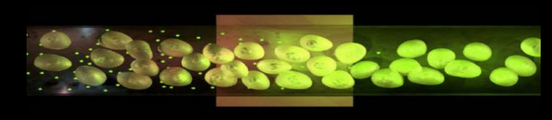
# GPCR and Ion Channel Functional Receptor Expression: Rapid & Reproducible Transient Transfection using a Scalable Electroporation Technology.



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## Abstract

Over expression of surface receptors such as GPCRs and Ion Channels is often used to facilitate their characterization and development as therapeutic targets. The MaxCyte® STX™ transfection system, based on a proprietary, scalable electroporation technology, can simultaneously transfect multiple plasmids into as few as $5x10^5$ cells in seconds or up to $1x10^{10}$ cells in less than thirty minutes. In this poster we present a series of case studies for the expression of three different GPCRs and three Ion Channels in commonly used CHO and HEK cell lines. We demonstrate their use in a variety of downstream functional assays including cAMP, FLIPR®, IonWorks® Quattro™ and PatchXpress® assays. Additionally, MaxCyte transient transfection is compared to lipid transfection reagents and stable cell lines. These data illustrate the utility of the MaxCyte STX as a means of reproducibly expressing GPCRs and complex multi-subunit ion channels and the superior performance of these transfected cells in downstream cellular assays. These data also demonstrate that MaxCyte electroporation eliminates the need for reliance on stable cell lines and costly transfection reagents, thus increasing laboratory productivity and maximizing budgets.

## MaxCyte Transfection

- 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.

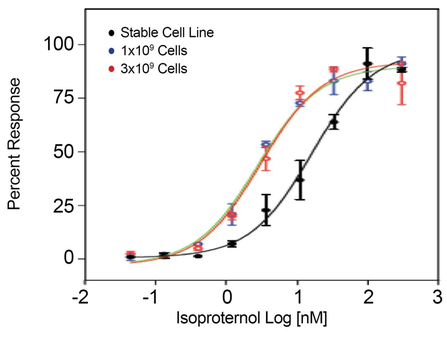
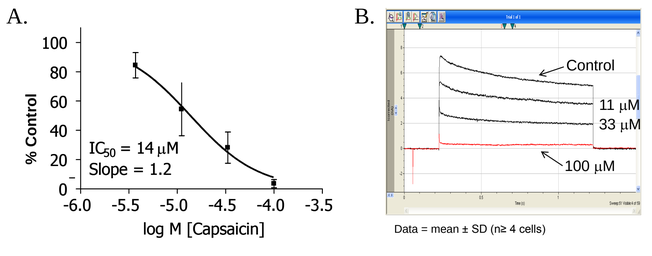



**Figure 4. β2 Adrenergic Receptor Transfection: cAMP Result Comparison to Stable Cell Line.** CHO K1 cells were transiently transfected with a plasmid encoding a β2 adrenergic receptor:eGFP fusion protein. Transfected cells and CHO cells stably expressing the β2A receptor (non-GFP fused receptor) were stimulated with various concentrations of isoproternol and functional responses assessed using the DiscoveRx cAMP kit. Two large-scale transfections produced high quality, consistent results with cell viability of greater than 98%. Electroporation did not significantly affect cell viability or assay quality as the level of PI exclusion and assay S/B ratios were nearly identical to those of a reference stable cell line. Importantly, transiently transfected cells performed similarly to the reference cell line in a cAMP assay as demonstrated by comparable isoproternol $EC_{50}$ values.

| Method | PatchPlate | % seals (>100MΩ) | Seal Resistance (mean±SD) | Expression (>0.5nA) | Current amplitude (mean±SD) |
|---|---|---|---|---|---|
| **Lipid-based Transfection** (20ug DNA + 60ul lipid reagent) | Single Hole | 77% | 191±46 MΩ | 4% | 1.1±1.0 nA |
| **150 ug/ml cDNA** (48 hr post-transfection) | Single Hole | 82% | 248±87 MΩ | 93% | 2.8±1.4 nA |
| | Population Patch Clamp | 100% | 72±31 MΩ | 98% | 1.3±0.30 nA |

PPC seals >20 MΩ

**Table 1. MaxCyte transfected cells perform better than lipid transfected cells for automated ion channel screening**. CHO K1 cells were transfected with $K_V1.5$ α-subunit plasmid DNA using a commercial lipid-based transfection reagent or with MaxCyte electroporation. Cells were assayed in the single hole and population patch clamp mode on the IonWorks Quattro system. Data courtesy of BioFocus.




