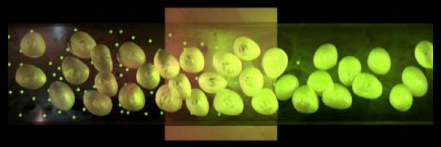

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MaxCyte STX Workshop

Optimizing Cell Based Assays from the Bench to HTS Using Scalable Transient Transfection


CERTIFICATE
TUV Rheinland of North America, Inc.
MaxCyte, Inc.
22 Firstfield Road, Suite 230
Gaithersburg, MD 20878 USA
Design and Manufacture of Electroporation Instrumentation and Assemblies.
ISO 9001: 2000
74 300 3071
ANAB

James P. Brady, PhD, MBA

LabAutomation2011, Palm Springs, CA

February 1, 2011

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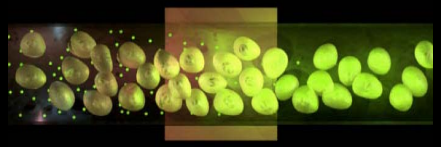
Drug Discovery Screening Challenges



Picking the *right target*	Testing therapeutic candidates in a *biologically relevant system*
Increasing and decreasing the *scale* of the testing from the bench to HTS to secondary and tertiary screening creates technical, scientific, and process complexity.	
speed in creating and conducting biologically relevant assays is critical to patients and to the financial success of a company.	

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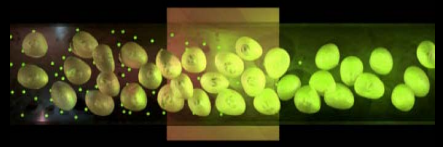
Ideal Attributes of a Transient Transfection System for Cell Based Assay Development



- Works with multiple cell types
- High efficiency & high viability
- Reproducible from day to day and scientist to scientist
- User friendly
- Loads multiple types of molecules & combinations of molecules
- Compatible with a variety of assay formats

- Allows cryopreservation of transfected cells
- Suitable for multiple classes of targets
- Scalable
- Process compatible
- Short assay development time
- Cost effective

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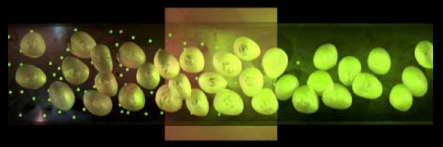
MaxCyte® STX™
Scalable Transfection System



Proprietary Electroporation Technology

Simple:	Fast and easy
High Yield:>90% viability	
High Efficiency:	>90% transfection efficiency
Safe:	Chemically defined buffer No added biological agents Sterile, closed system
Scalable & Rapid:	5 x10^5-4x10^7 (**Static** EP) in seconds 1 x10^{10} cells (**Flow** EP) in <30 min
Rugged:	Reproducible & consistent
Quality:	cGMP compliant ISO 9001 certification CE Marking

Small molecules
Antigens (proteins/lysates)
Nucleic acids (DNA, mRNA, siRNA)

