

Application Report:

# GABA-A $\alpha 1/\beta 3\gamma 2$

QPatch

PrecisION™ GABA-A  $\alpha 1/\beta 3\gamma 2$  HEK\* from Millipore



GABAA receptors are anionic channels and upon activation under physiological conditions, an inward chloride driven current is recorded. The channels were tested on QPatch and targeted with an agonist; GABA ( $\gamma$ -amino-butyric acid) and bicuculline (an antagonist).

AR\_PUBLIC14486-4

## Introduction

This report presents QPatch studies based on whole-cell current recordings from the ligand-gated ion channel hGABAA receptors ( $\alpha 1\beta 3\gamma 2$ ), expressed in HEK-293 cells from Millipore. GABAA receptors are anionic channels and upon activation under physiological conditions, an inward chloride driven current is recorded. The channels were targeted with an agonist; GABA ( $\gamma$ -amino-butyric acid) and bicuculline (an antagonist).

## Materials & Methods

### Cell culture

The cells were grown according to the SOP supplied by the vendor, Millipore. The cells were seeded out two days before reaching 70% confluency. Upon harvest, the cells were washed in PBS and detached from the flask using Detachin as described in the Sophion SOP for HEK cell harvest.

### Planar patch-clamping

The QPlate contains 16 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 GABAA receptors ( $\alpha 1\beta 3\gamma 2$ ) were kept in culture medium in the stirred reservoir for up to four hours. Prior to testing, the cells are 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 are formed upon execution of a combined suction/voltage protocol. Further suction lead to whole-cell configuration. Solutions and compounds are applied through the glass flow channels in the QPlate. All currents were recorded at a patch potential of -80 mV.

### Ringer's

Extracellular Ringer's solution: consisted of (in mM): 145  $\text{Na}^+$ , 4  $\text{K}^+$ , 2  $\text{Ca}^{2+}$ , 1  $\text{Mg}^{2+}$ , 154  $\text{Cl}^-$ , 10 HEPES (pH 7.4). Intracellular Ringer's solution consisted of (in mM): 120  $\text{K}^+$ , 1.8  $\text{Mg}^{2+}$ , 123.6  $\text{Cl}^-$ , 10 EGTA, 31.3 KOH, 10 HEPES (pH 7.2).

# PrecisION™ GABA-A $\alpha 1/\beta 3/\gamma 2$ HEK\* from Millipore



## Application Protocols

Agonist GABA 6-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 8x(2x6)

**Experiment cycles**

	Liquid	Volume [ $\mu$ l]	Wash	Washing liquid	Data acquisition
1	Res: Saline	5	<input type="checkbox"/>		<input checked="" type="checkbox"/>
2	MTP: GABA 0.5 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
3	MTP: GABA 1 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
4	MTP: GABA 5 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
5	MTP: GABA 10 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
6	MTP: GABA 50 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
7	MTP: GABA 100 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (2)	<input checked="" type="checkbox"/>
8	Res: Saline	5	<input type="checkbox"/>		<input checked="" type="checkbox"/>

Min. cycle duration:  s

Max. cycle duration:  s

Dose-response experiment

# PrecisION™ GABA-A $\alpha 1/\beta 3/\gamma 2$ HEK\* from Millipore



Antagonist Bicuculline 4-concentration DR:

Application protocol: HEK-GABA anta- and agonist  
Description: 4/1-2007 XLplates

Gating type  
 Voltage gated  
 Ligand gated

QPlate  
QPlate type: QPlate XL  
Waste reservoir: 250  $\mu$ l Free: 170  $\mu$ l

Compound plate layout  
MTP-96 8x12  
  
Edit...

