SOPHION BIOSCIENCE

Application Report

Stable response for ligand- and voltage-gated ion channels when tested on QPatch

Glu5R, GluR6 (GRIK2), ASIC3, $Na_V1.2a$, and hERG provide consistent currency signal before and after antagonist or blocker application

Summary

Confidence in the stability of your ion channel signal is an important starting point for any patch clamper.

This study shows data on several ligand- and voltage-gated ion channels recorded on QPatch. The data shows that assays on QPatch are flexible and easy to design such that a stable current response can always be obtained easily.

Introduction

The aim of this study is to observe the consistency of a current signal (elicited by an agonist or a voltage change) before and after antagonist or blocker application. This report presents data monitored on QPatch based on whole-cell current recordings from ligand-gated ion channels (LGIC); the glutamate-activated ligand-gated ion channels Glu5R expressed in HEK293 cells from Neurosearch and GluR6 (GRIK2) from Millipore as well as acid-sensing ion channel 3 (ASIC3) expressed in HEK293 cells. Similar experiments were performed on voltage-gated ion channels (VGIC); HEK293-$Na_V1.2a$ and CHO-hERG.

Pre- and post-response consistency

Tests were performed on ligand-gated ion channels in order to characterize the consistency of the receptor signal before and after antagonist application. The corresponding assays were set up for the voltage-gated ion channels with several runs of the voltage protocol before, during and after application of the blocker. The idea is then to compare the response level before and after antagonist/blocker was added to confirm that the signal level is consistent in the pre- and post-period.

Results

HEK-293-GluR5

In order to test the reproducibility of the response of GluR5, currents were elicited by 2 x 3 repeated additions of 100 µM kainate interspersed by an antagonist application of 10 µM NSX (raw data see Figure 1, IT-plot see Figure 2). This observation was consistent between the two rounds of agonist applications (Figure 3 b-d and f-h). The amplitude of the signal elicited by the first out of three agonist applications was slightly reduced compared to the last two signals (see Figure 3 b-d).

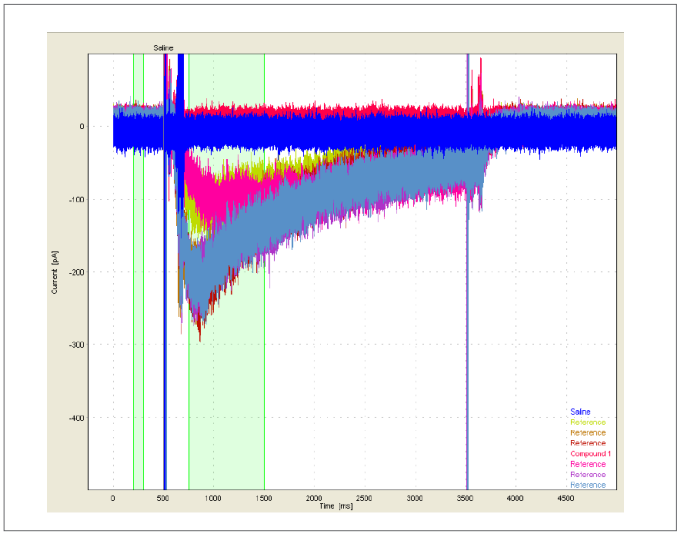


Fig. 1. Raw data of current elicited by GluR5.

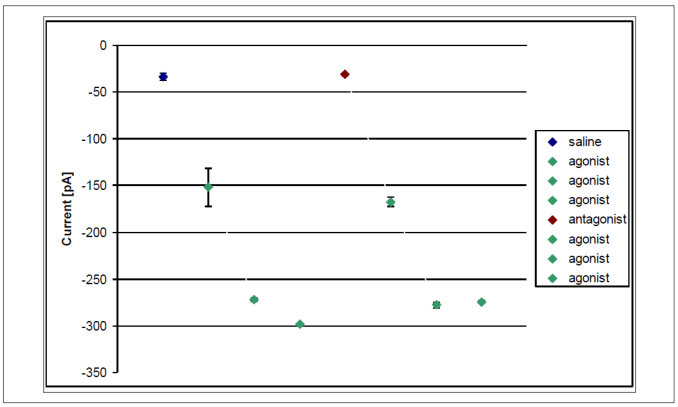


Fig. 2. IT plot of GluR5 currents elicited by kainite. Current amplitude elicited by saline is shown in blue, agonist activation is shown in green, and block by antagonist is in red.

