Simple and Effective Generation of Ion Channel Assays Using the MaxCyte STX® Scalable Transfection System

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MaxCyte Transfection: Diversity of Applications

Cell Therapy

Cell-based Assays

• Diversity of Targets: GPCRs, Ion Channels, Nuclear Receptors, Kinase, cAMP
• Diversity of Assay Formats: HCS, HTS & profiling, FLIPR, Automated Patch Clamp
• Rapid and flexible alternative to stable cell lines

Protein Production

• Gram-scale production of antibodies and recombinant proteins in CHO cells (also HEK, CAP-T, Vero, & others)
• VLPs, viral vectors, vaccines
MaxCyte STX Transient Transfection System

**High Yield:** >90% viability

**High Efficiency:** >90% transfection efficiency

**Universal:** (co)Transfect DNA, mRNA, siRNA, proteins, lysates

**Versatile:** Mammalian cell lines, primary cells, stem cells, insect cells, difficult-to-transfect cells

**Scalable & Rapid:**
- $5 \times 10^5$- $4 \times 10^7$ (Static EP) in seconds
- $2 \times 10^{10}$ cells (Flow EP) in <30 minutes

Sterile, closed processing assemblies
MaxCyte STX Electroporation Process

Cell Harvesting
- Collect cells
- Concentrate in buffer

Electroporation
- Mix Cells with DNA
- Transfer to PA
- STX electroporation

Recovery
- Remove cells from PA
- 30-40 min. recovery
- Add media
- Plate or cryopreserve
Flow Electroporation using a CL-2 Processing Assembly

Sample Bag (untransfected cells)

Collection Bag (transfected cells)

Air Bag

Pinch Valve

Pinch Valve

Peristaltic Pump

Electroporation Chamber
# Current (and Expanding) MaxCyte STX Protocol List

- 10T½
- 1321N1
- A549
- Ba/F3
- B16
- BHK-21
- C2C12
- C6
- CaCo-2
- CAP/CAP-T
- CHO
- CHO2 (Protein Expression)
- COS-1
- Cos-7
- CV-1
- DLD-1
- DT40
- EL4
- H1299
- HEK 293
- HEK2 (Protein Expression)
- Hela
- Hep G2
- HOS
- Huh-7
- HT1080
- Jurkat
- K562
- L5278Y
- LNCaP
- MCF7
- MDA-MB-231
- MDCK
- Mesenchymal Stem Cells
- Min-6
- Neuro2a
- NIH 3T3
- NIH3T3L1
- NS0
- Opossum Kidney
- P3U1
- Panc-1
- PC12
- PC-3
- Primary Fibroblasts
- Ramos
- RAW 264.7
- RBL
- Renca
- RLE
- SF21
- SF9
- SH-SY5Y
- SK-MES-1
- SK-N-SH
- SL3
- SP2/0
- SW403
- THP-1
- U2OS
- U937
- Vero
MaxCyte STX Transient Transfection of GFP

24 hrs post transfection with 2 µg/1e6 cells pGFP DNA
Transfecting Primary Cells (Neurons) with the MaxCyte STX

Day 0
Electroporate E18 rat hippocampal, cortical & ventricular neurons with 0 or 2 µg/1E6 cells pGFP

Day 5
Plate in multiwell plates @ high and low density (1.5e6 and 5e5 cells/cm²)
Assay viability & GFP expression

Day 5 High Density Photos
0 µg/1e6 cells DNA
2 µg/1e6 cells DNA

Day 5 FACS Data

Day 0
Day 5 FACS Data
Loading Agent Comparison: FACS Analysis of HEK 293 Cells 24 hrs Post EP

**FITC-Dextran (500 kD MW)**

**pGFP DNA**

**GFP mRNA**

Counts vs. FL1-Height

- No EP
- 100 µg/mL
- 300 µg/mL
- 600 µg/mL
- 600 µg/mL, No EP

Counts vs. FL1-Height

- No EP
- 50 µg/mL (MFI = 331)
- 200 µg/mL (MFI = 1669)

