Enabling rapid and sensitive GPCR assay development with the MaxCyte® STX™ Scalable Transfection System and the Codex ACTOne biosensor technology.

James Brady[1], Jimmy Lu[2], Rama Shivakumar[1], Angelia Viley[1], Madhusudan Peshwa[1], Karen Donato[1], and Krista Steger[1]. [1]MaxCyte Inc., Gaithersburg, MD, USA. [2]Codex BioSolutions, Inc., Gaithersburg, MD USA

Abstract

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 transfecting cells with the ACTOne CNG channel. Transfecting 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

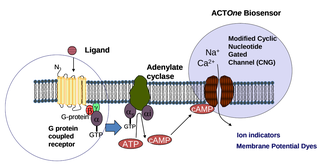
- Simple
- Rapid
- High efficiency
- Broad cell type compatibility
- Scalable

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 research and assay development or perform bulk transfections for use in full-scale, screening and profiling.

Codex ACTOne™ Biosensor Technology

Homogeneous, Live Cell Receptor Assay

A. ACTOne Biosensor detects physiological changes in cAMP levels



B. ACTOne is more sensitive than ELISA

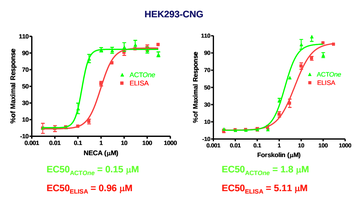


Figure 1. ACTOne Biosensor Technology. **A.** The ACTOne Biosensor technology is based upon a modified cyclic nucleotide-gated (CNG) ion channel which is used as a biosensor of cAMP activity in living cells. This channel responds in real-time to intracellular cAMP levels by altering ion flux (detectable by calcium-responsive dyes) and cell membrane depolarization (detectable with fluorescent membrane potential (MP) dyes). The ACTOne cAMP assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader. It is a simple homogenous assay involving only dye and compound addition steps, enabling easy implementation in a high-throughput environment. It has been successfully used to measure Gs and Gi coupled GPCR activity. A. Detection of cAMP with the ACTOne CNG channel. **B.** Measuring endogenous GPCR and adenylate cyclase activity in HEK cells stably transfected with the CNG channel and comparison to cAMP ELISA.

Cells to Results in 24 hours

High Throughput, Live Cell GPCR Assay

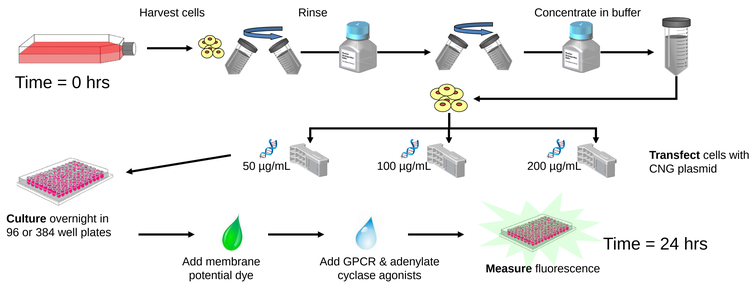


Figure 3. Rapid, small-scale GPCR assay development in HEK cells. HEK 293H cells were transfected in OC-100 processing assemblies with 3 different concentrations of plasmid encoding the ACTOne CNG biosensor. After overnight culture, cells were exposed to a dye that detects changes in membrane potential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

Concentration-Dependent GPCR Activation

cAMP Dose Response Curves

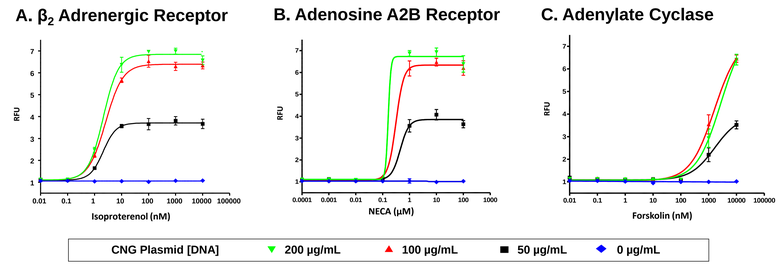


Figure 4. Endpoint GPCR and adenylate cyclase assays with transiently transfected cells. HEK 293H cells transfected with the ACTOne Biosensor CNG channel were treated with increasing concentrations of isoproterenol, NECA and forskolin to activate two different endogenous GPCRs and adenylate cyclase, respectively. The y-axis shows levels of fluorescence after compound addition relative to levels of fluorescence 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.