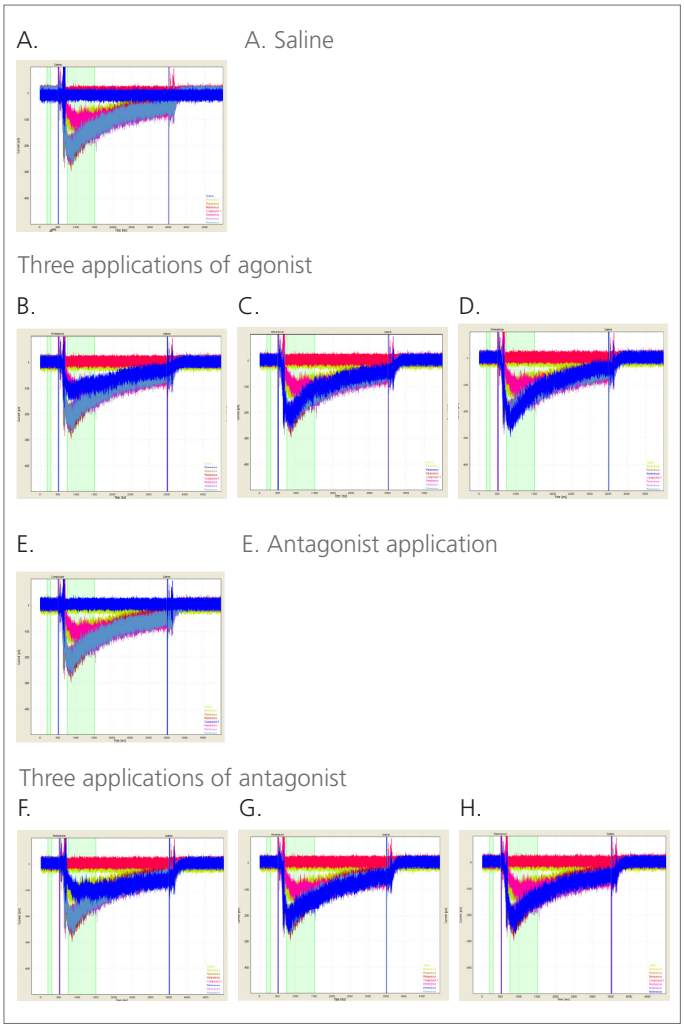


Fig. 3. Individual traces of GluR5 currents activated with agonist and antagonist.

Table 1. Current amplitude of the response of GluR5 to each application of agonist or antagonist. Calculated from the experiment shown in Figure 3.

	1 peak current [pA]	2 peak current [pA]	3 peak current [pA]
Saline	-30		
1st agonist applications	-169	-275	-300
Antagonist	-33		
2nd agonist applications	-165	-277	-276

Table 2. GluR5 agonist response relative to antagonist response before and after antagonist application.

1st agonist/antagonist	0.80	0.88	0.89
2nd agonist/antagonist	0.80	0.88	0.88

The relative block and wash-out of the antagonist was >80% in the individual measurements. On average the antagonist block was 82±2% (n=30).

HEK-GluR6

The reproducibility of the GluR6 current amplitude was investigated with 2 x 3 repeated additions of 1 mM L-glutamate interspersed by an antagonist application of 100 µM CNQX, see Figure 4. Individual traces are shown in Figure 5 and the corresponding IT-plot is shown in Figure 6.

