Rapid generation of cells for ion channel assays: efficient, large-scale transfection using the MaxCyte® STX™ system, convenient cell culture using Corning® HYPERFlask™ vessels, and robust target activity assayed by the Sophion QPatch.

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MaxCyte® TRANSFECTION SYSTEMS

Abstract

The MaxCyte® STX™ Scalable Transfection System uses a proprietary flow electroporation technology that can transfect up to 1E10 cells with target, reporter and protein expression plasmids, as well as other molecules, in less than 30 minutes. Transfected cells can be assayed immediately or cryopreserved for future use. Here we demonstrate the use of the MaxCyte STX system in coordination with Corning's® HYPER*Flask*® Cell Culture Vessel to provide an efficient and economical solution for culturing large numbers of adherent CHO cells before and after transfection. The HYPER*Flask* Cell Culture Vessel features Corning's HYPER (High Yield PERformance) technology, which utilizes a gas permeable film to provide gas exchange between the internal culture environment and the external atmospheric environment. The unique, space-saving, 10-layer film design results in 1720 cm^2 cell growth surface area, which is approximately 10 times that of a normal T-175 flask. We also show that cells transfected with a plasmid encoding a K_v1.3-GFP fusion protein exhibited good seal formation and strong potassium currents following large scale electroporation and cryopreservation. Ion channel activity was assayed on the Sophion QPatch automated patch clamp system. High viability and consistent levels of expression were obtained in three independent large scale electroporations, illustrating robustness and reproducibility of the transfection process. These results demonstrate that the MaxCyte STX system offers a time and labor saving alternative to stable cell line generation for ion channel assays.

MaxCyte® STX™ Scalable Transfection System

Transiently Transfect up to 1E10 Cells in <30 Minutes

- Simple
- Rapid
- High efficiency
- Broad cell type compatibility
- Scalable

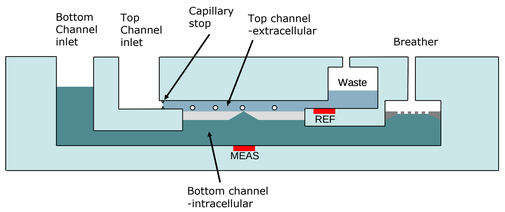
Figure 1. MaxCyte® STX™ Scalable Transfection System. The MaxCyte STX uses a proprietary, scalable electroporation technology to (co)transfect a variety of cell types, including primary cells, with DNA, RNA, siRNA, proteins or other biomolecules of interest. MaxCyte has developed electroporation protocols optimized for a wide range of cell types, simplifying assay development while maximizing performance and reproducibility. Transfection efficiencies are routinely greater than 85% and cell viability greater than 90%. Transfected cells can be used immediately following electroporation or cryopreserved for future use. The MaxCyte STX can perform small-scale transfections for basic Ion Channel research and assay development or perform bulk transfections for use in full-scale, screening and profiling.

Sophion QPatch

Fully Automated Patch Clamp System

QPatch Instrument

QPlate liquid handling



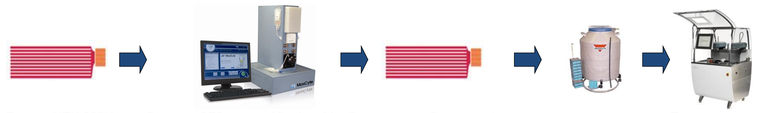
QPlates

16 or 48 patch clamp sites with intra- and extracellular flow channels

Figure 2. Sophion QPatch system. Sophion provides advanced products and integrated solutions for ion channel drug discovery and safety testing. Sophion's unique QPatch automated patch clamp system has an integrated cell preparation and QPlate exchange facilities enabling several hours of unattended operation. The QPlate microfluidic system enables fast liquid exchange and allows testing of multiple compounds or increasing concentrations on the same cell.

