

Application Report:

# HEK293-GRIK2

Glutamate receptor GluR6

QPatch

MILLIPORE



This report presents QPatch studies based on whole-cell current recordings from the ligand-gated ion channel GRIK2 receptors (Glu6R), expressed in HEK-293 cells from Millipore (CYL3049). Activation of GRIK2 receptors increases a  $\text{Ca}^{2+}$ -current. The channels were targeted with Glutamate or Kainate (agonist) and CNQX (an antagonist) at a holding potential at -60 mV. Activation of GRIK2 receptors increases a  $\text{Ca}^{2+}$ -conduction over the membrane.

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## Functional validation

The GRIK2 receptor was characterized pharmacologically using the whole-cell patch clamp technique on the automated QPatch16.

The average current amplitude in response to 100 mM glutamate was  $-424.4 \pm 43$  pA at a holding potential of  $-60$  mV. The  $EC_{50}$  value (peak current) was  $19.5 \pm 0.8$  mM in dose-response experiments (lit. value from Millipore datasheet. 19.5 mM). The corresponding  $EC_{50}$  (area under curve) for glutamate was measured to  $11.7 \pm 1.4$   $\mu$ M. The average current amplitude in response to 100 mM kainate was  $-348 \pm 40.7$  pA, which is slightly lower than for glutamate. The  $EC_{50}$  measured as the peak current was  $10.5 \pm 2.5$  mM ( $n=8$ ), (lit. value 36.3 mM). The  $EC_{50}$  value for area under the curve was  $2.3 \pm 0.5$   $\mu$ M ( $n=7$ ).

	$EC_{50}$ peak [ $\mu$ M]	$EC_{50}$ AUC [ $\mu$ M]	Lit. Value [ $\mu$ M]
Glutamate	$19.5 \pm 0.8$	$11.7 \pm 1.4$	19.5
Kainate	$10.5 \pm 2.5$	$2.3 \pm 0.5$	36.3

Table 1.  $EC_{50}$ s for glutamate and kainite.

## Materials & Methods

### Cells and Patch-clamping

The QPlate contains 16 or 48 individual patch-clamp sites that are operated asynchronously and in parallel. Ringer's solutions and compounds are applied by four pipettes. HEK-293 cells expressing the GRIK2 receptors acquired from Millipore were kept in culture medium in the stirred reservoir for up to four hours. Prior to testing, the cells were transferred to an on-board mini centrifuge, spun down and washed in Ringer's solution twice before being applied to the pipetting wells in the QPlate. Gigaseals were formed upon execution of a combined suction/ voltage protocol. Further suction lead to whole-cell configuration. Solutions and compounds were applied through the glass flow channels in the QPlate. All currents were recorded at a patch potential of  $-60$  mV.

### Ringer's

Extracellular Ringer's solution consisted of (in mM): 145 Na<sup>+</sup>, 4 K<sup>+</sup>, 2 Ca<sup>2+</sup>, 1 Mg<sup>2+</sup>, 154 Cl<sup>-</sup>, 10 HEPES (pH 7.4). Intracellular Ringer's solution consisted of (in mM): 120 K<sup>+</sup>, 1.8 Mg<sup>2+</sup>, 93.6 Cl<sup>-</sup>, 30 F<sup>-</sup>, 10 EGTA, 31.3 KOH, 10 HEPES (pH 7.3).

## Application Protocols

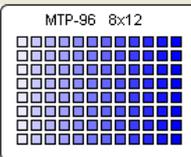
Agonist glutamate/kainate 12-concentration DR:

Application protocol:

Description:

Gating type  
 Voltage gated  
 Ligand gated

QPlate  
 QPlate type:   
 Waste reservoir:   $\mu$ l Free:   $\mu$ l

Compound plate layout  
 MTP-96 8x12 

Experiment cycles

	Liquid	Volume [ $\mu$ l]	Wash	Washing liquid	Data acquisition
1	Res: Saline	5	<input type="checkbox"/>		<input checked="" type="checkbox"/>
2	MTP: 0.01 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (1)	<input checked="" type="checkbox"/>
3	MTP: 0.03 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (1)	<input checked="" type="checkbox"/>
4	MTP: 0.1 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
5	MTP: 0.3 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
6	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
7	MTP: 1 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
8	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
9	MTP: 3 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
10	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
11	MTP: 10 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
12	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
13	MTP: 30 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
14	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
15	MTP: 100 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
16	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
17	MTP: 300 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
18	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
19	MTP: 1000 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
20	Res: Saline	5	<input type="checkbox"/>		<input type="checkbox"/>
21	MTP: 3000 $\mu$ M Glu	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>