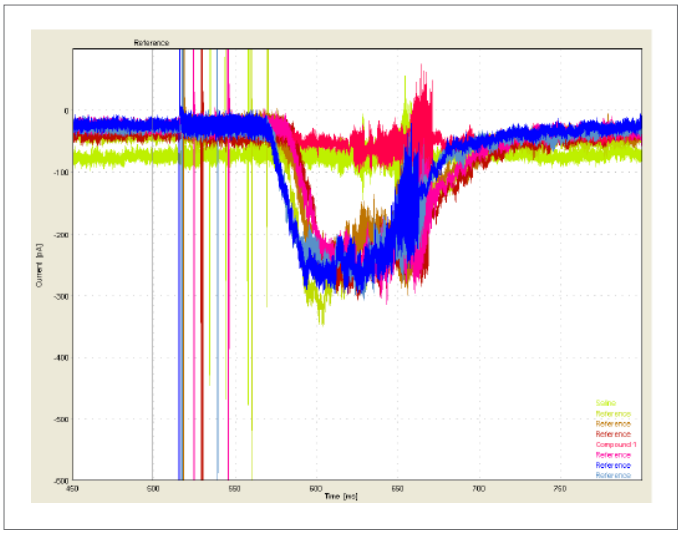


Fig. 4. Raw data showing current elicited by GluR6.

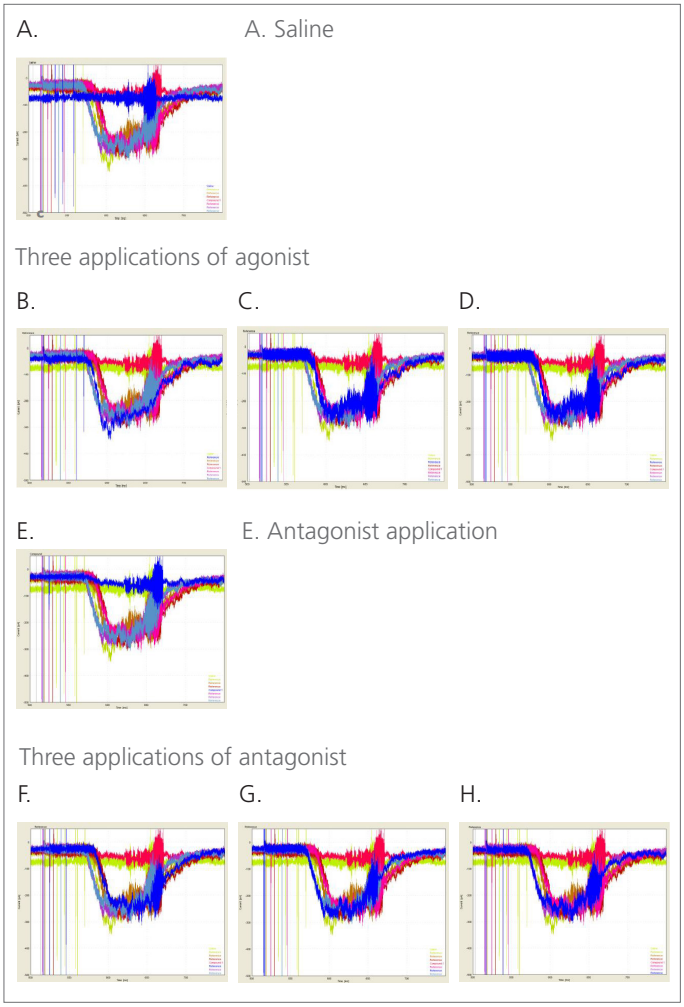


Fig. 5. Individual GluR6 current traces activated by agonist and antagonist.

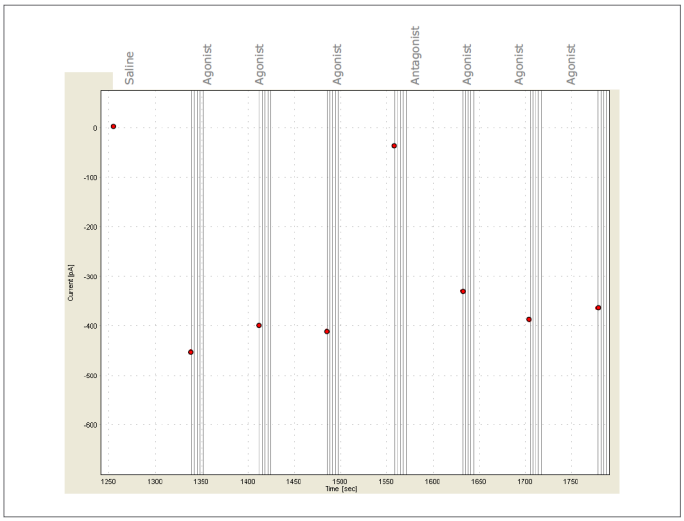


Fig. 6. IT plot of GluR6 currents elicited by glutamate.