Counts vs. FL1-Height

- No EP
- 67 µg/mL

MFI = 331

MFI = 1669

MaxCyte®
MaxCyte STX Cell-Based Assay Case Studies

MaxCyte Transfection for Functional Ion Channel Screening

Assay Challenges

• Transfection efficiency & cell health are critical for functional assays
• Co-expression of multiple subunits necessitates detection assay that may produce unhealthy and slow-growing cells
• Hard to generate stable cell lines; expression drifts over time

MaxCyte Advantages

• High transfection efficiency & viability; maintains membrane integrity
• Efficient multiple plasmid co-transfection
• Scalable; transfected cells can be cryopreserved
• Works with physiologically relevant cells
• Validated with a variety of channels (e.g., \( K_V \), \( Na_V \), \( Ca_V \), Cl, TRP, GABA)
  – 5 case studies: \( K_V 1.5 \), \( Na_V 1.5 \) & \( Ca_V \) assays
Simple Optimization with DNA Titration

- Non-transfected
- Mock-transfected
- 0.5 µg cDNA/1e6 cells
- 2 µg cDNA/1e6 cells

Yield:
- Current amplitude < 0.5 nA
- Current amplitude > 0.5 nA
- Seal resistance < 100 MΩ
- No seal

Single hole mode IonWorksQuattro™ data
Efficient Transient Transfection of hKv1.5 in CHOK1 Cells

- Mock-transfected cell

F10 Patch Plate, C5 Compound Plate

Pre-compound
Command Voltage

Current (nA)
Voltage (mV)
Time (msec)
Pre-compound
Command Voltage

- 2 µg cDNA/1e6 cells

E41 Patch Plate, C21 Compound Plate

Pre-compound
Command Voltage

Current (nA)
Voltage (mV)
Time (msec)
Pre-compound
Command Voltage

Single hole mode IonWorksQuattro™ data
## Good Seal Performance & Strong Functional Expression

<table>
<thead>
<tr>
<th>Condition</th>
<th>% seals (&gt;100 MΩ)</th>
<th>Seal resistance (mean±SD)</th>
<th>% expression (&gt;0.5 nA)</th>
<th>Current amplitude (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transfected</td>
<td>97%</td>
<td>322±136 MΩ</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 µg/1e6 cells cDNA</td>
<td>87%</td>
<td>295±101 MΩ</td>
<td>54%</td>
<td>1.8±1.5 nA</td>
</tr>
<tr>
<td>1 µg/1e6 cells cDNA</td>
<td>84%</td>
<td>278±83 MΩ</td>
<td>89%</td>
<td>2.9±1.8 nA</td>
</tr>
<tr>
<td>1.5 µg/1e6 cells cDNA</td>
<td>96%</td>
<td>246±78 MΩ</td>
<td>95%</td>
<td>3.2±2.1 nA</td>
</tr>
</tbody>
</table>

High seal efficiency & resistance  

Gene expression correlates with DNA concentration  

*Single hole mode IonWorksQuattro™ data*
Better Seal, and Higher Expression, MaxCyte STX vs. Lipid-based Transfection

<table>
<thead>
<tr>
<th>Condition</th>
<th>PatchPlate</th>
<th>% seals (&gt;100 MΩ)(^1)</th>
<th>Seal resistance (mean±SD)</th>
<th>% expression (&gt;0.5 nA)</th>
<th>Current amplitude (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-Mediated Transfection (20 µg DNA + 60 ul commercial lipid reagent)</td>
<td>Single Hole (SH)</td>
<td>77%</td>
<td>191±46 MΩ</td>
<td>4%</td>
<td>1.1±1.0 nA</td>
</tr>
<tr>
<td>1.5 µg/1e6 cells cDNA 48 hrs post-transfection</td>
<td>Single Hole (SH)</td>
<td>82%</td>
<td>248±87 MΩ</td>
<td>93%</td>
<td>2.8±1.4 nA</td>
</tr>
<tr>
<td></td>
<td>Population Patch Clamp (PPC)</td>
<td>100%</td>
<td>72±31 MΩ</td>
<td>98%</td>
<td>1.3±0.3 nA</td>
</tr>
</tbody>
</table>

\(^1\)PPC seals >20 MΩ

Data obtained from IonWorks Quattro™
Large Scale Transfection & Cryopreservation of Cells for Ion Channel Screening