Min. cycle duration:  s  Dose-response experiment

Max. cycle duration:  s

## Antagonist CNQX 4-concentration DR:

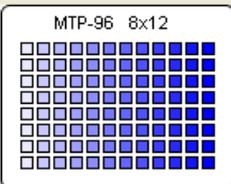
Application protocol:

Description:

Gating type  
 Voltage gated  
 Ligand gated

QPlate  
 QPlate type:

Waste reservoir:   $\mu$ l Free:   $\mu$ l

Compound plate layout  


Experiment cycles

	Liquid	Volume [ $\mu$ l]	Wash	Washing liquid	Data acquisition	
1	Res: Saline		5	<input type="checkbox"/>		<input checked="" type="checkbox"/>
2	Res: Saline		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input type="checkbox"/>
3	Res: Saline		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input type="checkbox"/>
4	Res: Saline		5	<input checked="" type="checkbox"/>	MTP: 0.1 $\mu$ M CNQX (2)	<input type="checkbox"/>
5	MTP: 0.1 $\mu$ M CNQX + 100 $\mu$ M Glu		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
6	Res: Saline		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input type="checkbox"/>
7	Res: Saline		5	<input checked="" type="checkbox"/>	MTP: 1 $\mu$ M CNQX (2)	<input type="checkbox"/>
8	MTP: 1 $\mu$ M CNQX + 100 $\mu$ M Glu		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
9	Res: Saline		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input type="checkbox"/>
10	Res: Saline		5	<input checked="" type="checkbox"/>	MTP: 10 $\mu$ M CNQX (2)	<input type="checkbox"/>
11	MTP: 10 $\mu$ M CNQX + 100 $\mu$ M Glu		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>
12	Res: Saline		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input type="checkbox"/>
13	Res: Saline		5	<input checked="" type="checkbox"/>	MTP: 100 $\mu$ M CNQX (3)	<input type="checkbox"/>
14	MTP: 100 $\mu$ M CNQX + 100 $\mu$ M Glu		5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>

## Voltage protocol

Voltage protocol:

Description:

Gating type  
 Voltage gated  
 Ligand gated

General Filtering Rseries

Holding potential (Vhold):  mV  Use constant voltage protocol at Vhold

Minimum seal resistance:  M $\Omega$   Enable C-slow measurement

Liquid dispense delay:  ms

Data acquisition time:  ms

General Filtering Rseries

Sampling frequency:  Hz

Filter type:

Filter order:

Cut-off frequency:  Hz

## 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 ( $C_{fast}$  and  $C_{slow}$ ). Drug effects were analyzed as function of concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software. For the currents used for concentration-response analyses the leak currents were subtracted off line.

## Results

### GRIK2 study

To test the reproducibility of the GRIK2 current signal, an experiment was made with 3 application of 100  $\mu$ M glutamate, by one application of the antagonist CNQX (100  $\mu$ M) followed by a re-activation with 3 application of 100  $\mu$ M glutamate, see Figure 1.