Table 3. Current amplitude of the response of GluR6 to each application of agonist or antagonist. Calculated from the experiment shown in Figure 6.

	1 peak current [pA]	2 peak current [pA]	3 peak current [pA]
Saline	-21		
1st agonist applications	-270	-264	-277
Antagonist	-45		
2nd agonist applications	-288	-284	-284

Table 4. GluR6 agonist response relative to antagonist response before and after antagonist application.

1st agonist/antagonist	0.73	0.72	0.74
2nd agonist/antagonist	0.75	0.73	0.74

The relative block and wash-out of the antagonist on the GluR6-current was on average 74%.

HEK-293-ASIC3

Currents were elicited by 2 x 3 repeated additions of extracellular solution with pH 5.3 interspersed by one antagonist application of 500 µM Gd3+ (for complete block an excess of 1 mM Gd3+ is needed). Raw data is shown in Figure 7. The corresponding IT-plot is shown in Figure 8.

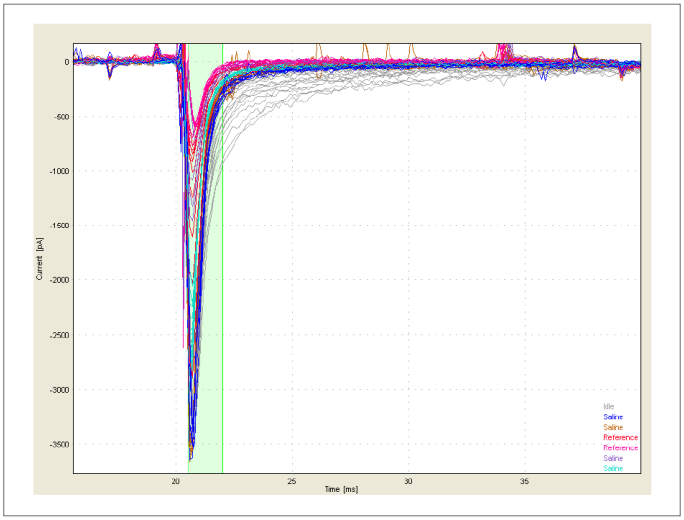


Fig. 7. Raw data from HEK-ASIC3.

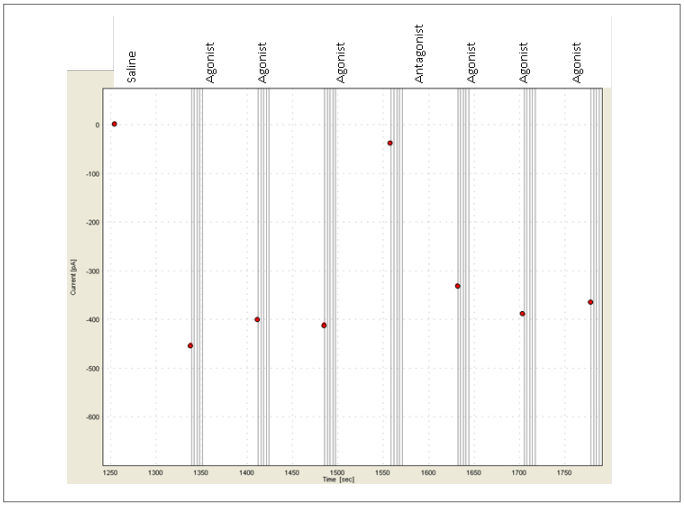


Fig. 8. IT plot of ASIC3 current.

Table 5. Current amplitude of the response of ASIC3 to each application of agonist or antagonist. Calculated from the experiment shown above in Figure 8.

	1 peak current [pA]	2 peak current [pA]	3 peak current [pA]
Saline	0		
1st agonist applications	-452	-400	-140
Antagonist	-36		
2nd agonist applications	-330	-390	-365

Table 6. ASIC3 agonist response relative to antagonist response before and after antagonist application.