Data = mean ± SD (n≥ 4 cells)

## GPCR Results

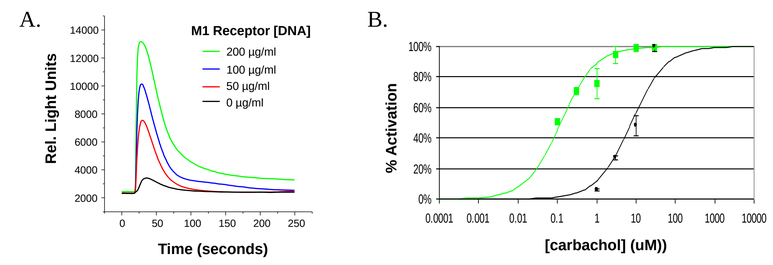



**Figure 2. FLIPR Assay using M1 Muscarinic Receptor Transfected HEK 293H cells. *A).*** Untransfected or cells transfected with three different concentrations of M1 muscarinic receptor DNA were treated with 0.1uM carbachol and FLIPR assays performed. The magnitude of calcium flux directly correlated with M1 plasmid DNA concentration. ***B).*** Untransfected or cells transfected with 200ug/ml of M1 receptor DNA were treated with varying concentrations of carbachol and FLIPR assays performed (n=8). Transfected cells demonstrated a dose-dependent response upon carbachol activation. The $EC_{50}$ value of transfected cells (0.12uM) is considerably lower than untransfected cells (7.6uM), indicating a clear distinction between M1 muscarinic receptor-specific and endogenous responses to carbachol. Data courtesy of Chantest.

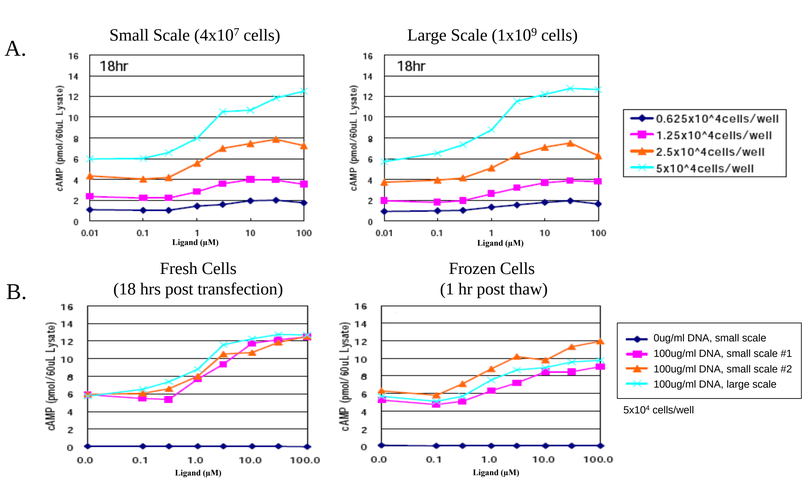



**Figure 3. cAMP GPCR Assay: Small vs. Large Scale Electroporation & Fresh vs. Frozen Cell Assay Performance.** HEK 293F suspension cells were transfected with a GPCR expression plasmid (100 µg/mL) using large or small scale MaxCyte electroporation. Half of the transfected cells were cryopreserved 18 hrs post transfection. GPCR activity was assayed by cAMP ELISA at 18 hrs post EP (fresh cells) or at 1 hr post thawing (frozen cells). ***A).*** Cells transfected at small and large scales showed comparable concentration-dependent responses to GPCR agonist. ***B).*** Cells transfected in two small scale experiments and in one large scale experiment exhibited reproducible ligand responses before and after cryopreservation.

