The MaxCyte STX Scalable Transfection System, which is based on a proprietary flow electroporation technology, provides a labor and cost saving alternative to generating stable cell lines for screening a variety of drug targets, including GPCRs. Up to 1E10 cells can be transfected with plasmid DNA, mRNA, siRNA, protein or other molecules in less than 30 minutes yielding viability and efficiency levels that exceed 90% with most cell types. The ACTOne™ platform is based upon a modified cyclic nucleotide-gated (CNG) ion channel that serves as a biosensor of cAMP activity in live cells, allowing sensitive detection of signaling by Gs, Gi or Gq-coupled receptors. Here we demonstrate that the STX system enables rapid development of cell-based GPCR assays by transfection to cells with the ACTOne CNG channel. Transfected the CNG channel by itself allowed detection of multiple endogenous receptors in HEK cells. Co-transfecting the CNG channel with GPCR expression plasmids yielded assay performance comparable to stable cell lines. Finally, assay scale up and cryopreservation of transfected cells were performed to illustrate that the MaxCyte STX System combined with the ACTOne Biosensor technology provide a rapid, flexible and economical alternative to stable cell line production for screening GPCRs.

MaxCyte® STX™ Scalable Transfection System
Transiently Transfection up to 1E10 Cells in <30 Minutes

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

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 cryopreservation for future use. The MaxCyte STX system can perform small-scale transfections for basic research and assay development or perform bulk transfections for use in full-scale, screening and profiling.

Figure 1. MaxCyte® STX™ Scalable Transfection System. The MaxCyte STX system 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’s technology provides optimized protocols 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 cryopreservation for future use.

Figure 2. GPCR assays following large-scale electroporation and cryopreservation. HEK cells were cryopreserved 20 min. or 20 hrs. prior to electroporation with the ACTOne Biosensor CNG plasmid. Both sets of cells showed concentration-dependent responses to isoproterenol and NECA that were comparable to the assay performances of freshly transfected cells (Figure 4). The Y axis shows levels of fluorescence after compound addition relative to levels before compound addition. Cells were cryopreserved at 20 min. exhibited greater assay sensitivity, reflecting the rapid kinetics of transgene expression post electroporation.

Figure 3. Rapid, small-scale GPCR assay development in HEK cells. HEK 293H cells were transfected in QC-100 processing assemblies with 3 different concentrations of plasmid encoding the ACTOne CNG biosensor. After overnight culture, cells were exposed to a dye that detects changes in membrane potential and treated with varying concentrations of GPCPs and adenylyl cyclase activators. Changes in intracellular cAMP levels were quantified using a standard fluorescent plate reader.

Figure 4. Endpoint GPCR and adenylyl cyclase assays with transiently transfected cells. HEK 293H cells transfected with the ACTOne Biosensor CNG channel were treated with increasing concentrations of isoproterenol, NECA and forskolin to activate two different endogenous GPCPs and adenylyl cyclase, respectively. The Y-axis shows levels of fluorescence after compound addition relative to levels before compound addition. All three assays exhibited concentration-dependent responses to agonists. Assay windows correlated with DNA concentrations, demonstrating that the STX system allows users to calibrate assay sensitivity simply by adjusting the DNA concentration.

Figure 5. Real-Time Analysis of GPCR Activity in Transiently Transfected Cells. Kinetic data for the CNG-dependent ACTOne Biosensor.

- A. CNG Negative Cells
- B. CNG Transfected Cells

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CodexACTOne™ Biosensor Technology
Homogeneous, Live Cell Receptor Assay

A. ACTOne Biosensor detects physiological changes in cAMP levels
B. ACTOne is more sensitive than ELISA

Concentration-Dependent GPCR Activation

Figure 6. Transfected HEK cells with the ACTOne Biosensor CNG plasmid at large-scale via flow electroporation. 3E8 HEK cells were suspended in 10 ml of MaxCyte’s electroporation containing 100 µg/mL of a plasmid encoding the CNG channel. Cells were aliquoted and cryopreserved in 90% FBS/10% DMSO either 20 minutes or 18 hrs. after flow electroporation in a CL-2 processing assembly. Cells were transferred to multidwell plates immediately after thawing, and assayed following overnight culture.

Sensitive GPCR Assay Responses with Cryopreserved Cells

Bulk transfection minimizes inter-assay variation

A. β2 Adrenergic Receptor
B. Adenosine A2B Receptor

Maximize Productivity Using Cell Cryopreservation

One Transfection, Multiple Experiments

- No Electroporation
- Cryo. 20 min post GP
- Cryo. 20 hrs post GP

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