1st agonist/ antagonist	0.92	0.91	0.91
2nd agonist/ antagonist	0.89	0.91	0.90

The relative block of the antagonist and the release by wash-out was on average > 90%.

HEK293-Na$_V$1.2a

Na^+-currents were elicited by depolarizations from -90 mV to 0 mV for 20 ms. The current is blocked by 30 nm TTX. Raw data is shown in Figure 9. The corresponding IT-plot is shown in Figure 10.

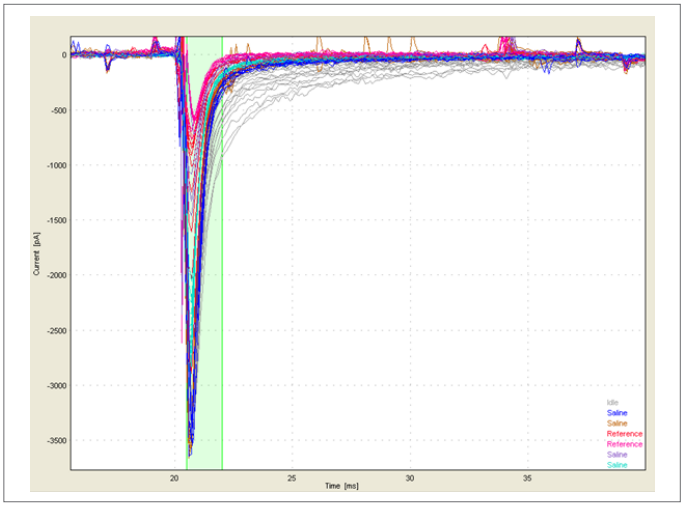


Fig. 9. Raw data from HEK-Na$_V$1.2a.

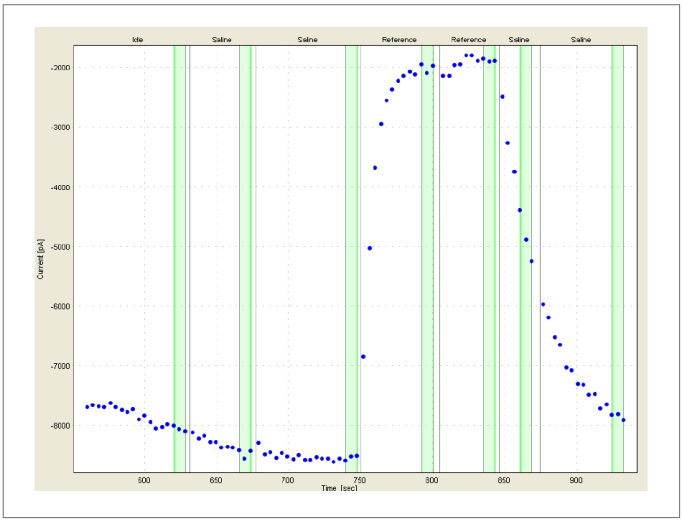


Fig. 10. Na^+ current elicited by depolarizations from -90 mV to 0 mV.

Table 7. Current amplitude of the full response of Na$_V$1.2a in comparison to block by 30 nM TTX. Calculated from the experiment shown in Figure 10.

	1 peak current [pA]	2 peak current [pA]	3 peak current [pA]	4 peak current [pA]
1st saline	-3500	-1820	-1810	-8380
Reference	-620	-250	-300	-1900
2nd saline	-3040	-1460	-1800	-7850

Table 8. Na$_V$1.2a response relative to the response when blocked by 30 nM TTX.

Δfull response/ block	0.82	0.94	0.83	0.78
Washout	0.87	0.99	0.89	0.94

The relative block by TTX was 84% and the wash-out level was 92% of the initial saline level (n=4).

CHO-hERG

K^+-currents were elicited by a voltage protocol stepping from -80 to -50, +20, -50 and finally to -80 mV. The current is blocked by 10 µM quinidine. Raw data is shown in Figure 11. The corresponding IT-plot is shown in Figure 12.

