Keys to Successful Cell-Based Assay Development with the MaxCyte® STX™ Scalable Transfection System

Introduction

The MaxCyte® STX™ Scalable Transfection System allows users to transfect up to $1x10^{10}$ cells with single or multiple plasmids encoding GPCRs, ion channels and other targets. The technology overcomes the time and cost constraints of assay development with stable cells. It also allows expression of toxic proteins and enables assay development with physiologically relevant cell types, including primary cells, stem cells and cells of hematopoietic origin. Below, we discuss some of the factors that are important for developing a successful cell-based assay using the MaxCyte STX.

DNA Preparation

DNA quality has a significant impact on both transfection efficiency and cell viability. Plasmid DNA can be prepared using commercially available endotoxin-free plasmid isolation kits. However, it is important to suspend the DNA in water rather than in TE buffer because EDTA can interfere with conductivity of the electroporation buffer. The $OD_{260/280}$ ratio should be greater than 1.8, and the DNA should be analyzed on an agarose gel to confirm that at least 85% of the plasmid is in a covalently closed, circular conformation and that the DNA does not show signs of degradation. Finally, when preparing plasmid stock solutions, the DNA should be suspended at high concentrations (preferably > 5 mg/mL) to avoid diluting the electroporation buffer after mixing DNA with the cells.

Cell Culture and Handling

The most critical factor for ensuring successful and consistent transfection results is to work with cells that are healthy and in log phase growth. Cells should be split one day prior to transfection, and adherent cells should be subconfluent when harvested for electroporation. Freshly thawed cells generally do not transfect as well as cells that have been passaged at least once between thawing and electroporation. Conversely, late passage cells are more likely to yield poorer transfection results compared to early passage cells. In addition, it is important to make sure that cells are completely dissociated during the trypsinization and washing steps prior to electroporation because cell clumping can reduce transfection efficiency.

Cells should be removed from the processing assembly immediately after electroporation and allowed to recover at 37°C for 20 minutes. It is important not to add media or otherwise dilute the cells during the recovery period. Also, it is not advisable to try to recover more cells by rinsing out the processing assembly. Allowing the cells to spread out in a multiwell plate or empty culture vessel during the 20 minute recovery period may increase oxygen transfer and improve cell viability.

DNA Titration

The MaxCyte electroporation process has minimal impact on cell viability. However, viability is correlated inversely with the amount of DNA that is loaded into the cell due to a phenomenon known as DNA toxicity. Figure 1 illustrates how increasing concentrations of plasmid DNA lead to higher transgene expression but lower viability in HEK 293H cells.

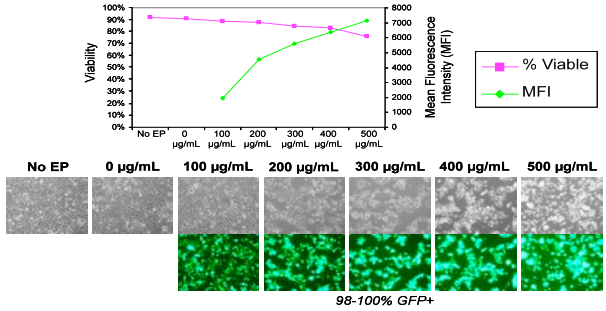

Figure 1. Inverse correlation between transgene expression and cell viability in transiently transfected HEK 293H cells. Cells were transfected with increasing concentrations of GFP expression plasmid DNA in OC-100 processing assemblies and analyzed by FACS at 24 hours post electroporation. Viability was assessed by propidium iodide exclusion.

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When developing an assay with a new cell type or loading agent, users should perform several small scale transfections in OC-100 or OC-400 processing assemblies to identify a DNA concentration that yields optimal levels of assay responsiveness and cell viability. After optimizing the DNA concentration, the reaction can be scaled up using the same concentration of DNA to transfect cells via flow electroporation in a CL-2 processing assembly.

