

## Activation and Inhibition of human ASIC3 Channels on Nanion's SyncroPatch 384PE

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### Summary

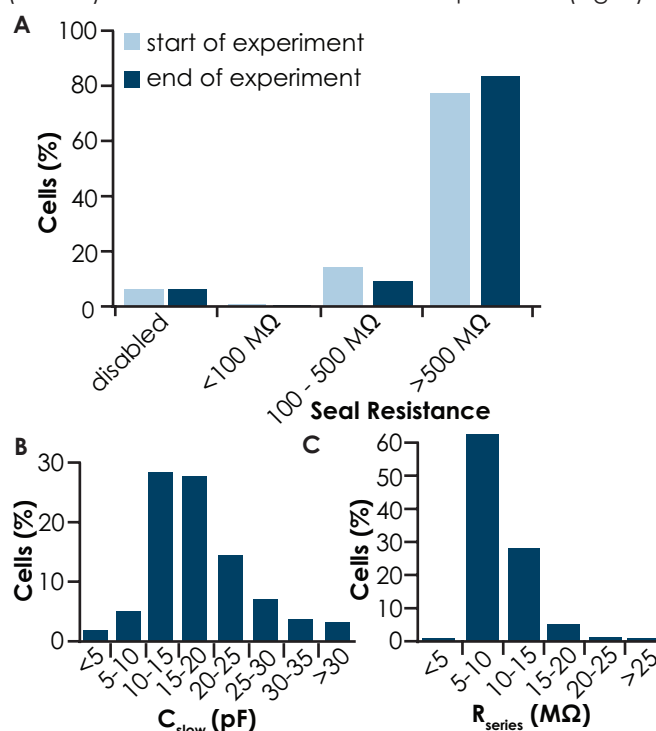
Acid-sensing ion channels (ASICs) are ligand-gated ion channels activated by protons. They are members of the sodium-selective cation channels belonging to the epithelial sodium channel/degenerin (ENaC/DEG) family<sup>1</sup>. ASICs are highly sensitive to extracellular acidosis and are permeable to cations<sup>2</sup>, predominantly Na<sup>+</sup>.

So far, 6 different ASIC subunits have been identified encoded by 4 genes<sup>3</sup>. They are found expressed throughout the CNS and PNS and have a proposed role in nociception and pain, and other neurological diseases such as ischaemia and inflammation<sup>4</sup>. The ASIC3 channel was first identified in the late 1990's<sup>5</sup>. It was found to be localized to primary afferent nociceptive fibers innervating the skin, muscles, joints and viscera, in agreement with a role in pain perception<sup>4</sup>. Furthermore, ASIC3 is expressed in higher amounts in nociceptive neurons innervating muscle (~ 50%) compared to skin (~ 10%), which indicates that ASIC3 may play an important role in detecting muscle acidosis<sup>4</sup>.

Here we present high quality data at a high throughput collected on the SyncroPatch 384PE showing activation and inhibition of ASIC3 expressed in HEK cells. The pH which elicited a half-maximal response was in good agreement with the literature<sup>3,6</sup>. The IC<sub>50</sub> for block of the ASIC3 current by amiloride, a known blocker of ASIC and ENaC channels, was also in good agreement with the literature<sup>2,6,7</sup>. Success rates of over 80% for completed experiments were recorded.

### Results

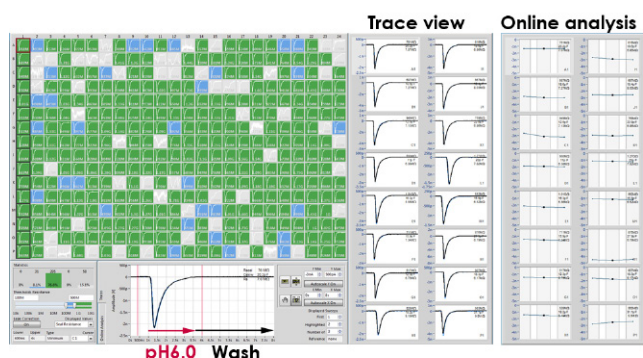
For the evaluation of the performance of HEK-ASIC3 cells, Seal Resistance, C<sub>slow</sub> and Series Resistance (R<sub>series</sub>) were determined from one experiment (Fig. 1).