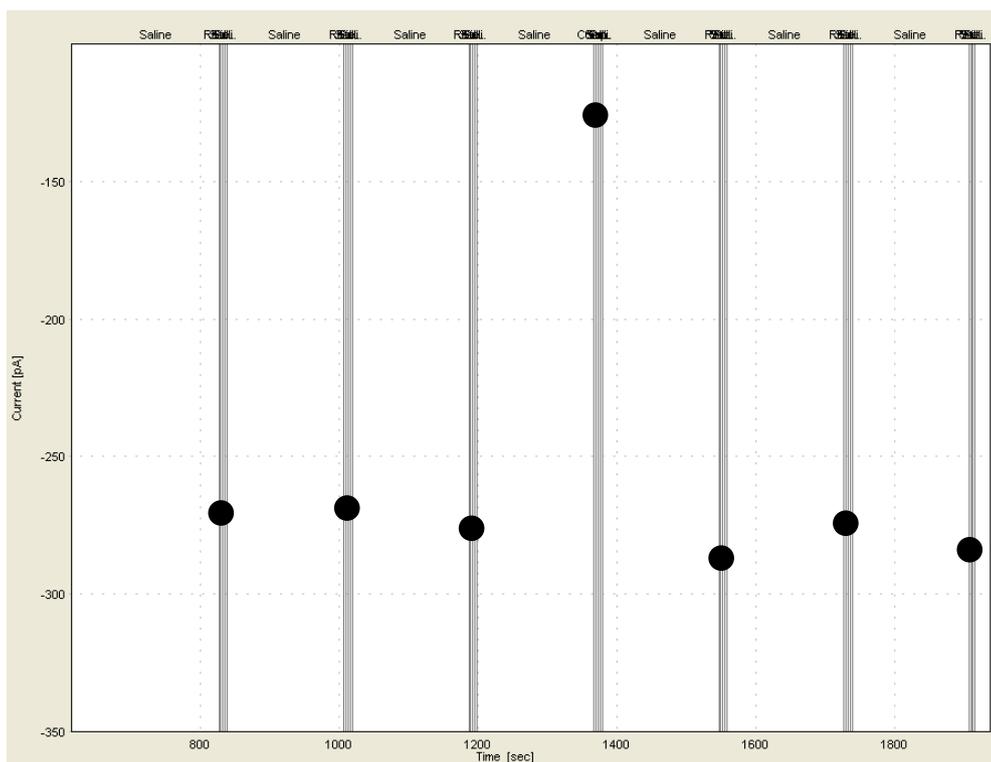


Figure 1. Reproducibility of GRIK2-current amplitude.

The figures below show GRIK2 currents in response to 12 increasing concentrations of glutamate (Fig. 2A) and the corresponding Hill fit (Figure 2B).

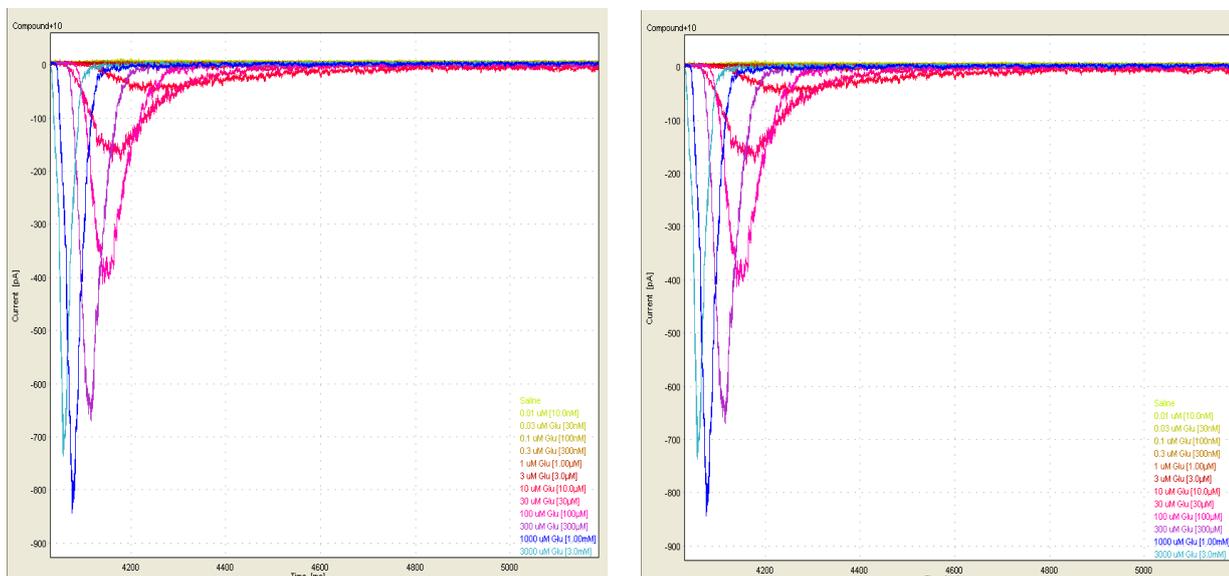


Figure 2. Left: Raw data for glutamate 12-point dose-response, and right: corresponding Hill fit.

The average rise-time for Glu6R currents measured at 3 mM glutamate was  $23.2 \pm 1.2$  msec. The fastest rise time measured to 19 msec at 3 mM glutamate.

The individual current traces showing dose-response for kainate (Figure 3). The corresponding Hill fit is presented in Figure 4.

