SOPHION BIOSCIENCE

Application Report

# Developing a stable high-throughput assay using electroporation transfected cells

The Neon® Transfection System offers a gentle transfection method, allowing high-throughput pharmacological evaluation of the monomeric α1 glycine receptors on Qube.


## Summary

- A stable Qube assay with high success rates (88%) using electroporation transfected cells
- Simultaneous testing of up to 16 cell preparations allows fast electroporation protocol optimization
- Multi-hole technology enhances signals in cases of low transfection levels.


## Introduction

### Electroportation

Electroporation is a technique allowing chemicals, drugs, or DNA to be introduced into the cell by applying an electrical field to the cells, thereby increasing the permeability of the cell membrane. The Neon® Transfection System is a novel benchtop electroporation device that employs the pipette tip as an electroporation chamber to easily and efficiently transfect mammalian cells.

In this study, native HEK 293 cells were transfected with a plasmid encoding the human glycine receptor α1 (hGlyRα1), to demonstrate how a Qube can be used for:

1. optimization of electroporation transfection
2. pharmacological evaluation of transfected cells with varying transfection levels

### Glycine

The amino acid glycine is an important inhibitory transmitter in the spinal cord, brainstem, and other regions of the central nervous system[1,2]. Upon binding and opening of the glycine receptor, glycine causes neuronal hyperpolarization through permeability to chloride ions[3,4].

Here we describe the development of an automated patch clamp (APC) assay with high patching success rates (up to 88%) using single gene transfections of hGlyRα1. The APC assay was first used to optimize the electrical parameters and DNA concentration of the transfection and subsequently for pharmacological evaluation of the glycine receptor.

## Results and discussion

In the following we:

1. Design an assay to evaluate electroporation efficiency
2. Use this assay to optimize the electroporation protocol
3. Demonstrate a pharmacological assay on electroporation transfected cells

### 1. Designing a glycine receptor assay to evaluate electroporation efficiency

#### Glycine receptor assay

To evaluate transfection efficiency (the fraction of cells expressing the receptor) and experimental success rate (number of single-cell experiments passing through the experiment), a glycine receptor assay was developed. The assay consisted of 8 repetitions of glycine additions: 7 additions of 100 µM glycine ($EC_{80}$ values from literature[5,6]) and 1 mM glycine (saturated concentration). The assay employs the stacked delivery feature, where both the glycine-containing solution and the washout solution are stacked in the pipette. In this way, the exposure time is reduced to less than one second.

The assay was evaluated using cells transfected under Neon systems recommended settings for HEK 293 cells (100 µL tip, pulse voltage: 1100 V, pulse width: 20 ms, pulse number: 2, cell density: 5 x $10^7$, see ref 7, Table 1).

100 µM glycine was able to activate the expressed glycine receptors and elicit a current response (5.1 nA ± 2.2 nA, Figure 1A) that over seven repetitive applications did not show rundown

or desensitization (Figure 1B). The 8th glycine application (1 mM) showed a slightly larger peak (6.1 nA ± 2.3 nA) and a faster rise time (25 ms ± 1 ms vs 64 ms ± 3 ms). The intracellular ringer contained fluoride to enhance the seal resistance and no chloride ions ($Cl^-$), hence the outward going current.

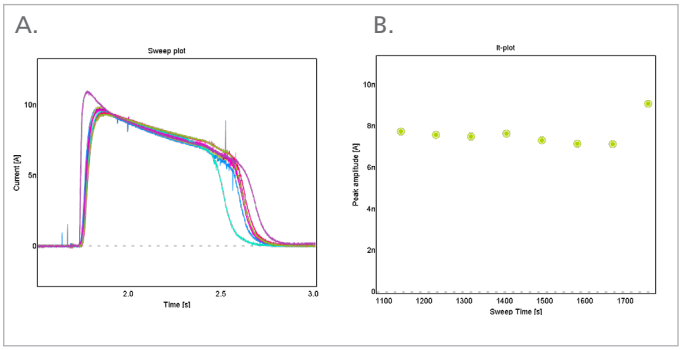


**Fig. 1:** Glycine-induced current verified the expression of functional hGlyRα1. A) Current trace from one cell showing the response to 7 consecutive applications of 100 µM glycine followed by a 1mM glycine application. Note that the current is outward going, as the intracellular ringer is $Cl^-$ free. B) Plot of peak current over time from the same cell.