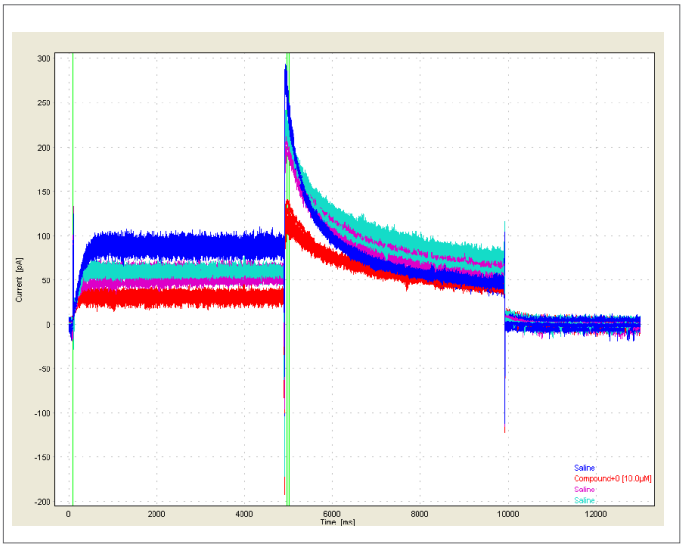


Fig. 11. Raw data from CHO-hERG.

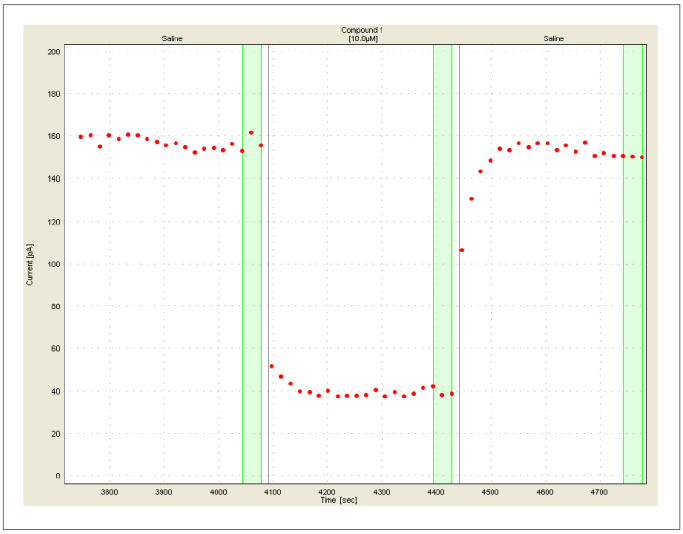


Fig. 12. IT plot showing K^+ current from CHO-hERG.

Table 9. Current amplitude of the full response of hERG in comparison to block by 10 µM quinidine. Calculated from the experiment shown in Figure 12.

	1 peak current [pA]	2 peak current [pA]	3 peak current [pA]
1st saline	250	160	204
Reference	100	40	55
2nd saline	211	150	153

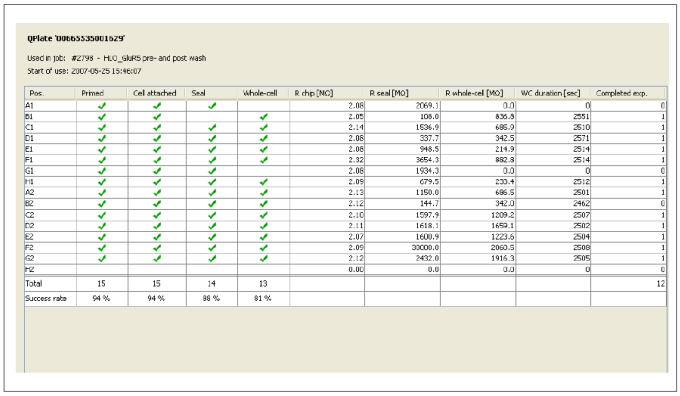
Table 10. hERG response relative to the response when blocked by 10 µM quinidine.

Δfull response/ block	0.60	0.75	0.73
Washout	0.84	0.86	0.78

The relative block of quinidine was 70% and the wash-out current level was 83% of the initial saline level (n=3).

Success rate

The QPlate data is a representative QPlate run with an average success rate of 80% completed experiments, which was the general success rate.