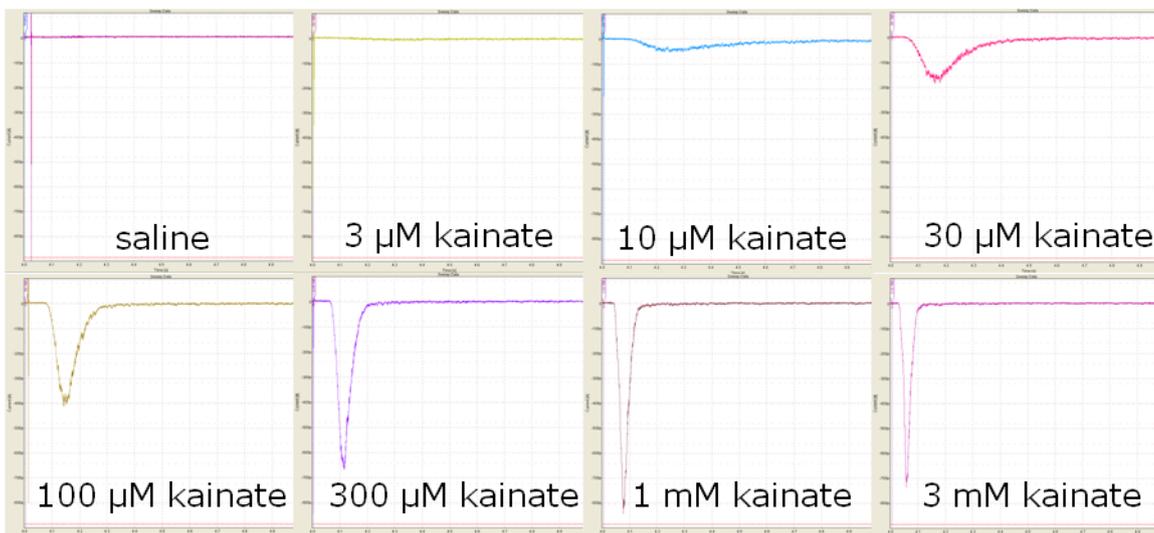


Figure 3. Individual current traces from GRIK2 elicited by kainate.

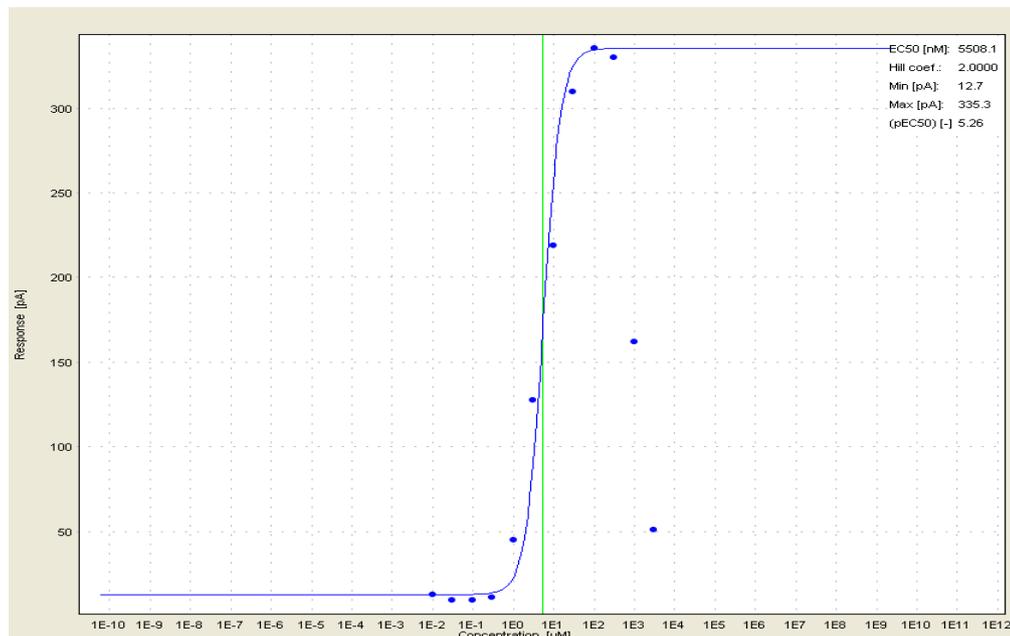


Figure 4. Hill fit showing 12-concentration dose-response for kainate. The maximum current was obtained at 100 µM kainate.