**Figure 1: Statistics of ASIC3 expressing HEK293 cells recorded on one NPC-384 chip on the SyncroPatch 384PE.** Success rate (seal resistance) of individual HEK cells on the SyncroPatch® 384. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B** Bar graph of cell capacitance (C<sub>slow</sub>) and **C** Series Resistance (R<sub>series</sub>) values for HEK293 cells expressing ASIC3.

# Application Note

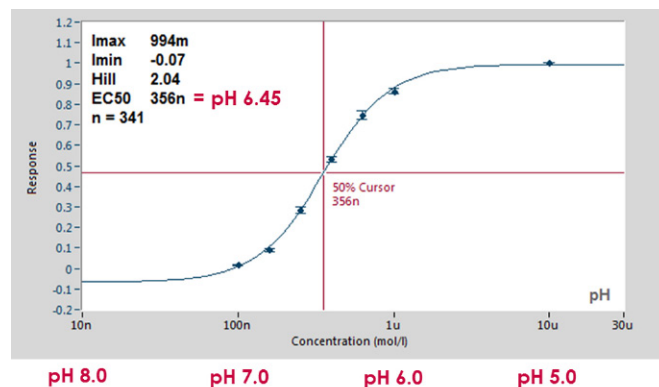
Currents mediated by ASIC3 could be reliably recorded on the SyncroPatch 384PE with a high success rate. Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch® 384PE during an experiment recording the current response to 3 applications of pH 6.0 solution. In this experiment, 76.8% of cells had a seal resistance > 500 MΩ with a further 8.1% with a seal resistance > 100 MΩ.



**Figure 2: Typical recording from ASIC3 expressed in HEK cells on the SyncroPatch 384PE.** The screenshot shows the data acquisition and analysis software used on the SyncroPatch 384PE. Three hundred and eighty-four small color-coded pictures shown in the upper left part from A1 to P24 display 384 recordings. Wells are color-coded based on seal resistance (green: Rmemb > 500 MΩ, blue: Rmemb = 100 - 500 MΩ, light blue or grey: Rmemb < 100 MΩ or disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. On the far right, the online analysis view showing peak current amplitude is provided. Vertical lines and blue shaded area show application of low pH solution and white shows incubation in wash solution. The experiment shows current activation in response to acidified solution at pH 6.0. This step was repeated 3 times (with wash between applications) to show reproducibility of the signal. Holding potential was -60 mV.

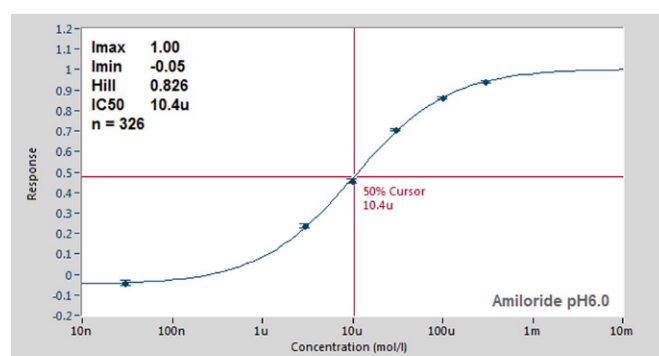
ASIC3 could be reliably and reproducibly recorded on the SyncroPatch 384PE as shown in Figure 2. The current responses to multiple applications of pH 6.0 solution could be exactly overlaid.

Figure 3 shows the response curve to decreasing pH. Cells were activated by pH 5.0 to obtain maximum peak amplitude and to check reproducibility of the signal. Following this, the test pH was applied and this was repeated 3 times with washout between each addition. The responses were normalised to maximum current elicited by pH 5.0 and the pH response curve calculated across the whole plate. The pH which elicited the half-maximal current,  $pH_{0.5}$ , was  $pH 6.45 \pm 0.08$  ( $n = 341$ ), in excellent agreement with the literature value of pH 6.4 - 6.6<sup>3,6</sup>.