Before using the assay to optimize the transfection protocol, we investigated which QChip configuration should be used: one patch hole or multiple patch holes per recording site:

## QChip optimization

### Patch hole number

To accommodate cell lines with low expression, heterogeneous expression, or low transfection rate, QChips with multiple patch hole numbers per site have been designed, where the membrane conductance of several cells will be summed. Increasing the number of patch holes per site will magnify and homogenize the signal, but both the leak and noise will also increase and the chance of having one low resistance seal, which will cause the entire site to fail.

We evaluated the impact of different numbers of patch holes per site by using a special QChip with varying number of patch holes (1, 2, 6, 10, 16, 36) in different measurement sites.

The experiments were filtered using the following settings:

**Resistance**: Site resistance x number of patch holes ≥ 10 mΩ
**Current:** Peak amplitude ≥ 500 pA

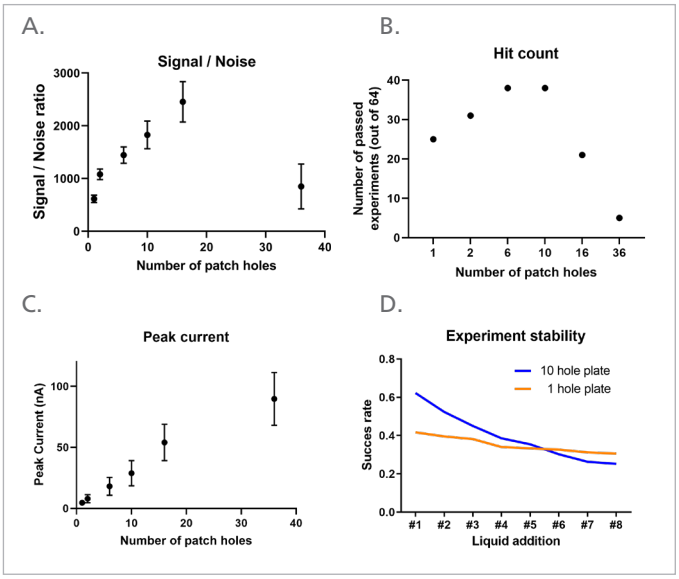


**Fig. 2: Evaluation of patch hole number**
A) The signal to noise ratio increased with increasing hole numbers, except for sites with 36 holes. The noise was calculated as RMS B) the success rate increased with increasing hole numbers until 10 holes, where it started decreasing dramatically. C) As expected, the peak amplitude increased proportionally to the number of patch holes. D) 10 patch holes per site will magnify and homogenize the current signal but will also increase the chance of losing a cell through the course of the experiment. Despite starting at a lower success rate, in the end, more experiments were successfully completed using a single hole QChip Compared to a multi-hole QChip.

Using the unoptimized transfection protocol, the low transfection efficiency favoured the use of QChips with 10 patch holes per site. However, experiments using 10-hole QChips can suffer from losing a cell as it will cause the site resistance to drop. We therefore investigated the success rate over 8 liquid additions (Figure 2D) and found that with the current experimental settings, the single hole QChip had more successful experiments at the end of the experiment.

Hence, the final assay was performed using a single hole QChip.

## 2. Optimizing electroporation transfection protocol

Using the designed glycine assay, the transfection protocol could be optimized with experimental success (patch rate) and ion channel expression (hit rate).

Cells transfected under different conditions were run simultaneously using 4, 8 or 16 mode ccCTPs (see Figure 3).

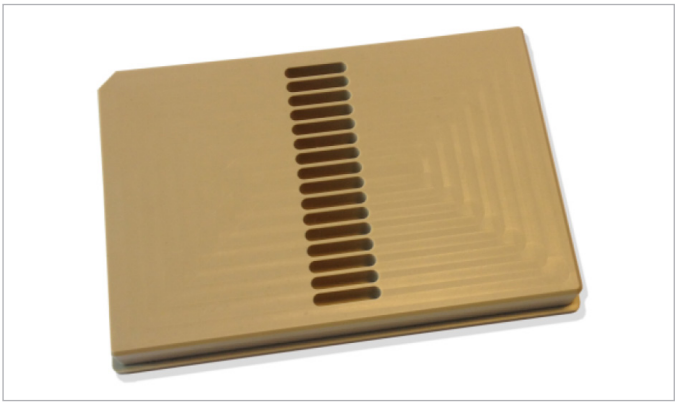

**Figure 3:** Cell clone Cell Transfer Pate (ccCTP) with 16 chambers. Qube can be used for testing different cell clones or different cell lines on the same QChip. To do this, the optional CTPs with either 4, 8 or 16 (shown here) chambers should be used.