- 8e8 CHO K1 cells transfected with 1.5 µg/1e6 cells Kv1.5 plasmid DNA using flow electroporation (CL2)
- Cultured @ 37°C for 24 hrs and @ 28°C for additional 24 hrs
- Cryopreserved @ 2e6 cells/vial

<table>
<thead>
<tr>
<th>Date</th>
<th>PatchPlate</th>
<th>% seals (&gt;100 MΩ)(^1)</th>
<th>Seal resistance (mean ± SD)</th>
<th>% expression (&gt;0.5 nA)</th>
<th>Current amplitude (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 2009</td>
<td>SH</td>
<td>50%</td>
<td>202 ± 77 MΩ</td>
<td>86%</td>
<td>2.7 ± 1.3 nA</td>
</tr>
<tr>
<td></td>
<td>PPC</td>
<td>98%</td>
<td>38 ± 11 MΩ</td>
<td>99%</td>
<td>1.5 ± 0.3 nA</td>
</tr>
<tr>
<td>January 2010</td>
<td>SH</td>
<td>53%</td>
<td>197 ± 80 MΩ</td>
<td>81%</td>
<td>2.4 ± 1.2 nA</td>
</tr>
<tr>
<td></td>
<td>PPC</td>
<td>93%</td>
<td>30 ± 8 MΩ</td>
<td>97%</td>
<td>1.4 ± 0.3 nA</td>
</tr>
<tr>
<td></td>
<td>PPC</td>
<td>90%</td>
<td>32 ± 8 MΩ</td>
<td>100%</td>
<td>1.9 ± 0.2 nA</td>
</tr>
<tr>
<td></td>
<td>PPC</td>
<td>99%</td>
<td>34 ± 8 MΩ</td>
<td>100%</td>
<td>1.4 ± 0.2 nA</td>
</tr>
</tbody>
</table>

Data obtained from IonWorksQuattro™
Functional Assay hKv1.5 Pharmacology Validation

A.

Data = mean ± SD (n ≥ 4 cells)

B.

IC₅₀ = 14 µM
Slope = 1.2

- 2 SealChips
- 19 whole-cell recordings (possible 32)
- 17 (89%) recordings with current > 0.5nA
- Mean current amplitude 5.96 ± 3.27nA

Data acquired from PatchXpress®
### hKv1.5 Assay: Cryopreserved Cells Pharmacology

<table>
<thead>
<tr>
<th>Compound</th>
<th>MaxCyte transfected cells</th>
<th>BioFocus stable cell line</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End Step IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>End Step IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>12 µM</td>
<td>48 µM</td>
<td>23 µM</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>10 µM</td>
<td>16 µM</td>
<td>27 µM</td>
</tr>
<tr>
<td>Bupivicaine</td>
<td>49 µM</td>
<td>66 µM</td>
<td>13 µM</td>
</tr>
</tbody>
</table>

*Data acquired from PatchXpress®*
Patch Clamp Data on Cells Transiently Transfected with Naᵥ1.5 cDNA Confirmed Ion Channel Property

Sweep Plot

Displayed liquid periods
- Break-in
- Saline
- Saline
- Saline
- TTX [20.0 μM]

IV Plot

Min(IV) [A]
Step Voltage

IT Plot

Saline
Saline
TTX [20.0 μM]

Min(Cl⁻⁻) [A]
Sweep Time [S]

Data acquired from QPatch®
HEK 293 cells were transfected with hNa<sub>v</sub>1.5 plasmid in OC-100 PAs. Cells were assayed on the QPatch® in single hole mode 48 hrs post EP.

<table>
<thead>
<tr>
<th>[DNA]</th>
<th>Transfection efficiency</th>
<th>Average current level*</th>
<th>TTX block**</th>
</tr>
</thead>
<tbody>
<tr>
<td>[µg/1e6 cells)]</td>
<td>[%]</td>
<td>[nA]</td>
<td>[%]</td>
</tr>
<tr>
<td>37° C 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57</td>
<td>-6.3</td>
<td>65</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>-5.7</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>-3.6</td>
<td>82</td>
</tr>
<tr>
<td>2.5</td>
<td>75</td>
<td>-3.5</td>
<td>86</td>
</tr>
<tr>
<td>37° C 48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>82</td>
<td>-2.5</td>
<td>77</td>
</tr>
<tr>
<td>1.5</td>
<td>89</td>
<td>-2.0</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>-3.8</td>
<td>79</td>
</tr>
<tr>
<td>2.5</td>
<td>42</td>
<td>-3.0</td>
<td>82</td>
</tr>
</tbody>
</table>

