# EVALUATION OF THROUGHPUT AND DATA QUALITY ON THE QPATCH HTX

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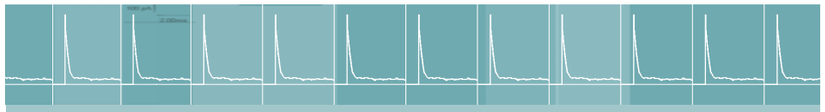


The QPatch HTX is the newest member of the QPatch family of high-end fully automated patch clamp systems from Sophion Bioscience. The QPatch HTX builds on the proven technology from the QPatch 16 and the QPatch HT, where cells are patched on a silicon chip with integrated microfluidic flow channels made in glass. The measurement plate, called a QPlate, allows true gigaseals to be formed and high quality data to be recorded.

For the QPatch HTX modifications have been made to the QPlate and to the amplifier. To diminish problems with low-expressing cell lines, the QPlate HTX contains multiple holes per recording site and to accommodate the increased current amplitudes, the amplifier has been built with a broader current range.

In this study we present data obtained during the assay validation and optimization of a high throughput screening assay using a HEK293 cell line expressing the voltage gated sodium channel Nav1.2a. This stable cell line is characterized by low functional expression of the Nav1.2a channel with approximately 30% expressing cells in the population resulting in a relatively low success rate in data recording on the QPatch HT. We found that with the QPlate HTX the success rate could be dramatically improved. After assay optimization on the QPatch HTX the effective success rate approaches 100%. We present data to compare throughput and assay quality between the QPatch HTX in multi hole mode and traditional single cell patch clamp on the QPatch HT.

Furthermore we have evaluated the compound profiling properties of the QPatch HTX by characterizing known blocker against the Nav1.2a channel - a challenging channel for electrophysiological recording due to the extremely fast current activation time constants elicited upon voltage stimulation. Surprisingly, our biophysical and pharmacological data suggest that the data quality obtained on the QPatch HTX is overall comparable to equivalent data obtained on the QPatch HT.

## MATERIALS AND METHODS

**Cells:** HEK293 cells stably expressing Nav1.2a. (generously supplied by Neurosearch).

**Ringer's solutions:** Intracellular Ringer (in mM): 135 CsF, 1/5 EGTA/CsOH, 10 HEPES, 10 NaCl. Osmolarity adjusted to approximately 320 mOsm with sucrose, pH 7.4.

Extracellular Ringer (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 TEA-Cl, 10 Glucose. Osmolarity adjusted to approximately 320 mOsm with sucrose, pH 7.4

**Compound:** Tetrodotoxin (Alomone Labs, Jerusalem, Israel).

**Voltage protocols:** For all concentration-response experiments a depolarization to +20 mV for 10 msec from a holding potential of -90 mV was used (Figure 1). For IV relationships a voltage protocol with incremental steps of +10 mV from -120 to +70 mV of 1000 ms duration was used (Figure 2).

**Data analysis:** Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). IV-relationships for current activation/inactivation as well as concentration-dependent drug effects (Hill fit and $IC_{50}$) were analyzed using the QPatch Assay Software.