Experiment cycles

	Liquid	Volume [ $\mu$ l]	Wash	Washing liquid	Data acquisition
1	Res: Saline	5	<input checked="" type="checkbox"/>	MTP: 100 nM Bicuculline (2)	<input checked="" type="checkbox"/>
2	MTP: 100 nM Bicuculline + GABA 10 $\mu$ M	5	<input checked="" type="checkbox"/>	MTP: 1 $\mu$ M Bicuculline (2)	<input checked="" type="checkbox"/>
3	MTP: 1 $\mu$ M Bicuculline + GABA 10 $\mu$ M	5	<input checked="" type="checkbox"/>	MTP: 10 $\mu$ M Bicuculline (2)	<input checked="" type="checkbox"/>
4	MTP: 10 $\mu$ M Bicuculline + GABA 10 $\mu$ M	5	<input checked="" type="checkbox"/>	MTP: 100 $\mu$ M Bicuculline (2)	<input checked="" type="checkbox"/>
5	MTP: 100 $\mu$ M Bicuculline + GABA 10 $\mu$ M	5	<input checked="" type="checkbox"/>	Res: Saline (3)	<input checked="" type="checkbox"/>

Min. cycle duration: 60.0 s  
Max. cycle duration: 300.0 s  
 Dose-response experiment  
Edit Wash... Add Remove

## Data Analysis

Recorded ion channel whole-cell currents are stored in an integrated Oracle database along with data on suction pressure, series resistance, seal resistance and capacitances (Cfast and Cslow). Drug effects are analyzed as function of time (I-t plot) and concentration (dose-response relationship). Data analyses are accomplished with the QPatch Assay Software. For the currents used for I-t and concentration-response analyses the leak currents are subtracted off-line.

## Results

### GABA study

In order to validate the consistency of the signal amplitude, 4 subsequent application of 10  $\mu$ M GABA were applied to the cells followed by two washes per application (Figure 1).

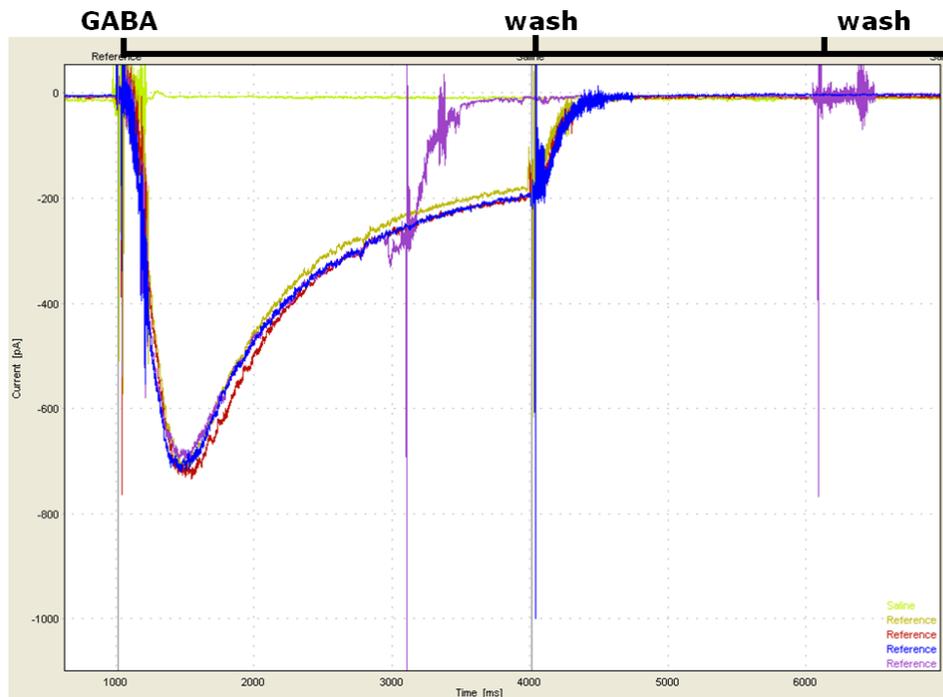


Figure 1. The GABA response is stable and reproducible. No desensitization can be observed between the first (green trace) and last (purple trace) application of 10  $\mu\text{M}$

Figure 2 shows GABA currents in response to 6 increasing concentrations of (GABA), each followed by 2 washes.