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 µM TTX single addition
Na\textsubscript{v}1.5 Assays: Cryopreservation Cells Performed Consistently

Static EP in OC-400 PA
[\text{cell}] = 1e8/mL
[\text{DNA}] = 1.5 µg/1e6 cells

Na\textsubscript{v}1.5 Activity Before Cryopreservation

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Transfection efficiency</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Temp. [° C]</td>
<td>[%]</td>
<td>[nA]</td>
<td>[%]</td>
</tr>
<tr>
<td>28</td>
<td>93</td>
<td>-6.5</td>
<td>85</td>
</tr>
<tr>
<td>37</td>
<td>80</td>
<td>-5.2</td>
<td>85</td>
</tr>
</tbody>
</table>

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

Na\textsubscript{v}1.5 Activity Post Cryopreservation

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Transfection efficiency</th>
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<tr>
<td>Temp. [° C]</td>
<td>[%]</td>
<td>[nA]</td>
<td>[%]</td>
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<tr>
<td>28</td>
<td>89</td>
<td>-5.4</td>
<td>81</td>
</tr>
<tr>
<td>37</td>
<td>88</td>
<td>-4.8</td>
<td>79</td>
</tr>
</tbody>
</table>

* Measured @ 0 mV in simple depolarizing step protocol
** Percentage block compared to saline period of a 20 mM TTX single addition
Ca\textsubscript{\text{V}} Channels Studies using MaxCyte STX \textsuperscript{\textregistered}

- Transfect HEK 293 cells with equimolar ratio of four cDNAs:
  - 4 different Ca\textsubscript{\text{V}} pore-forming \(\alpha\) subunits (Ca\textsubscript{\text{V}}1.2, Ca\textsubscript{\text{V}}2.2, Ca\textsubscript{\text{V}}2.1, and Ca\textsubscript{\text{V}}3.2)
  - Modulatory \(\beta\) subunit
  - Modulatory \(\alpha2\delta\) subunit
  - Inward rectifier potassium channel (Kir2.1) to allow modulation of resting membrane potential by external \(K^+\)

- Plate in 384 well plate 20 min. post EP
- Perform FLIPR\textsuperscript{TETRA\textsuperscript{\textregistered}} calcium flux assays @ 24 & 48 hrs post EP
**Ca\textsubscript{v} Ion Channels: Ca Influx in HEK Cells Measured 24-48hrs Post Transfection**

**Cav1.2 + β2 + α2δ + Kir2.1**  
Untransfected

**Cav2.1 + β4 + α2δ + Kir2.1**  
Untransfected

**Cav2.2 + β3+ α2δ + Kir2.1**  
Untransfected

**Cav3.2 + β2+ α2δ + Kir2.1**  
Untransfected

Controls: n = 8; antagonist groups: n = 4; Error bars = Std. Dev.
Other Cell-Based Assay Applications: MaxCyte Customer Presentations & Publications

**HTS for CFTR Modulators**
- Co-transfection of CFBE human bronchial epithelial cells with CFTR & YFP sensor plasmids
- Iodide flux assay

**HCS for mPER Modulators**
- Co-transfection of COS-7 cells with mPER-GFP & CK1 plasmids
- High content imaging on Cellomics Array Scan

**Screening for GABA_A Channel Activators & Modulators**
- Four plasmid co-transfection
- Iodide flux assay
MaxCyte STX Advantages for Cell Based Assays

• High transfection efficiency and cell viability
• Scalability
• Cryopreservation option provides flexibility and consistency in cell supply for HTS screening
• Highly effective for multiple plasmid co-transfection
• Works with multiple cell types (physiological relevant)
• High assay success rate on various HTS platforms
Acknowledgements

AstraZeneca

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ChanTest

Flatley Discovery Lab

Pfizer

sophion

MaxCyte
Advancing Drug Discovery & Candidate Screening

MaxCyte® STX™
SCALABLE TRANSFECTION SYSTEM


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