Rapid and Scalable Approach to Generating Cells for Ion Channel Assays

Expansion in HYPERFlask Vessels and Transfection via Flow Electroporation



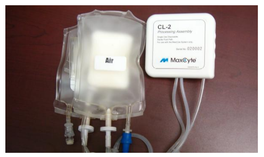
Expand HEK 293H cells in **Corning HYPER*Flask* vessel**

Suspend 3E8 cells in 10 mL of MaxCyte buffer with 150 µg/mL of plasmid encoding Kv1.3-GFP fusion protein, load into CL-2 processing assembly and transfect by **flow electroporation using ther MaxCyte STX.**

Seed transfected cells into Hyper*Flasks* (1.7E8 cells per flask), culture 24h and cryopreserve

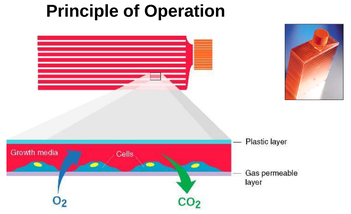
Thaw cells and assay on **Sophion Qpatch**

MaxCyte STX CL-2 Processing Assembly



- Sterile, closed single use design prevents contamination
- Variable capacity enables transfection of 1E8 to 1E10 cells in < 30 minutes
- Levels of transfection efficiency and viability are equivalent to those achieved by small-scale, static electroporation

Corning HYPER*Flask* Vessel Principle of Operation



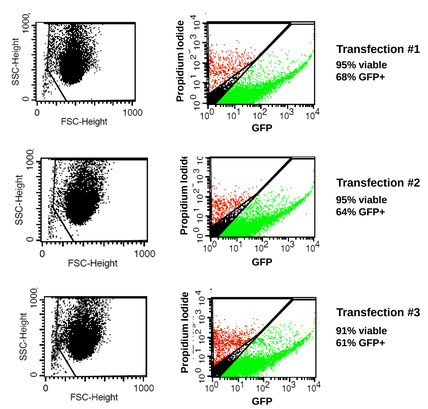
- A "Flaskette" is a cell culture compartment
- 10 Flaskettes are grouped together to form the HYPER*Flask* Vessel.
- The air gap is the space open to the atmosphere beneath each Flaskette. Gas exchange occurs between the Flaskette & the air gap through a film

Figure 3. Large-scale electroporation of HEK cells cultured in HYPER*Flask*® vessels pre- and post-transfection. HEK 293H cells were expanded in Corning HYPER*Flask* vessels, harvested with trypsin and resuspended in MaxCyte's electroporation buffer at 3E7 cells/mL. Hyper*Flasks* contain ten interconnected polystyrene film growth surfaces that allow for gas exchange directly into the medium, and offer 1720 cm^2 growth area in the footprint of a traditional 175 cm^2 flask. In this experiment, five HYPER*Flask* vessels yielded sufficient cells for three large scale transfections (3E8 cells per sample). Transfections with a K_v1.3-GFP expression plasmid were performed with the MaxCyte STX system in sterile, single-use CL-2 processing assemblies. Following a 20 min. recovery, cells were returned to a HYPER*Flask*, cultured for 24 hrs and cryopreserved in 90% FBS/10% DMSO.

Expression of a K_v1.3-GFP Fusion Protein Following Flow Electroporation

High Viability, Reproducible Efficiency and Functional Ion Channel Activity

A. FACS Analysis Prior to Cryopreservation



B. QPatch Assay with Frozen Cells

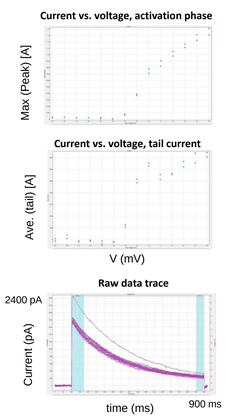


Figure 4. Analysis of GFP expression, viability and ion channel activity in HEK cells transfected with K_v1.3-GFP DNA. HEK 293H cells were harvested 24 hrs post electroporation with a K_v1.3-GFP expression plasmid using MaxCyte flow electroporation. **A.** FACS analysis of GFP expression and viability prior to cryopreservation showed reproducible levels of transfection efficiency and high viability in three sets of transfected cells. **B.** Representative data generated on the Sophion QPatch in single hole mode with thawed cells illustrate that cryopreservation does not impact the performance of transfected cells on the QPatch platform.