It should also be noted that some target proteins can reduce cell viability when expressed at high levels. Therefore, DNA optimizations should be performed with the target of interest rather than with GFP or empty vector. However, it is recommended to perform parallel electroporations with a constitutive reporter plasmid and with no DNA to determine the relative effects of electroporation, DNA toxicity and target toxicity on cell viability.

Plating and Analysis

Cells transfected with the MaxCyte STX can be plated in a variety of multiwell plates or assay chambers. Assay responsiveness will depend on the number of cells plated per well and on the length of time between transfection and analysis. Therefore, when developing a new assay, it is advised to plate varying numbers of cells in different wells and to assay the cells at several different time points after electroporation. In some cases, cells can be assayed within 6 hours after transfection, whereas other assays may require multiple days to reach peak sensitivity. When screening ion channels, some users report that placing transfected cells in a 28°C incubator for a period of time will slow the rate of cell division, producing a greater concentration of ion channels on the cell surface and leading to higher expression levels in automated planar patch clamp assays.

Cryopreservation

Another advantage to the MaxCyte STX is that cells can be transfected in bulk, aliquoted, and cryopreserved for future use. However, different cell types, targets and assay applications will require different cryopreservation regimens. In some cases, cells can be cryopreserved immediately after the 20 minute post electroporation recovery period. In other cases, cells need to be plated or cultured for a period of time before cryopreservation. Culturing adherent cells overnight allows users to wash away any nonviable cells prior to freezing. Also, cells can be cryopreserved when gene expression is at its peak, allowing the cells to be assayed soon after thawing. A third alternative is to put the cells in medium and culture them without plating for one or two hours prior to cryopreservation. This often will improve viable cell recovery after thawing with cell types or targets that do not respond well to immediate freezing. It is recommended that users test several different cryopreservation regimens for each new cell type or target. Figure 2 shows a typical experimental plan for optimizing cryopreservation conditions.

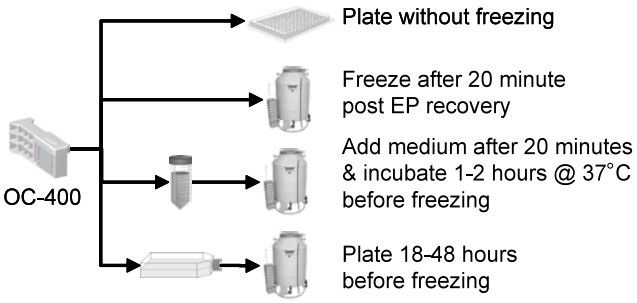

Figure 2. Optimizing cryopreservation conditions with transfected cells. After transfecting $4x10^7$ cells in an OC-400 processing assembly and allowing the cells to recover at 37°C for 20 minutes, the cells are divided into four treatment groups: 1) plate without freezing and assay at 2-3 time points after transfection; 2) freeze immediately; 3) incubate in medium for 1-2 hours prior to freezing; and 4) plate 18-48 hours before freezing.

MaxCyte STX users commonly freeze cells in 10% DMSO with serum concentrations ranging from 20-90%. Commercially available cryopreservation solutions also work well with MaxCyte STX transfected cells. Most users cryopreserve cells in ethanol jacketed, polycarbonate containers that are placed at -80°C for 18-48 hours prior to long term storage in liquid nitrogen.

Summary

- ✓ High quality DNA and healthy cells are critical for achieving optimal results with the MaxCyte STX scalable transfection system.
- ✓ To minimize the effects of DNA toxicity and to maximize assay performance, MaxCyte STX users should perform several small scale, static electroporations with varying concentrations of DNA in OC-100 or OC-400 processing assemblies prior to conducting a large scale, flow electroporation using a CL-2.
- ✓ A systematic approach to optimizing post electroporation analysis and cryopreservation conditions will allow users to maximize the capabilities of the MaxCyte STX technology and achieve the best possible assay results with their transfected cells.

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