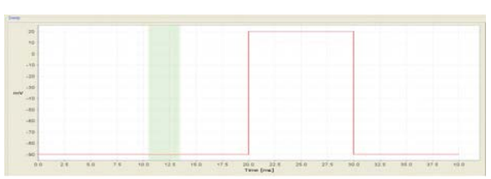


**Figure 1.** Voltage protocol for concentration-response experiments

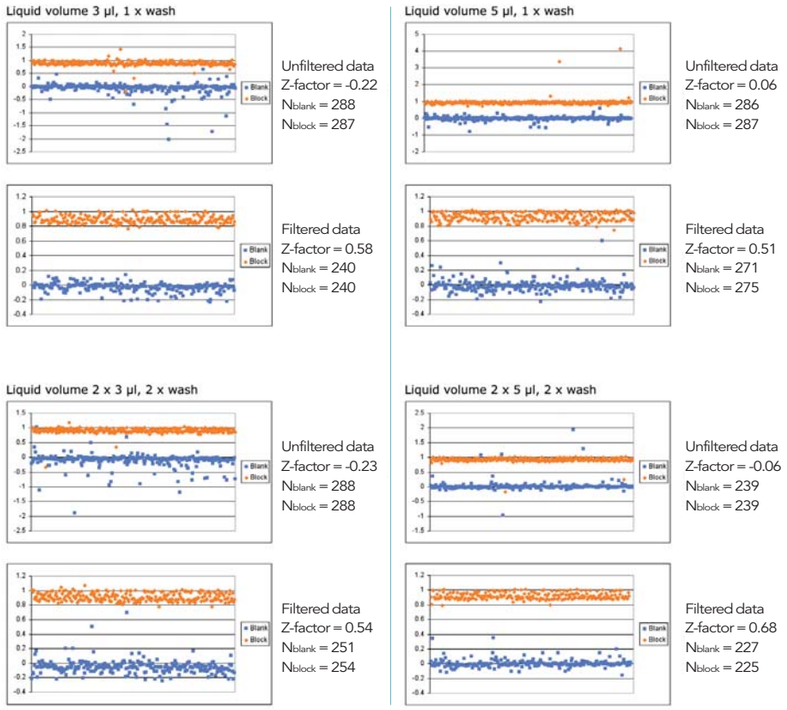
**Figure 2.** Voltage protocol for determining IV-relationship

$$\text{Z-factor} = 1 - \frac{(3SD_{BLANK} + 3SD_{BLOCK})}{Avg_{BLANK} + Avg_{BLOCK}}$$

| Z-factor | Interpretation |
|---|---|
| Z = 1.0 | Ideal |
| 0.5 < Z < 1 | An excellent assay |
| 0 < Z < 0.5 | A marginal assay |
| Z < 0 | The signal from the positive control and the negative controls overlap, making the assay unsuitable for screening purposes |

**Table 1.** Z-factor values and the corresponding assay quality interpretation. The Z-factor is a measure of the suitability of a particular assay for use in high-throughput screening. The Z-factor is calculated from results of a screening assay with negative (=blank) and positive (=block) stimulation multiple times. The Z-factor calculation uses four parameters; average values and standard deviations of both positive and negative controls.

**Figure 3.** Assay optimization and Z-factor the QPatch HTX was used for HEK293-Nav1.2a screening assay validation and optimization. TTX (100nM) was used as positive control (block) and saline as negative control (blank). Four different assays were performed testing different test parameters: 1) compound volume 2) single compound application 3) double compound application 4) single or double wash. Effects of the compounds were normalized to the baseline current level. Different filter types were used e.g to exclude experiments with small initial current and experiments with unstable current. The assay software automatically excluded the experiments failed by the filters.
To automatically remove non-biological artifacts we post-experimentally applied user-determined filters. Z-factor values were calculated before (top) and after (below) filtering of the data.



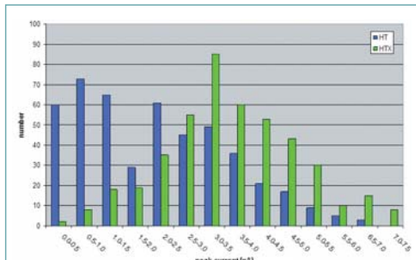




**Figure 4.** Histogram showing peak current distribution from the QPatch HTX and the QPatch HT. Peak current was measured from stimulation of HEK293-Nav1.2 at +10 mV using either the QPatch HT single hole measurements (blue bars) or the QPatch HTX multi hole measurements (green bars). The figure shows the distribution of the peak current (QPatch HTX, n=473; QPatch HT, n=473).