## Antagonist study with CNQX

Figure 5 shows GRIK2 currents elicited by 100 µM glutamate in response to four increasing concentrations of the inhibitor CNQX (100 µM, 10 µM, 1 µM to 0.1 µM).

Figure 6 shows the dose-concentration relationship for CNQX as determined with the QPatch Assay Software. The mean IC<sub>50</sub> was 5.0±2.0 µM (n=6) at 100 µM Glutamate (lit. value: 4.74 µM).

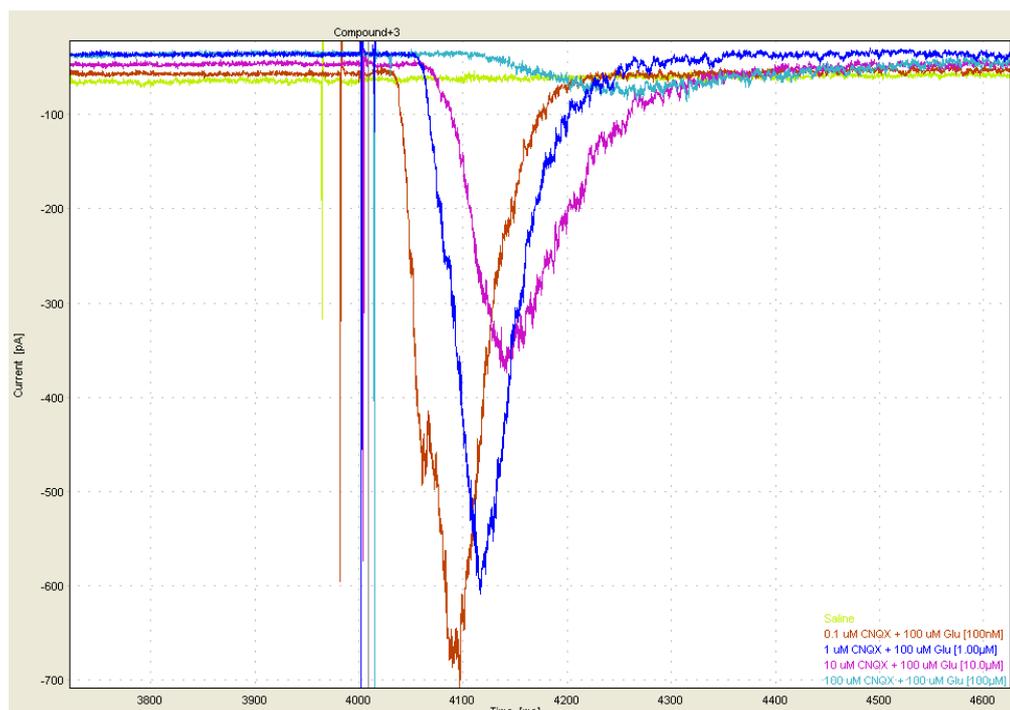


Figure 5. Raw data for antagonist CNQX block of GRIK2-currents.

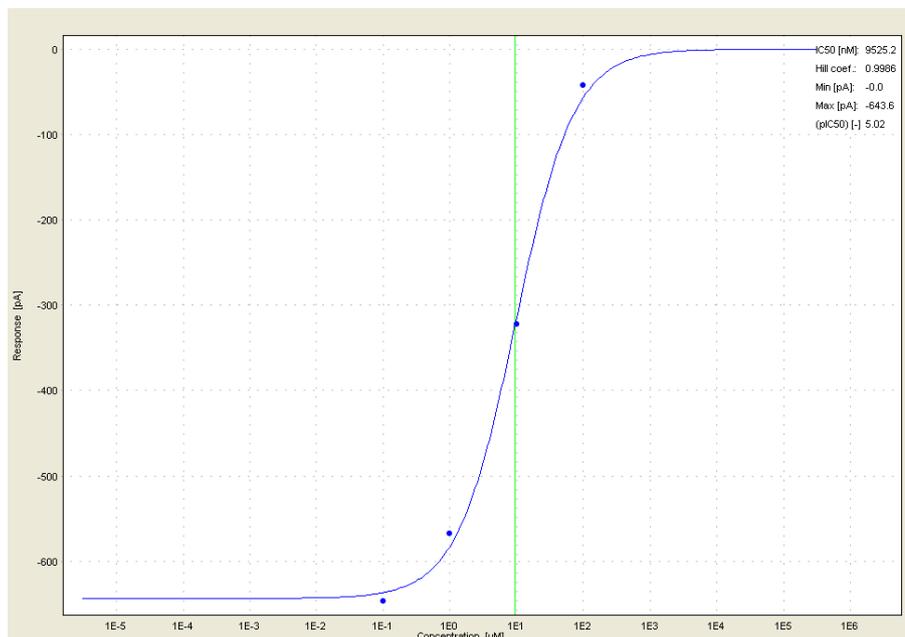


Figure 6. Hill fit of the dose-response relationship of CNQX on GRIK2

## Success rate

5 QPlates was used to obtain the data presented in this report. The tables below shows data from the individual QPlates. The average success rate was 69% whole cells pr. QPlate.