**Figure 3: Activation of ASIC3 by decreasing pH.** Currents were normalised to the maximum peak amplitude elicited by pH 5.0 and the pH response curve calculated across the whole plate. The curve was fitted with a Hill equation and the  $pH_{0.5}$  calculated to be  $pH 6.45 \pm 0.08$  ( $n = 341$ ), in excellent agreement with the literature<sup>3,6</sup>.

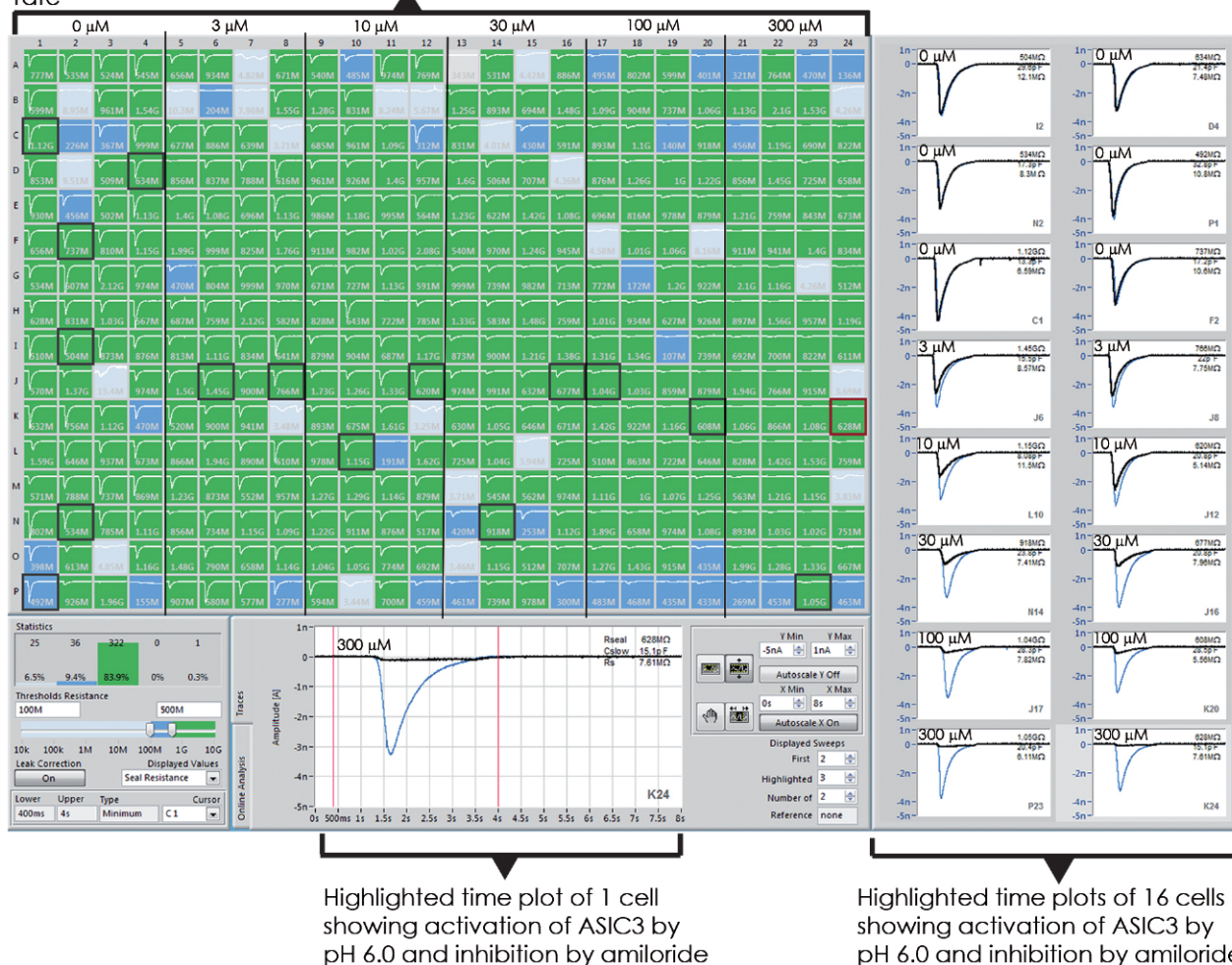
Amiloride is a potassium-sparing diuretic used to treat hypertension and congestive heart failure. It is a known blocker of ASICs and ENaC, acting as a pore-blocker of ASICs<sup>6</sup>. Figure 4 shows the concentration response curve for amiloride block of ASIC3. ASIC3-mediated currents were activated using pH 6.0 solution and the concentration response curve was calculated across the whole plate (as shown in Figure 5). The  $IC_{50}$  for amiloride block of ASIC3 was  $10.4 \pm 0.1 \mu M$  ( $n = 326$ ) in excellent agreement with the literature ( $16 \mu M^2$ ;  $5 - 100 \mu M^6$ ;  $60 \mu M^7$ ).