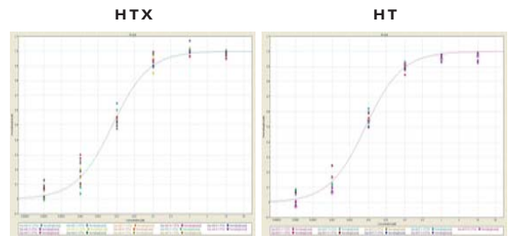



**Figure 5.** Concentration-response characteristics of HEK293-Nav1.2a. Left side panel shows Hill fits based on the current at the steady-state compound response for several cells (group Hill fit) for HEK293-Nav1.2a tested on the QPatch HTX (N = 14). The corresponding group Hill fit measured on the QPatch HT is shown on the right (N = 9). TTX was added in increasing concentrations (0.1 – 10.000) nM.

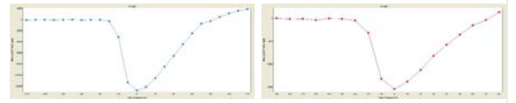


**Figure 6.** Representative IV-plots of HEK293-Nav1.2a. Left side panel shows IV plot of HEK293-Nav1.2a recorded on the QPatch HTX. Right side panel shows IV plot of HEK293-Nav1.2a recorded on the QPatch HT.

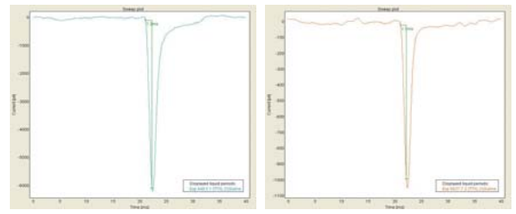


**Figure 7.** HEK293-Nav1.2a raw data currents from the QPatch HTX and the QPatch HT. Raw data current sweeps recorded from saline periods. Left side panel data from the QPatch HTX. Right side panel data from the QPatch HT. The current time to peak was calculated using 2-98% of the extreme current value within a defined cursor interval (the cursor interval is not shown).

**Table 2.** Biophysical characteristics of HEK293-Nav1.2a obtained from the QPatch HTX and the QPatch HT. The results from the average maximal current ($I_{max}$) and the voltage for $I_{max}$ ($V_{Imax}$) were obtained from stimulation with the voltage step protocol. Data were not corrected for liquid junction potential.

| | HTX | HT |
|---|---|---|
| Sites with current [%] | 93.7 | 40.6 |
| TTX $IC_{50}$ [nM] | 7.6 ± 0.6 | 8.3 ± 0.6 |
| $I_{max}$ [nA] | -10.9 ± 0.1 | -1.4 ±0.8 |
| $V_{Imax}$ [mV] | 3.7 ± 0.7 | -0.6 ± 2.4 |
| Time to peak (2-98%) [ms] | 1.3 ± 0.07 | 1.1 ± 0.03 |
| $V_{0.5activation}$ [mV] | -29±6.0 | -31±3.0 |
| $V_{0.5inactivation}$ [mV] | -55±7.7 | -62±2.1 |

## CONCLUSION

In statistics, the Z-factor is a measure of the quality of a high-throughput assay. During assay optimization of HEK293-Nav1.2a on the QPatch HTX platform we focused on obtaining a high Z-factor while maintaining a cost-efficient assay.

In an experimental setup with 288 blockers of the Nav1.2a channel, we varied application parameters to find the ideal assay set-up with the lowest discard rate. To automatically remove non-biological artifacts we post-experimentally applied user-determined filter functionalities in the QPatch Assay Software. As a result we were able to develop a highly reliable high-throughput assay with a Z-factor near 0.7 and a discard rate at only 5% using an assay set-up with double compound application and double wash. Compared to the results obtained on the QPatch HT with this particular HEK293-Nav1.2a cell line, QPatch HTX gives more than a two-fold increase in overall success rate.

As the QPatch HTX builds on the multi-hole technology, we wanted to compare the biophysical properties of the Nav1.2a receptor obtained on the QPatch HTX versus traditional single hole recordings obtained on the QPatch HT. For the tested parameters we found a very high correlation.

In conclusion these studies clearly demonstrate that the assay established for Nav1.2a on the QPatch HTX has an overall quality that makes it suitable for a cost-efficient high-throughput screening campaign.