**QPlate '00665535001086'**

Used in job: #1447 - HLO\_AMPA 4-conc Glu  
 Start of use: 2007-02-19 14:54:56

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	2.17	535.1	640.1	921	1
B1	✓	✓	✓	✓	2.18	102.8	278.5	985	1
C1	✓	✓	✓	✓	2.18	270.7	444.3	1606	1
D1	✓	✓	✓	✓	2.18	393.4	1279.9	966	1
E1	✓	✓	✓	✓	2.19	283.4	394.0	318	0
F1					0.00	0.0	0.0	0	0
G1	✓	✓	✓		2.19	2170.5	0.0	0	0
H1					0.00	0.0	0.0	0	0
A2	✓	✓	✓	✓	2.16	432.9	560.3	918	1
B2	✓	✓	✓	✓	2.15	946.0	1112.1	932	1
C2	✓	✓	✓	✓	2.16	659.8	571.9	975	1
D2	✓	✓	✓	✓	2.19	334.7	587.4	969	1
E2	✓	✓	✓	✓	2.21	787.8	739.9	939	1
F2	✓	✓	✓	✓	2.19	263.2	525.5	176	0
G2	✓	✓	✓	✓	2.22	743.9	761.7	981	1
H2	✓	✓	✓	✓	2.23	272.8	444.0	1007	1
Total	14	14	13	13					11
Success rate	88 %	88 %	81 %	81 %					

## QPlate '00665535001057'

Used in job: #1479 - HLO\_AMPA 12-conc DR Kainate  
 Start of use: 2007-02-21 10:08:52

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	1.84	867.5	995.9	2064	1
B1	✓	✓	✓	✓	2.00	1567.8	1074.8	1994	1
C1	✓				2.34	0.0	0.0	0	0
D1	✓				1.84	0.0	0.0	0	0
E1	✓	✓			1.85	95.8	0.0	0	0
F1					271.30	0.0	0.0	0	0
G1	✓	✓	✓	✓	1.85	1083.9	222.9	2092	1
H1	✓	✓	✓	✓	1.84	871.8	862.0	1553	0
A2	✓	✓	✓	✓	1.85	1572.0	1038.5	2056	1
B2	✓	✓			1.83	116.1	0.0	0	0
C2	✓	✓	✓	✓	1.86	4103.3	974.9	2039	1
D2	✓	✓	✓	✓	1.84	1695.6	810.6	2046	1
E2	✓				1.83	0.0	0.0	0	0
F2	✓	✓	✓	✓	1.83	1132.6	1041.5	197	0
G2	✓	✓	✓	✓	1.84	1020.8	743.9	2064	1
H2	✓	✓		✓	1.84	100.3	182.8	2108	1
Total	15	12	9	10					8
Success rate	94 %	75 %	56 %	63 %					

## QPlate '00665535001059'

Used in job: #1471 - HLO\_AMPA GluCNQX II  
 Start of use: 2007-02-20 15:46:41

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	2.15	213.6	334.6	1058	0
B1	✓	✓	✓	✓	2.16	541.8	428.3	1647	1
C1	✓				2.21	0.0	0.0	0	0
D1	✓	✓	✓		2.20	552.8	0.0	0	0
E1	✓	✓	✓		2.20	526.0	0.0	0	0
F1	✓	✓	✓	✓	2.14	786.2	1963.7	415	0
G1	✓	✓	✓	✓	2.15	367.7	506.5	1700	1
H1	✓	✓	✓	✓	2.15	452.4	600.1	1645	1
A2	✓	✓	✓	✓	2.24	356.4	230.6	499	0
B2	✓	✓	✓	✓	2.14	1275.3	572.7	1655	0
C2	✓	✓	✓	✓	2.25	644.3	638.4	1721	1
D2	✓	✓	✓	✓	2.29	968.6	621.8	1686	1
E2	✓	✓	✓	✓	2.21	393.9	211.4	1663	1
F2	✓	✓	✓		2.18	531.1	0.0	0	0
G2	✓	✓	✓	✓	2.15	709.5	488.0	1735	1
H2	✓	✓	✓	✓	2.15	1236.6	744.1	915	0
Total	16	15	15	12					7
Success rate	100 %	94 %	94 %	75 %					