## Electroporation transfection parameters

Electroporation is mainly dependent on the combination of three electric parameters: the electric field, pulse width, and pulse number. Based on the initial results, using the standard settings for HEK 293 cells, an optimization of the electroporation parameters was conducted to finetune the settings (see Table 1). The following combination of settings was tested:

**Table 1:** Electroporation transfection parameter settings. The electroporation parameters: electric field, pulse width, and pulse number were finetuned by testing 2-3 different values.

| Reference | Pulse Voltage | Pulse Width | Pulse No | |
|---|---|---|---|---|
| #1 | 1100 | 20 | 1 | |
| #2 | 1100 | 20 | 2 | * |
| #3 | 1100 | 30 | 1 | |
| #4 | 1100 | 30 | 2 | |
| #5 | 1200 | 20 | 1 | |
| #6 | 1200 | 20 | 2 | |
| #7 | 1200 | 30 | 1 | |
| #8 | 1200 | 30 | 2 | |
| #9 | 1500 | 30 | 1 | |
| #10 (no DNA) | 1100 | 20 | 2 | |

*Recommended by manufacturer

### Experimental success (Patch rate)

The impact of the electroporation and receptor expression on cell health and viability might not only reduce the number of cells in the culture flask but also render the cells more fragile, potentially lowering the seal quality, gigaseal rate and experimental lifespan. A set of quality control filters was set up to evaluate experimental success (resistance ≥ 100 MΩ, capacitance ≥ 4, successful sweeps in experiment ≥ 8). Note, an experiment was considered successful despite an unsuccessful transfection. The result is listed in Table 2.

### Ion channel expression (Hit rate)

The glycine receptor current amplitude varies between the transfected cells. Despite the actual transfection rate being higher, only the cells displaying a glycine receptor-mediated current larger than 200 pA was considered a hit (see Table 2).

### Success rate

The hit rate is highly dependent on the electroporation parameters. Still, the same applies to patch rate (the transfection can hamper cell health and lower the number of cells successfully passing through an experiment). Therefore, to determine the optimal electroporation parameters, the success rate was corrected for experiment success and ion channel expression (hit rate divided by patch rate, see Table 2 for example).

**Table 2:** Electroporation transfection parameter optimization. Parameter reference (Table 1), experimental success (Patch rate), Ion channel expression (Hit rate) and overall success rate (hit rate divided by patch rate) when the parameter settings from Table 1 was applied. Note that the DNA concentration has not yet been optimized.

| Group Name | Patch rate (%) | Hit rate (%) | Success rate (%) | n |
|---|---|---|---|---|
| Group #1 | 77.8 | 11.8 | 15.2 | 144 |
| Group #2 | 68.8 | 25.0 | 36.4 | 48 |
| Group #3 | 72.9 | 20.8 | 28.6 | 48 |
| Group #4 | 75.0 | 33.3 | 44.4 | 48 |
| Group #5 | 58.3 | 10.4 | 17.9 | 48 |
| Group #6 | 60.4 | 14.6 | 24.1 | 48 |
| Group #7 | 79.2 | 29.2 | 36.8 | 48 |
| Group #8 | 61.8 | 35.4 | 57.3 | 144 |
| Group #9 | 42.7 | 22.9 | 53.7 | 96 |
| Group #10 | 88.5 | 1.0 | 1.2 | 96 |
| **Sum** | | | | **768 (2 QChips)** |

Based on these optimization experiments, the electroporation transfection parameters were set to (group #8):

| | |
|---|---|
| Pulse Voltage (V) | 1200 |
| Pulse Width | 30 |
| Pulse No | 2 |

### DNA content optimization

With the electroporation transfection parameters set, the DNA amount could be optimized. 4 different DNA amounts were tested (for each 100 µL tip): 2 µg, 5 µg, 10 µg and 30 µg. When using 10 µg DNA, the viability increased, and with that, both the hit rate and transfection efficiency increased:

**Table 3:** Result from DNA concentration optimization

| Group Name | Patch rate (%) | Hit rate (%) | Success rate (%) | n |
|---|---|---|---|---|
| 10 µg | 87.5 | 77.1 | 88.1 | 96 |

## 3. Pharmacological evaluation of Glycine receptor transfected cells

We tested the pharmacological properties of the monomeric α1 glycine receptors to demonstrate that the electroporation transfected cells indeed show high performance in a patch-clamp study. First, the concentration-response relationship of glycine was evaluated and second, using one QChip, two positive and two negative modulators were characterized: alfaxalone (pos), Ethanol (pos), Picrotoxin (neg), and Bicuculline (neg).