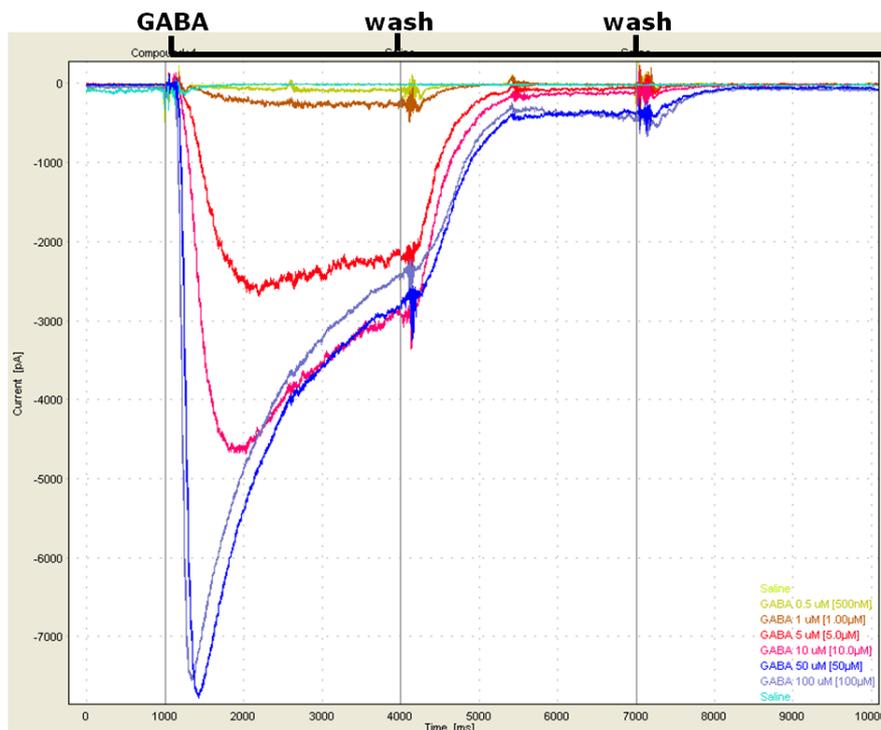


Figure 2. Raw data with 6 increasing concentrations of GABA.

Figure 3 shows the complete concentration-response relationship as determined with the QPatch Analysis Software.

