SOPHION BIOSCIENCE

Application Report

Reduced cell consumption on QPatch II and Qube 384 while maintaining high success rates

Cell density can be reduced with a factor 6 without compromising throughput and assay quality.

Summary

Recommended cell concentration on both QPatch II and Qube depends on both assay complexity, assay longevity and especially on cell quality. We generally recommend having a cell density of $3x10^6$ cells/ml on both Qube and QPatch II. However, the cell consumption for both systems can be reduced significantly, with a factor of at least 6, and still ensure success rates above 90%. We recommend that optimal cell concentrations are evaluated when working with cells that are expensive or in short supply. Furthermore, QPatch II can now be loaded with as little as 50 µl cells for a single experiment.

Introduction

We recommend the use of an excessive number of cells in the onboard cell hotel (QStirrer) on our automatic patch-clamp systems to avoid that low cell numbers are the limiting factor of an experiment. However, with the progressive development in cell technologies, e.g. induced stem cells and primary cell cultures, there is sometimes a wish to reduce the cell consumption to preserve cells that are expensive to produce and often low in number. We currently advise using $1.2\text{-}1.5x10^4$ cells per measuring site. In this study, we evaluate the effect of lower cell numbers on the overall success rate, to identify the lowest quantity of cells per measurement site that will still ensure a success rate above 90%. As model cell, we used a Rhabdomyosarcoma cell line, which endogenously expresses a Na_V.

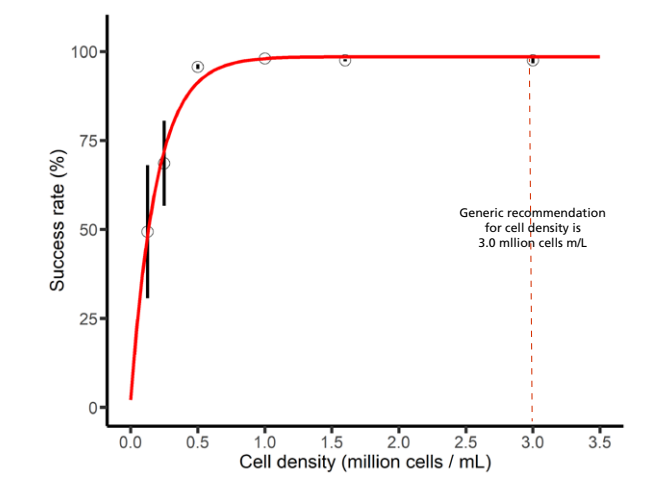

Fig. 1: QStirrer cell density vs success rate plot for Qube 384 in a Na_V assay.

Table 1: Cell density and success rate on Qube 384.

n	QStirrer cell density (10^6/ml)	Cells added per measuring site	Success rate ± STDEV (%)
4	0.125	500	49 ± 32
4	0.250	1,000	69 ± 27
3	0.500	2,000	96 ± 1
3	1.0	4,000	98 ± 0
5	1.5	6,000	98 ± 1
3	**3.0**	**12,000**	**98 ± 2**

The overall success rate of the Na_V assay on Qube (Fig. 1) is at least 90%, when the cell density is 500,000 cells/ml or more, i.e. down to a factor of 6 times lower than standard density. This corresponds to as little as 2,000 cells per site. Still, with the recommended use of $3x10^6$ cells/ml (bold in Table 1), we obtain a 98% success rate after all quality filters have been applied.

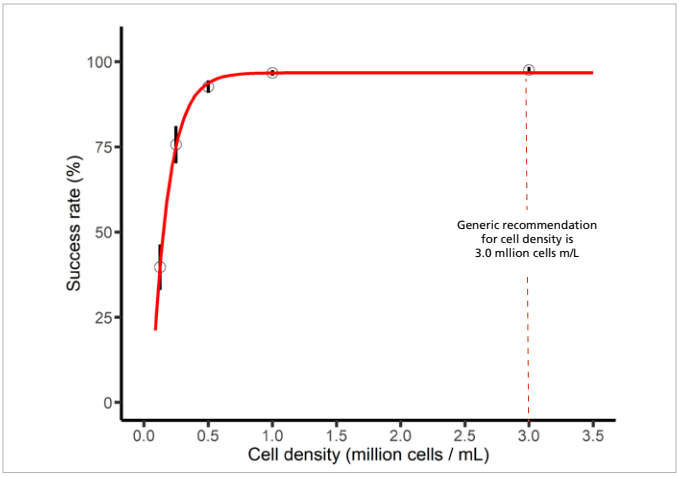

Fig. 2: Cell density vs. success rate on QPatch II in a Na_V assay.