## Ion Channel Results

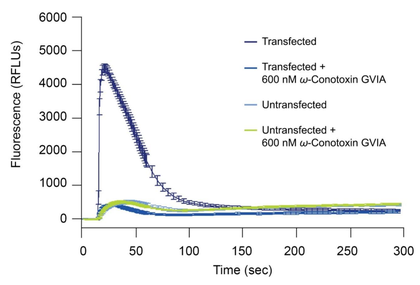



**Figure 5. Multi-subunit Calcium Channel Expression: Functional Responses Measured using FLIPR.** HEK293 cells were transfected in bulk with multiple cDNA constructs encoding 3 calcium channel subunits ± a plasmid expressing the inward rectifier potassium channel, Kir2.1. Calcium influx FLIPR assays were conducted 24 hrs post transfection. Dye was added in a low potassium, low calcium solution and loaded for 30 mins. Vehicle control or a calcium channel specific antagonist (600 nM ω-Conotoxin GVIA) was added for an additional 30 mins. Cells were depolarized with high external $K^+$ (up to 135 mM). Strong calcium flux was evident in cells transfected with all four plasmids, but not in cells transfected without the inward rectifier plasmid or in untransfected control cells. Inclusion of the inward rectifier was essential for the FLIPR assay. The Cav2.2 channel was inhibited by ω-conotoxin, illustrating the functionality of the multi-subunit ion channel. Data courtesy of Chantest.

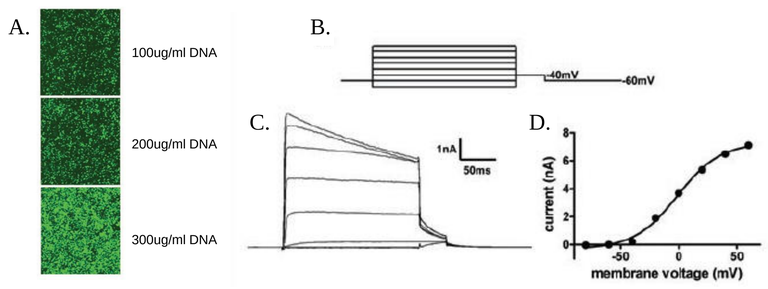



**Figure 6. $K_V1.3$ Expression and PatchXpress Automated Electrophysiology Assays. *A).*** HEK 293 cells were transfected with three concentrations of the human $K_V1.3$ cDNA containing a C-terminal GFP tag (OriGene). Channel expression was assessed by FACS. At 300ug/ml DNA, approximately 80% of cells were $GFP^+$. 36 hours post transfection, cells were assessed using the PatchXpress 7000A. ***B).*** Voltage protocol. ***C).*** Sample $K_V1.3$ currents recorded from a transfected cell. ***D).*** Current-voltage relationship of $K_V1.3$ channel extracted from the same cell as in C. Each data point represents the peak outward current elicited by its respective voltage step. 50-65% Gigaohm seal rate was achieved, comparable to results of a stable cell line (data not shown). Data courtesy of Molecular Devices.

| Compound | MaxCyte transfected cells End Step $IC_{50}$ | Stable cell line End Step $IC_{50}$ | Literature $IC_{50}$ |
|---|---|---|---|
| Capsaicin | 12 μM | 48 μM | 23 μM |
| Nifedipine | 10 μM | 16 μM | 27 μM |
| Bupivicaine | 49 μM | 66 μM | 13 μM |

**Figure 7. Pharmacological assays with transiently transfected cells. Table:** CHO K1 cells transiently transfected with a $K_V1.5$ expression plasmid were incubated with varying concentrations of three ion channel inhibitors and assayed on the PatchXpress. $IC_{50}$ values for each compound compared favorably to data obtained using a stable cell line (data not shown). ***A)***. Pharmacological analysis of transiently transfected cells using the PatchXpress instrument. ***B).*** Representative current tracings that depict a step-wise loss of $K_V1.5$ activity in response to increasing concentrations of the inhibitor capsaicin. Data courtesy of BioFocus.

## Summary

- MaxCyte electroporation can be used to (co)transfect expression constructs for the expression of functional GPCRs, calcium-gated & potassium-gated ion channels.
- The MaxCyte STX Scalable Transfection System is fully scalable, allowing researchers to rapidly transfect from $5x10^5$ to $1x10^{10}$ cells using the same protocol.
- The MaxCyte STX provides a time- and cost-effective alternative to stable cell line or lipid reagent usage for GPCR and Ion Channel expression.
- Cells transfected using the MaxCyte STX have proven results when used in a variety of cellular assays including cAMP, calcium flux and automated electrophysiology assays.
- Transfected cells can be used immediately in cell based assays or cryopreserved for use in future downstream functional assays without a significant loss in performance.

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