## QPlate '00665535001082'

Used in job: #1458 - HLO\_AMPA 8-conc DR  
 Start of use: 2007-02-20 12:55:17

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓				2.21	0.0	0.0	0	0
B1	✓				2.25	0.0	0.0	0	0
C1	✓	✓	✓	✓	2.21	306.1	362.1	2111	1
D1	✓	✓	✓	✓	2.23	174.2	396.1	2078	1
E1	✓	✓	✓	✓	2.21	285.2	368.2	2180	1
F1					6617.77	0.0	0.0	0	0
G1	✓	✓	✓	✓	2.19	782.6	579.5	2200	1
H1	✓	✓	✓	✓	2.19	388.0	421.8	2138	1
A2	✓	✓	✓	✓	2.21	990.5	331.0	2163	1
B2	✓				2.21	0.0	0.0	0	0
C2	✓	✓	✓		2.22	786.3	0.0	0	0
D2	✓	✓	✓		2.24	86.0	0.0	0	0
E2	✓	✓	✓	✓	2.24	460.1	534.3	2201	1
F2	✓	✓	✓	✓	2.25	462.4	436.2	2146	1
G2	✓	✓	✓	✓	2.27	154.7	231.8	894	0
H2	✓	✓	✓	✓	2.28	9892.7	561.0	808	0
Total	15	12	12	10					8
Success rate	94 %	75 %	75 %	63 %					

**QPlate '00465535030479'**

 Used in job: #2784 - HLO\_GluR5 4 applications II  
 Start of use: 2007-05-24 12:51:06

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓		2.38	546.4	0.0	0	0
B1	✓	✓	✓	✓	2.43	2099.4	722.8	1693	1
C1	✓	✓	✓		2.41	360.1	0.0	0	0
D1	✓	✓	✓	✓	2.48	516.6	251.7	941	1
E1	✓	✓	✓		2.44	162.5	0.0	0	0
F1	✓	✓	✓	✓	2.45	2888.2	594.4	1687	1
G1	✓	✓	✓	✓	2.41	167.8	417.5	1696	1
H1	✓	✓	✓	✓	2.41	264.8	485.3	971	1
A2	✓	✓	✓		2.36	4220.7	0.0	0	0
B2	✓	✓	✓	✓	2.36	214.0	690.3	1714	1
C2	✓	✓	✓	✓	2.40	104.0	144.0	234	0
D2	✓	✓	✓		2.40	178.9	0.0	0	0
E2	✓	✓	✓	✓	2.38	138.0	341.7	984	1
F2	✓	✓	✓	✓	2.41	1392.7	935.5	1735	1
G2	✓	✓	✓	✓	2.40	405.3	675.4	2484	1
H2					0.00	0.0	0.0	0	0
Total	15	15	12	10					9
Success rate	94 %	94 %	75 %	63 %					

## References

Paternain AV., Cohen A., Stern-Bach Y., Lerma J. A role for extracellular Na<sup>+</sup> in the channel gating of native and recombinant kainite receptors. *The journal of neuroscience*. 2003 Sept. 23(25):8641-8648

Lerma J., Paternain AV., Rodríguez-Moreno A., López-García JC. Molecular physiology of kainite receptors. *Physiological reviews*. 2001 July 81(3): 971-998

Everts I., Petroski R., Kizelsztein P., Teichberg VI., Heinemann SF., Hollmann M. Lectin-induced inhibition of desensitization of the kainite receptor GluR6 depends on the activation state and can be mediated by a single native or ectopic N-linked carbohydrate side chain. *The journal of neuroscience*. 1999 Feb. 19(3): 916-927

## Conclusion

The experiments show that the QPatch 16 automated patch-clamp system efficiently can target effects of agonists, agonists and modulators on the fast ligand-gated ion channel GluR6. The characterizations were based on IT and concentration-response relationships, and on rise-time determinations. The EC50 and IC50 values determined from the concentration-response relations in the present study are comparable to values listed in the literature..