**Figure 4: Average concentration response curve for amiloride block of ASIC3.** The concentration response curve was constructed across the whole plate as shown in Figure 5. The SyncroPatch 384PE analysis software (DataControl 384) was used to calculate the average concentration response curve, normalized to maximum block and fitted with a standard Hill-equation. The  $IC_{50}$  for amiloride block of ASIC3 was  $10.4 \pm 0.1 \mu M$  ( $n = 326$ ), in excellent agreement with the literature<sup>2,6,7</sup>.

# Application Note

384 color coded depictions of data traces eases judgement of success rate



**Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE.** Screenshot of depiction of raw data traces of ASIC3-expressing HEK cells as recorded on one NPC-384 patch clamp chip. A single compound (in this case amiloride) was applied to individual wells at varying concentrations (including control wells) across the plate. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green ( $R_{\text{memb}} > 500 \text{ M}\Omega$ ), blue ( $R_{\text{memb}} = 100 - 500 \text{ M}\Omega$ ), light blue or grey ( $R_{\text{memb}} < 100 \text{ M}\Omega$  or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show raw data traces of ASIC3 channels following activation by pH 6.0 (blue trace) and inhibition by amiloride at the concentrations indicated (black trace).



**Figure 6: Timeline of an experiment on the SyncroPatch 384PE.** The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single point concentration response curve on ASIC3-mediated currents took approximately 17-19 min.

# Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, raw traces are chosen and the graphs show control responses to pH 6.0 (blue) and current responses in the presence of amiloride at the concentrations indicated (black). An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, ASIC3 expressed in HEK cells can be recorded on the SyncroPatch 384PE with high success rates for completed experiments (typically >80%). The timeline of each experiment was about 17-19 minutes (start – end) and included 3 control applications with pH solution followed by pre-incubation with blocker and activation of currents in the presence of 1 concentration of blocker. The pH response curve for ASIC3 recorded

on the SyncroPatch 384PE is in good agreement with the literature<sup>3,6</sup>. The  $IC_{50}$  for amiloride block of ASIC3-mediated currents calculated using the SyncroPatch 384PE's analysis software, DataControl 384, was in excellent agreement with the literature<sup>2,6,7</sup>.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording ASIC3 currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

## References

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## Methods

### Cells

HEK293 cells expressing hASIC3.

### Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

### Electrophysiology

Perforated patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384PE using an internal solution containing 25  $\mu$ M Escin. Cells were held at -60 mV for the duration of the experiment. To calculate the pH response curve, cells were activated 3 times with pH 5.0 to monitor reproducibility of the signal and to elicit maximum peak amplitude. Three test pH solutions were applied subsequently to each cell with washout between additions. The responses were normalised to the maximum response (pH 5.0) and the pH response curve calculated across the whole plate. Amiloride was pre-incubated in pH 8.0 solution for 150 s prior to co-application at pH 6.0.