Real Time Analysis of GPCR Activity in Transiently Transfected Cells

Kinetic data for the CNG-dependent ACTOne Biosensor

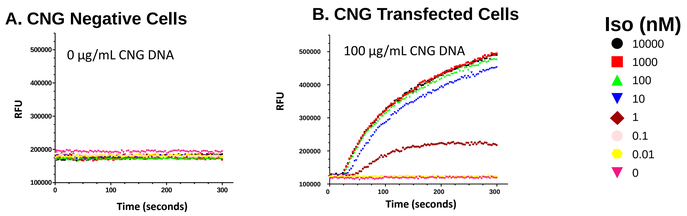


Figure 5. Kinetic GCPR assay with transiently transfected cells. HEK cells transfected with 100 μg/mL of the ACTOne Biosensor CNG plasmid were treated with a membrane potential dye and increasing concentrations of isoproterenol. A kinetic plate reader was used to measure the time course of fluorescence. Time- and concentration-dependent activation of the endogenous β_2-adrenergic receptor in response to isoproterenol is clearly evident in cells transfected with the CNG plasmid, but not in control cells that were electroporated in the absence of DNA.

Maximize Productivity Using Cell Cryopreservation

One Transfection, Multiple Experiments

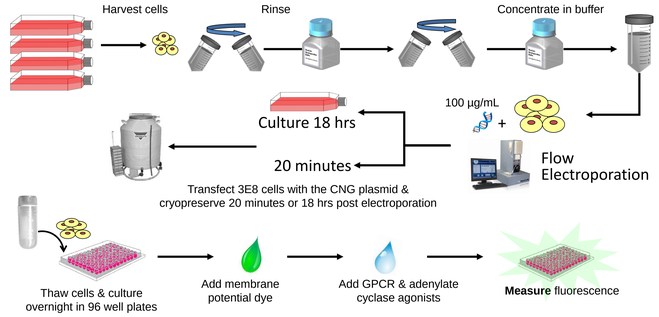


Figure 6. Transfecting HEK cells with the ACTOne Biosensor CNG plasmid at large-scale via flow electroporation. 3E8 HEK cells were suspended in 10 mL of MaxCyte's electroporation containing 100 μg/mL of a plasmid encoding the CNG channel. Cells were aliquoted and cryopreserved in 90% FBS/10% DMSO either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

Sensitive GPCR Assay Responses with Cryopreserved Cells

Bulk transfection minimizes inter-assay variation

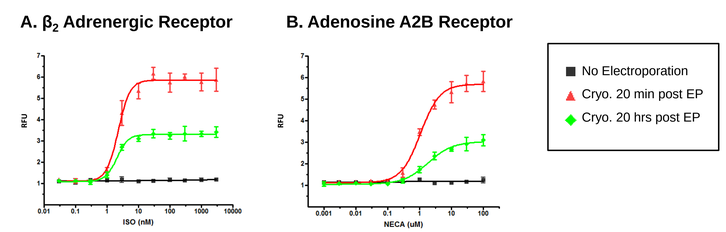


Figure 7. GPCR assays following large-scale electroporation and cryopreservation. HEK cells were cryopreserved 20 min. or 20 hrs. post electroporation with the ACTOne Biosensor CNG plasmid. Both sets of cells showed concentration-dependent responses to isoproterenol and NECA that were comparable to the 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 that were cryopreserved at 20 min. exhibited greater assay sensitivity, reflecting the rapid kinetics of transgene expression post electroporation.

Summary

- The MaxCyte STX scalable transfection system combined with the Codex ACTOne Bionsensor technology enables rapid generation of cells for GPCR assays and eliminates the need to create stable cell lines.
- Assay sensitivity can be adjusted simply by altering the concentration of DNA in the electroporation reaction.
- Large scale (flow) electroporation allows users to generate cells for multiple assays in a single transfection run.
- Cells can be cryopreserved following electroporation without impacting assay performance. Users have the flexibility to run assays at their convenience, and they can be assured of consistent assay responses by aliquoting cells from a single, bulk transfection.

Correspondence: Jim Brady at jamesb@maxcyte.com

MaxCyte®
Tel: (301) 944-1700
info@maxcyte.com
www.maxcyte.com