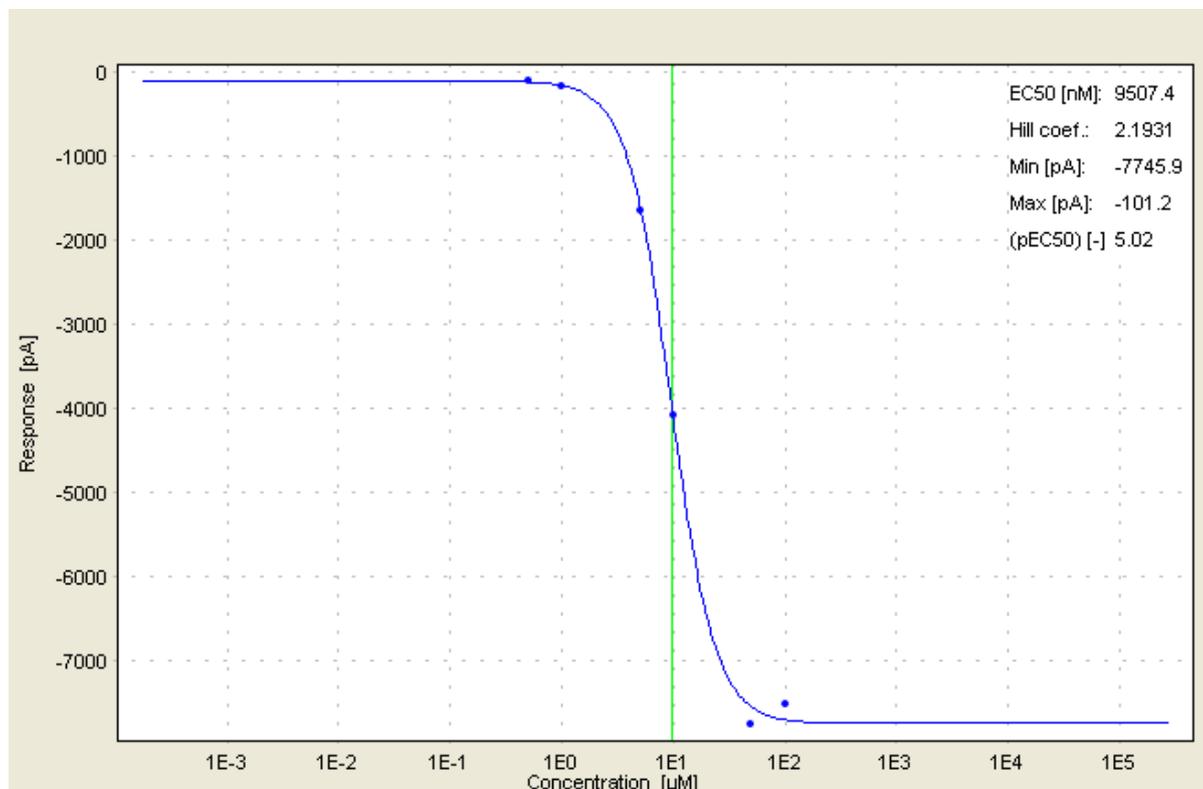


Figure 3. Hill fit representing 6 concentrations of GABA.

The mean  $EC_{50}$  was  $5.9 \pm 2.9 \mu M$  ( $n=5$ ). The mean Hill slope =  $1.6 \pm 0.3$ . The mean rise-time for GABA<sub>A</sub> receptor currents measured at  $10 \mu M$  GABA was 167.1 msec.

## Bicuculline study

The figure below shows GABA<sub>A</sub> currents elicited by  $10 \mu M$  GABA in response to four increasing concentrations of the inhibitor bicuculline ( $100 \mu M$ ,  $10 \mu M$ ,  $1 \mu M$  to  $0.1 \mu M$ ).

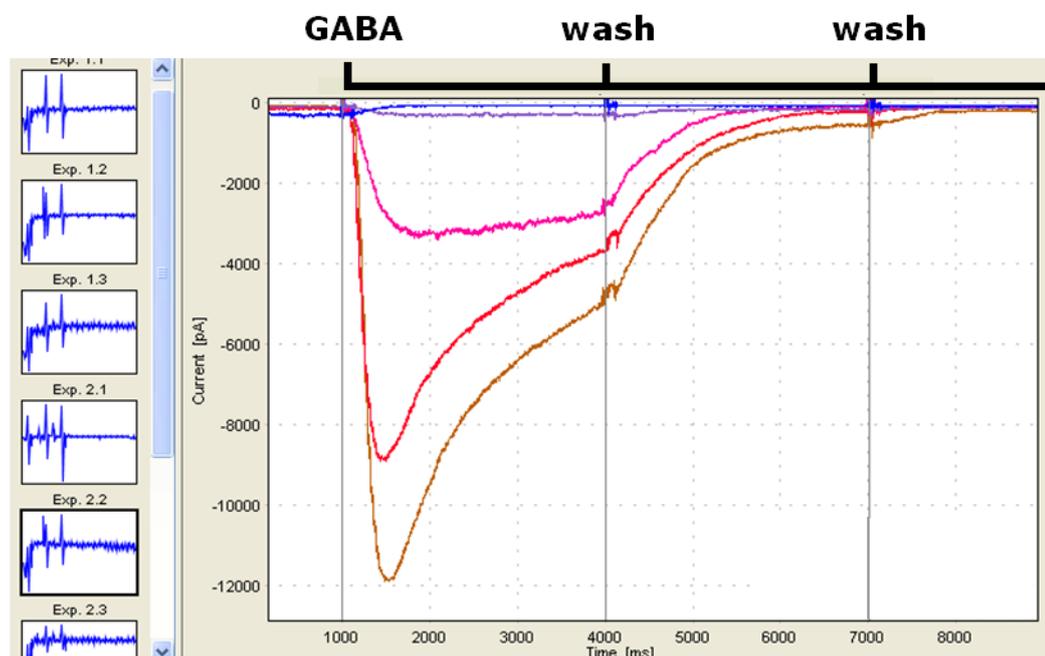


Figure 4. Raw data sjpwomg dose-response for bicuculline in response to 10  $\mu\text{M}$  GABA.

Figure 5 shows the concentration-relationship for bicuculline as determined with the QPatch Assay Software. The mean  $\text{IC}_{50}$  was  $2.4 \pm 0.8 \mu\text{M}$  ( $n=6$ ) at 10  $\mu\text{M}$  GABA (lit. value 1.3  $\mu\text{M}$ ). The mean Hill slope = 0.96.