Conclusion

The experiments show that QPatch efficiently can activate currents from fast ligand-gated ion channel GluR5, GluR6 and ASIC3. Following antagonist block, the current elicited with agonist is returned to the same current level as before the block. In experiments performed on voltage-gated $Na_V1.2a$ and hERG channels, the current was activated by a change in the holding potential and then blocked by specific inhibitors, TTX and quinidine, respectively. The current amplitude was returned to the basic level upon wash with saline after block.

Methods

Cells

Cells were cultured according to Sophion SOP.

Data analysis

Recorded ion channel whole-cell currents were stored in an integrated Oracle database along with data on suction pressure, series resistance, seal resistance and capacitances (Cfast and Cslow). Data analysis was accomplished with the QPatch Assay Software using online leak subtraction for HEK-Nav1.2a and offline leak subtraction for the remainder of the cell lines.

Voltage protocols

HEK293-GluR5/HEK293-GluR6 (LGIC)

V_{hold} = -90 mV

Sampling frequency: 50,000 Hz

Cut-off frequency: 10,000 Hz

HEK293-ASIC3 (LGIC)

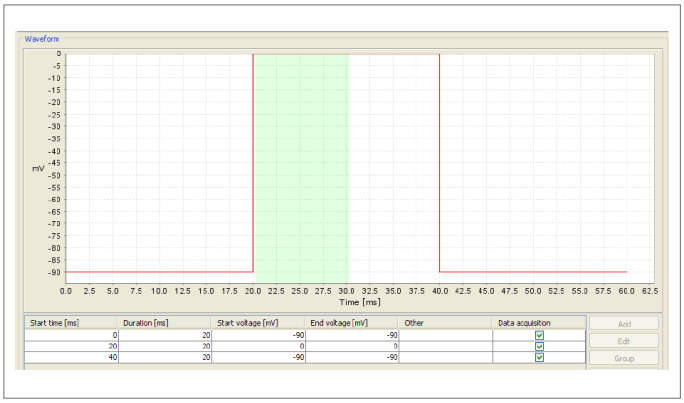
V_{hold} = -90 mV

Sampling frequency: 1,000 Hz

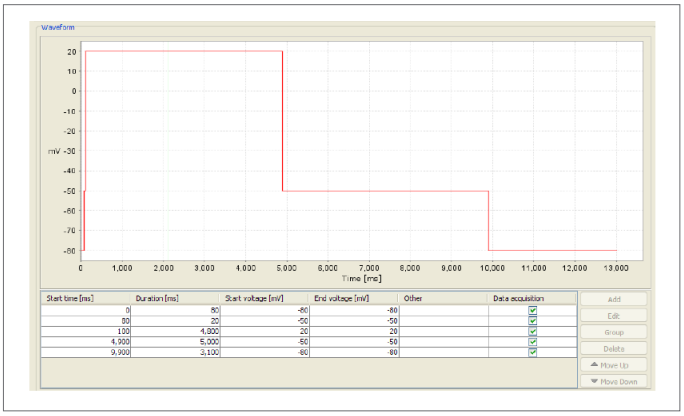
Cut-off frequency: 100 Hz

HEK293-Nav1.2a (VGIC)

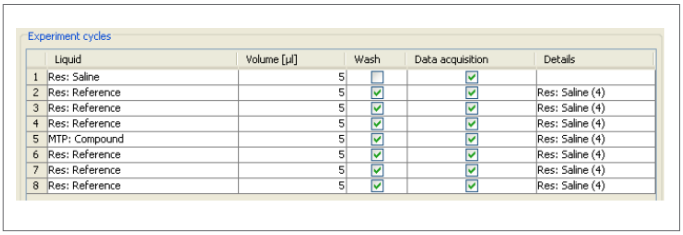
V_{hold} = -90 mV



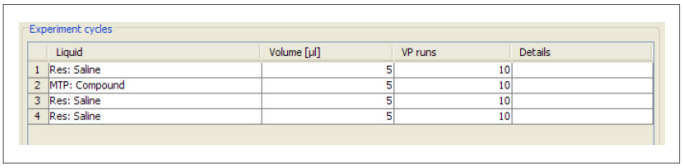
CHO-hERG (VGIC)



Application protocol - ligand-gated ion channels



Application protocol - voltage-gated ion channels



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