### Cumulative Glycine concentration-response

The concentration-response relationship of glycine on the monomeric α1 glycine receptor was evaluated on Qube. Glycine was applied in increasing concentrations (3-fold dilution from 1000 µM, Figure 4).

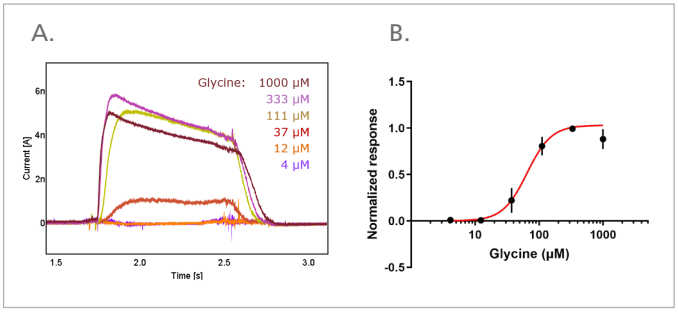


**Figure 4:** Cumulative concentration-response relationship of glycine. A) Typical recorded currents in response to increasing concentrations of glycine. B) Average peak current (normalized to the highest value in the experiment, average ± SD, n=49) as a glycine concentration function. The $EC_{50}$ value for the cumulative concentration-response was found to be 57 µM (CI95%: 54 to 60 µM), and the Hill slope was 2.8 (±0.2, SD).

### Pharmacological evaluation

We designed a layout of the compound plates that allowed the evaluation of four compounds simultaneously, using one QChip. The experiment results include cumulative ethanol, alfaxalone, picrotoxin and bicuculline (see Figure 5 to Figure 8). The positive modulators (ethanol and alfaxalone) were evaluated in the presence of 30 µM glycine (app. $EC_{20}$) and the negative modulators (picrotoxin and bicuculline), were evaluated in the presence of 100 µM Glycine (app. $EC_{80}$).

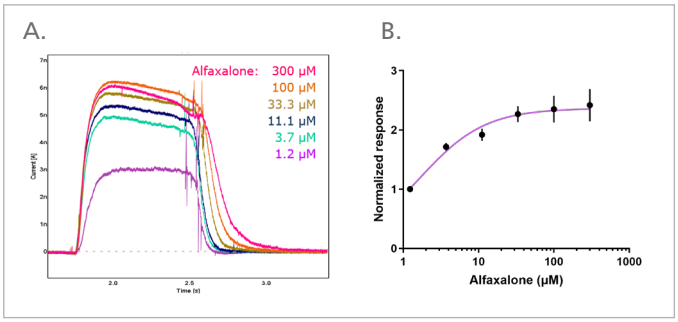


**Figure 5:** Potentiation of the glycine response by increasing alfaxalone concentrations in the presence of 30 µM glycine. A) Typical recorded currents traces. B) Average peak current (normalized to 30 µM glycine response, average ± SD, n=48). The $EC_{50}$ value was found to be 4.4 µM (CI95%: 2.6 to 8.8µM).

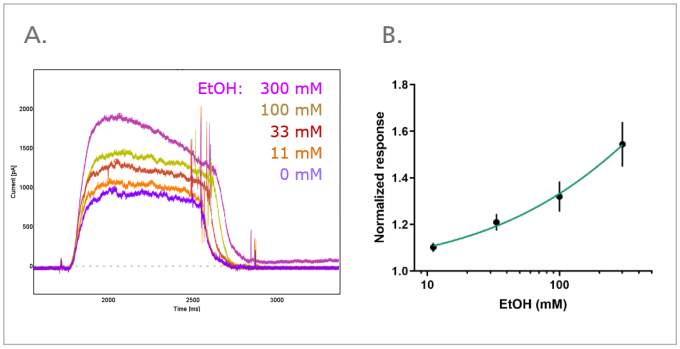


**Figure 6:** Potentiation of a 30 µM glycine response by increasing concentrations of ethanol. A) Typical recorded currents traces. B) Average peak current (normalized to 30 µM glycine response, average ± SD, n=41). The $SC_{50}$ value (stimulatory concentration 50%, the concentration giving a 50% increase in response) was found to be 250 mM (CI95%: 160 to 364 mM).