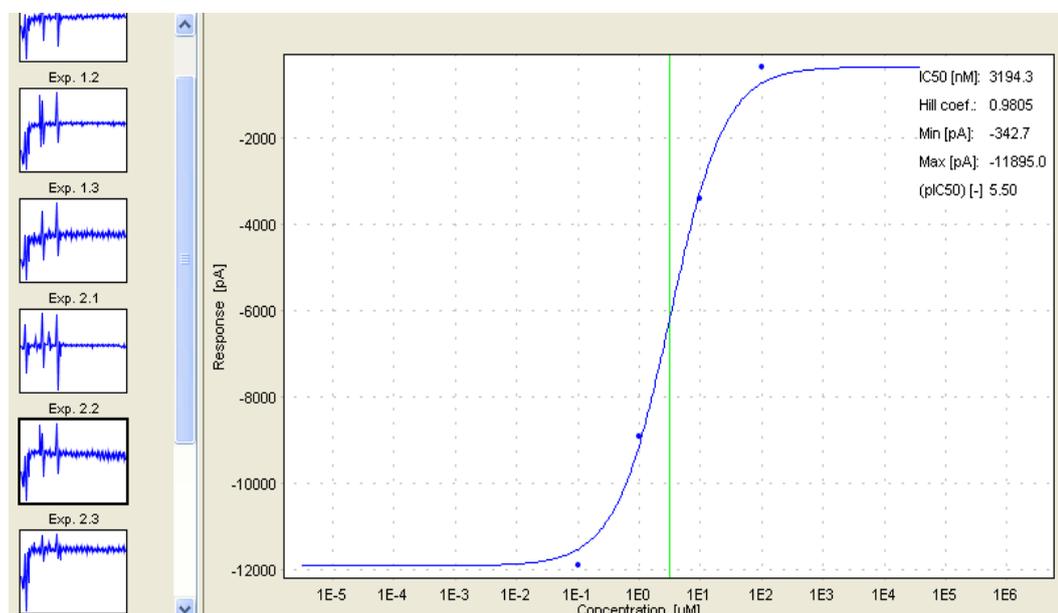


Figure 5. Hill fit representing 4 increasing concentrations of bicuculline.

## Reversal Potential

The reversal potential was investigated for the GABA-receptor by applying a ramp from a holding potential of -80 mV to 60 mV for 2000 ms (Figure 6). The reversal potential was measured in the presence of 10  $\mu$ M GABA.