Table 2: Cell density and success rate on QPatch II.

n	QStirrer cell density (10^6/ml)	Cells added per measuring site	Success rate ± STDEV (%)
3	0.125	1,875	40 ± 12
3	0.25	3,750	75 ± 9
6	0.5	7,500	93 ± 5
6	1.0	15,000	97 ± 2
4	**3.0**	**45,000**	**98 ± 2**

The generic recommended cell concentration for QPatch II is $3x10^6$ cells/ml corresponding to 15,000 cells per measurement site. Lowering the cell concentration with a factor of 6, to 0.5x106 cells/ml did not decrease the success rate significantly (Fig. 2) and resulted in a success rate of 93% ± 5% (Table 2). This clearly underlines the option of using lower cell numbers and still maintain high success rates.

Total cell volume down to 50 µl

Qube uses 4 ml starting volume per QChip and QPatch II uses 1,4 ml per QPlate. They add 4 µl respectively 5 µl per experiment site. This has all been designed to obtain high stable success rates, as demonstrated above, and reduces risk of human errors leading to loss of experiments. However, QPatch II can execute automatic experiments with as little as 50 µl starting volume of cell suspension, thus reducing cell consumption more than 25 times compared to standard conditions. So even with the most inaccessible cell types, it is still possible to conduct automatic patch clamping.

Conclusion

The cell density can be drastically lowered on both Qube and QPatch II without compromising the success rate. Using 500,000 cells/ml in the QStirrer is sufficient to ensure success rates above 90% on both Qube and QPatch II. While this study demonstrates that we can reduce the cell consumption on both platforms, our generic recommendation remains unaltered, since the optimal cell density can vary for different cells. We continue to recommend evaluating cell density for each individual cell line and assay before running a larger screen or setting up standard assays.

Methods

The human Muscle Rhabdomyosarcoma (RD) cells endogenously expressing a voltage-gated current (Na_V) were prepared.

We used an assay setup resembling the Quality Control for our QChips assay on Qube for testing and assessing the success by looking at the overall membrane resistance throughout the experiment. The same set of experiments were performed on QPatch II.

We initially assessed at which cell density the success rate dropped below our acceptance level and selected a new test matrix to be carried out using the automatic cell preparation.

The RD cells were placed in the QStirrer in the density desired for testing according to the new test matrix. The Instruments automatic cell preparation system prepared the cells, and the experiments were carried out automatically by the system.

The cell densities in the QStirrer for Qube were:

1. $3.0x10^6$/ml
2. $1.5x10^6$/ml
3. $1.0x10^6$/ml
4. $0.5x10^6$/ml
5. $0.25x10^6$/ml
6. $0.125x10^6$/ml

The cell densities in the QStirrer for QPatch II were:

1. $3.0x10^6$/ml
2. $1.0x10^6$/ml
3. $0.5x10^6$/ml
4. $0.25x10^6$/ml
5. $0.125x10^6$/ml

Solutions used in the experiments:

Extracelluar Ringer solution EC000 (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose. The pH value was set to 7.4 with 5 M NaOH, and the osmolarity was adjusted to 300 mOsm with sucrose before use.

Intracellular Ringer solution IC500 (in mM): 140 CsF, 1 EGTA/ 5 CsOH, 10 NaCl, 10 HEPES. The pH value was adjusted to 7.3 with 3 M CsOH and the osmolarity was adjusted to 320 mOsm with sucrose.

The experimental time course was constructed with three liquid periods; two with saline and one with 30 µM tetracaine. To demonstrate reproducibility, the experiments were carried out on different days, so no identical cell densities were tested on the same day.

The experiment results were analyzed in Sophion Analyzer adhering to the criteria used in our quality control for our QChips.

- Minimum seal resistance: 300 MΩ
- Baseline current amplitude should be greater than 500 pA.
- Experiment duration after whole cell obtained ≥ 25 min.
- Residual current after application of 30 µM tetracaine should be less than 20% of baseline.
- Rundown max 20%

The success criteria for the assay on QPatch II were:

- Minimum seal resistance throughout the experiment: 100 MΩ
- Baseline current amplitude should be greater than 500 pA.
- Experiment duration after whole-cell obtained ≥ 25 min.
- Residual current after application of 30 µM tetracaine should be less than 20% of baseline.
- Rundown max 20%

References:

Andrew Randall, Nicole McNaughton, Paula Green, 2006. Properties of voltage-gated Na^+ channels in the human rhabdomyosarcoma cell-line SJ-RH30: Conventional and automated patch clamp analysis, Volume 54, Issue 2, August 2006, Pages 118-128

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