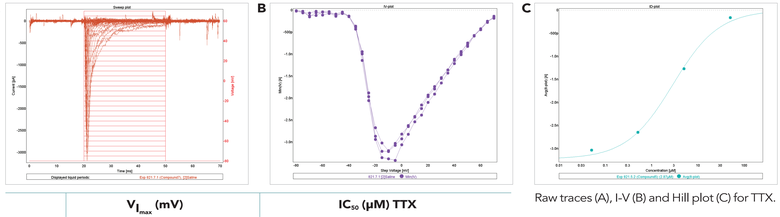
### hERG (*KCNH2*)




| | $V_{½}$ (mV) | | $IC_{50}$ (nM) E-4031 | |
|---|---|---|---|---|
| | Measured | Literature | Measured | Literature |
| hERG | -25.1±0.4 (n=39) | -26.2 | 4.4±0.3 (n=8) | 18-200 (Ref. 1,3) |

Raw traces (A), I-V (B) and Hill plot (C) for E-4031.

### Nav1.5 (*SCN5A*)




| | $V_{I_{max}}$ (mV) | $IC_{50}$ (µM) TTX | |
|---|---|---|---|
| | Measured | Measured | Literature |
| Nav1.5 | -13.2±5.7 (n=4) | 1.7±0.5 (n=7) | 1-2 (Ref. 2) |

Raw traces (A), I-V (B) and Hill plot (C) for TTX.

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## SUMMARY


The QPatch HTX technology has opened a variety of opportunities for exploring multiple cell lines expressing different ion channels on a single multi-hole chip unit. Provided the individual ion channel currents can be satisfactorily separated either temporally or pharmacologically, recordings from several ion channels can be obtained in the same experiment using appropriate voltage protocols.

We have demonstrated this capability by conducting simultaneous recordings on a mix of cell lines expressing various ion channels involved in cardiac risk assessment:

- Kv1.5 and Nav1.5 currents taking advantage of the fact that Nav1.5 is a fast voltage-gated ion channel (full acivation within 0.5 msec) and Kv1.5 is a slowly activating voltage-gated ion channel (full activation in the order of 100 msec)
- KvLQT1, hERG and Nav1.5 currents taking advantage of the fact that the three channels can be selectively blocked by specific inhibitors (XE991, E-4031 and TTX, respectively)

This strategy resulted in a doubling or even a tripling of the system throughput. Importantly, the biophysical and pharmacological data obtained on QPatch HTX were consistent with literature values and with values obtained on QPatch HT single-hole.

The QPatch HTX multi cell line approach may even prove more effective in targeting ligand-gated ion channels, because the high specificity of ligands (agonists and antagonists) ensures an excellent pharmacological current separation.