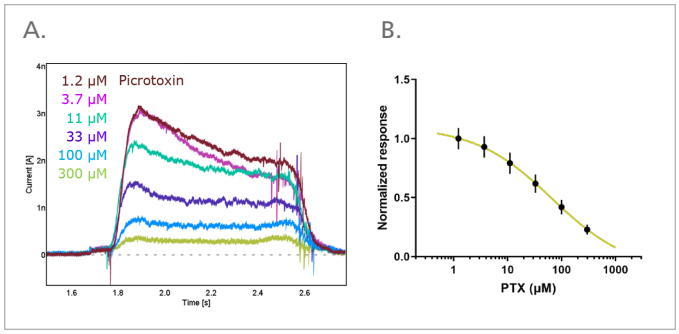


**Figure 7:** Inhibition of a 100 µM glycine response by increasing concentrations of picrotoxin. A) Typical recorded currents traces. B) Average peak current (normalized to 100 µM glycine response, average ± SD, n=30). The $IC_{50}$ value was found to be 69 µM (CI95%: 61 to 78 µM).

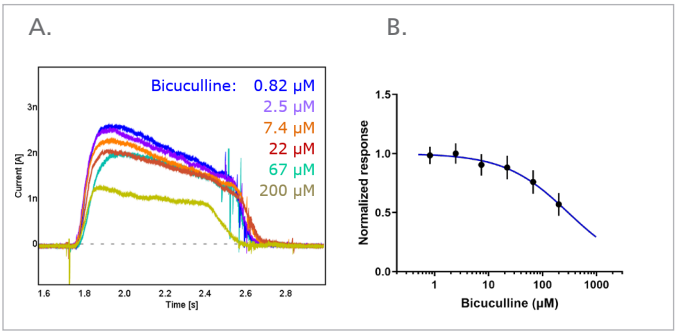


**Figure 8:** Inhibition of a 100 µM glycine response by increasing concentrations of picrotoxin. A) Typical recorded currents traces. B) Average peak current (normalized to 100 µM glycine response, average ± SD, n=30). The $IC_{50}$ value was found to be 69 µM (CI95%: 61 to 78 µM).

## Methods

### Cells and cell culture

Experiments in this study were performed using HEK 293 cells. HEK 293 cells were harvested with Detachin, spun down and resuspended in a serum-free medium with HEPES. For more information, contact your application scientist.

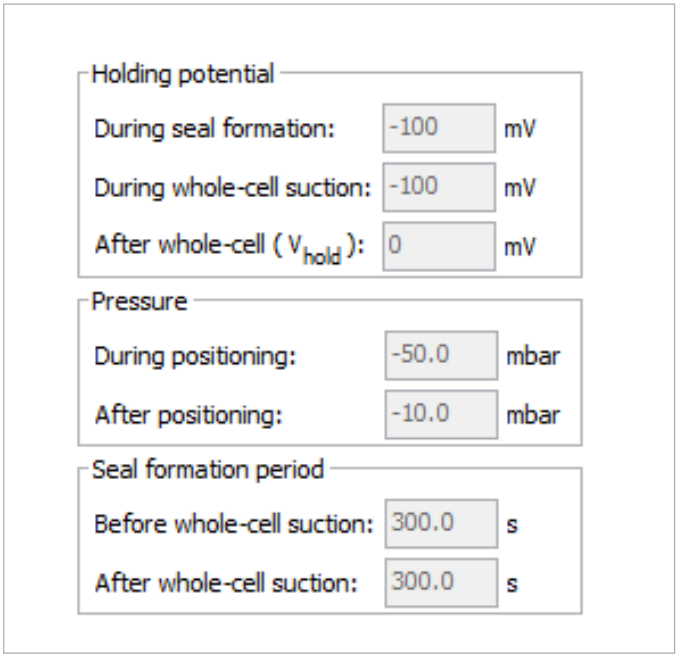


**Fig. 9:** Detailed parameters of the whole-cell protocol.

$IC_{50}$ values were estimated using the "Normalized Group Hill Fit with Error" method. All results and figures are presented as average values ± Standard Error (SD), n = number of QChip sites which passed QC-filters.

### Whole-cell protocol

A two-second suction pulse from -10 mbar to -250 mbar was followed by 10 seconds at -10 mbar, and after that a two-second suction pulse from -10 mbar to -350 mbar was applied. For more parameters, see Figure 9

### Experiment protocol

Cells were held at 0 mV throughout the entire experiment. The intracellular ringer contained fluoride to enhance the seal resistance and no chloride, hence the outward going current.

Liquid stacking in the pipettes enables ligand exposure times down to 0.8 s; however, the exposure time can be precisely customized. For more information, see reference 8 or contact your application scientist.

Quality control filters (if not otherwise stated)
Resistance (Sweep filter) ≥ 100MΩ per cell
Passed sweeps ≥ 1
Current ≥ 500 pA
Whole-cell capacitance ≥ 4 pF

### Data Analysis

**References:**

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