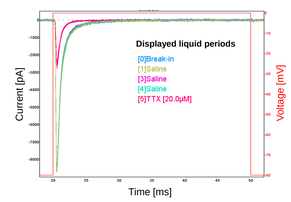
Robust Sodium Channel Activity in Transiently Transfected Cells

High expression and strong currents detected by Sophion QPatch

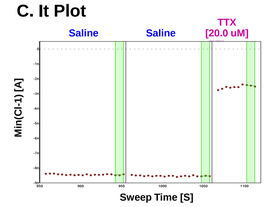
A. Na_v1.5 Activity 48 hrs post electroporation

[DNA]	Test condition	Transfection efficiency	Average current level*	TTX block*
[µg/ml)]	Temp. [°C]	[%]	[nA]	[%]
100	28	57	-6.3	65
150	28	100	-5.7	71
200	28	80	-3.6	82
250	28	75	-3.5	86
100	37	82	-2.5	77
150	37	89	-2.0	79
200	37	83	-3.8	79
250	37	42	-3.0	82

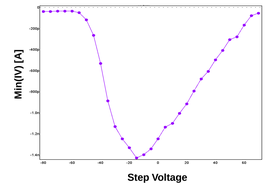
B. Sweep Plot



C. It Plot



D. IV Plot



* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 mM TTX single addition

Figure 5. Analysis of Na_v1.5 ion channel activity. Cells were transfected with four concentrations of a plasmid encoding the voltage-gated sodium channel Na_v1.5 (kindly provided by Thomas Jespersen, Copenhagen University). Transfected cells were cultured for 24 hrs at 37°C. Half were transferred to 28°C and half were kept at 37°C for an additional 24 hrs. **A.** QPatch assays in single hole mode showed good expression and robust currents in all four sets of cells. Optimal results were obtained with 150 µg/mL of DNA and shifting the temperature to 28°C for 24 hrs prior to analysis. **B & C.** Raw current traces and time-current plot from a single cell illustrate typical Na channel activity and an expected response to a Na channel blocker (TTX). **D.** An IV curve in a voltage protocol stepped from -80 to +80 mV with 5 mV steps illustrates typical sodium channel activity in a transiently transfected cell. The IV curve was not liquid-junction corrected.

Analysis of Na_v1.5 Activity in Freshly Transfected and Frozen Cells

Cryopreservation does not inhibit ion channel activity

A. Na_v1.5 Activity Before Cryopreservation

Test condition	Transfection efficiency	Average current level*	TTX block**
Temp. [° C]	[%]	[nA]	[%]
28	93	-6.5	85
37	80	-5.2	85

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 mM TTX single addition

B. Na_v1.5 Activity Post Cropreservation

Test condition	Transfection efficiency	Average current level*	TTX block**
Temp. [° C]	[%]	[nA]	[%]
28	89	-5.4	81
37	88	-4.8	79

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 mM TTX single addition

Figure 6. Na_v1.5 channel activity in freshly transfected & cryopreserved cells. HEK 293 cells were transfected by static electroporation with 150 µg/mL of a Na_v1.5 expression plasmid. Transfected cells were cultured for 24 hrs at 37°C. Half of the cells were transferred to 28°C and half were kept at 37°C for an additional 24 hrs. Cells were harvested 48 hrs post transfection. Some cells were assayed on the Qpatch in single hole mode immediately after harvest; remaining cells were cryopreserved and assayed immediately after thawing. The overall success rates for fresh and frozen cells were 61% and 60%, respectively. Levels of transfection efficiency and average current levels were also consistent between the freshly transfected and frozen cells.

Summary

- Corning HYPER*Flask* vessels and MaxCyte's scalable electroporation technology enable the rapid generation of large numbers of cells for ion channel assays with minimal consumption of resources.
- MaxCyte's flow electroporation process produces consistent and reproducible results. Transfected cells exhibit high viability, robust transgene expression and good membrane integrity.
- Cells transfected with either potassium or sodium channels formed tight seals and exhibited robust currents on the Sophion QPatch. Assay performance was not impacted by cryopreservation.

MaxCyte®

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