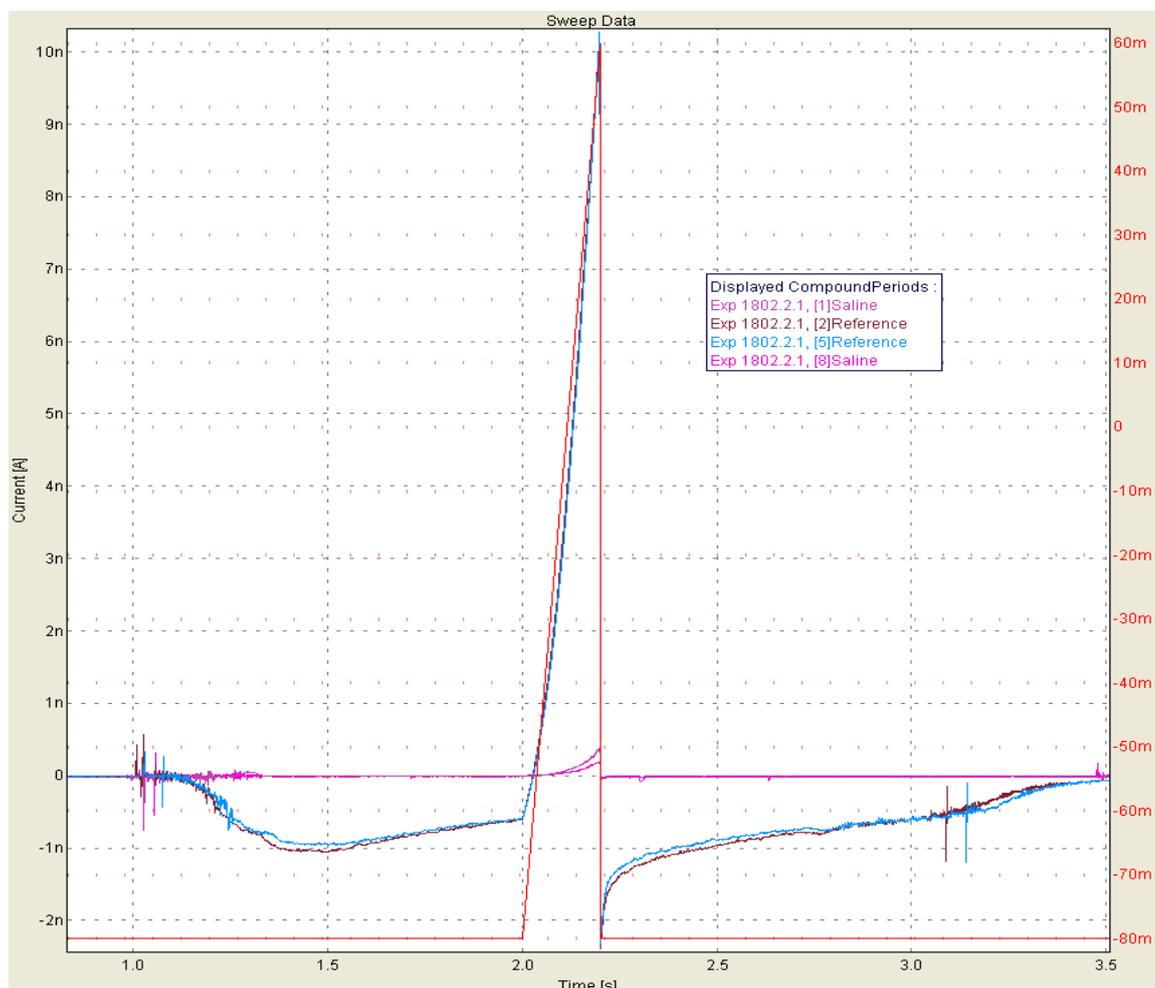


Figure 6. Raw data in the absence (purple and red line) and presence of 10  $\mu$ M GABA (blue and brown line). The voltage ramp is represented by a red line. The insert shows where the GABA elicited currents crosses zero. The small increase in control traces during the ramp is due to an endogenous channel.

The reversal potential was estimated to -61 mV (n=18). The theoretical  $E_{rev}$  for chloride is calculated to -75.21 mV. The liquid junction potential for the Ringer solution used is 8.416 mV. Taken the junction potential into the equation results in an estimated reversal potential of -69.4 mV, which is close to the calculated reversal potential for chloride.

## Success rate

After only two weeks of optimization, we reached an average rate of over 50% completed experiments with whole-cells lasting about 30 min. Of the whole cells obtained, 100% expressed recordable currents on QPatch.

## References

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Javors MA, King TS, Chang X, Ticku MK, Levinson C. Characterization of chloride efflux from GT1-7 neurons: lack of effect of ethanol on GABAA response. *Brain Res.* 1998 Jan 12;780(2):183-9.

Rosas-Arellano A, Ochoa-de la Paz LD, Miledi R, Martinez-Torres A. Brain distribution and molecular cloning of the bovine GABA rho1 receptor. *Neurosci Res.* 2006 Dec 21.

Feigenspan A, Weiler R. Electrophysiological properties of mouse horizontal cell GABAA receptors. *J Neurophysiol.* 2004 Nov;92(5):2789-801. Aslfkjaslfj

## Conclusion

The analyses presented here demonstrate that compound screening on the ligand-gated PrecisION GABA-A  $\alpha 1/\beta 3/\gamma 2$  HEK cells\* from Millipore can be performed efficiently with the QPatch 16 automated patch-clamp system. We have successfully characterized the effects of an agonist, an agonist and a modulator. The characterizations are based on I-t and concentration-response relationships, and on tau determinations. The  $EC_{50}$  and  $IC_{50}$  values determined from the concentration-response relations in the present study are comparable to values listed in the literature. We therefore argue that the QPatch and the GABA<sub>A</sub> cell line are apt for both agonist and antagonist experiments.