Primary cells
Stem cells
Mammalian cell lines

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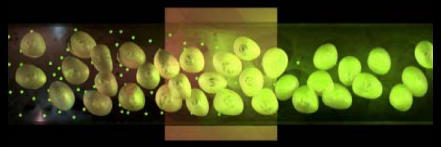
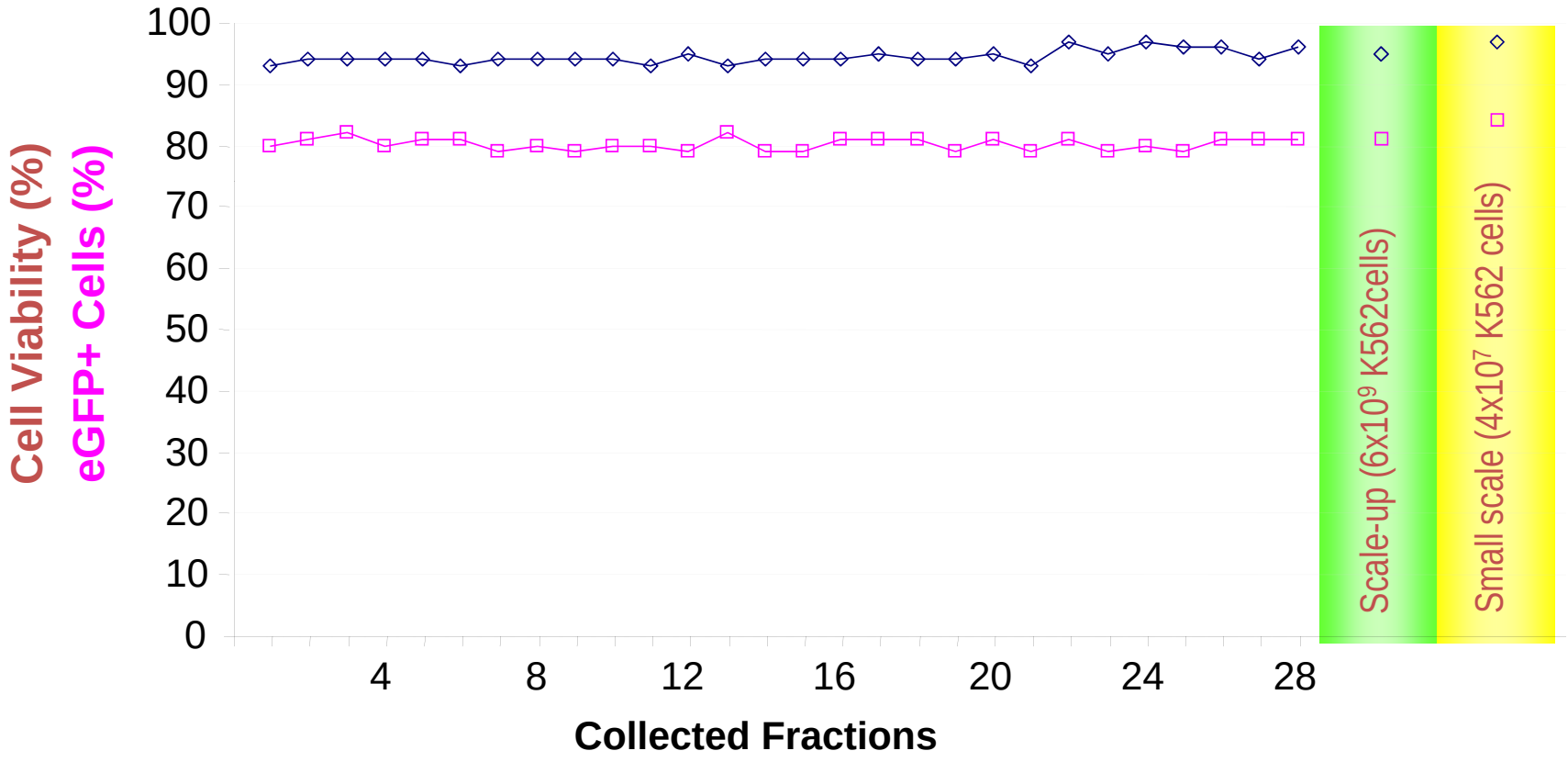
MaxCyte STX:
Reproducible, Consistent, and Scalable





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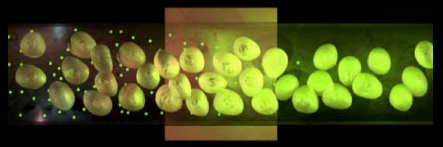
Representative Sample of MaxCyte STX Transfected Cell Lines & Primary Cells



Cell Type	Efficiency % GFP +	Viability
293T	**95%**	**95%**
CHO Cells	**90%**	**90%**
VERO	**90%**	**90%**
K562	**90%**	**90%**
NIH 3T3	**90%**	**90%**
Jurkat	**90%**	**90%**
Huh-7 Cells	**80%**	**90%**
Renca	**80%**	**97%**
Human Mesenchymal Stem Cells	80%	80%
Human Myoblasts	90%	90%
Human Lymphocytes – B Cells	85%	90%
Human Lymphocytes – T Cells	50%	70%
Human HSC ($CD34^+$ cells)	60%	60%
Human Dendritic Cells	50%	80%

Results of processing cell lines with DNA plasmid encoding for Green Fluorescent Protein (GFP). Efficiency expressed as % cells GFP+ at 24 to 48 hrs post process; viability as % cells excluding propidium iodide (PI).

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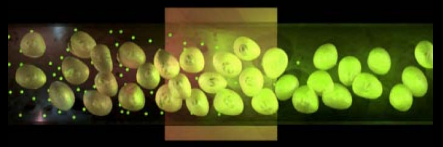
Current (and Expanding) MaxCyte STX Protocol List



- CHO
- CHO2 (Protein Expression)
- Hela
- HEK 293
- HEK2 (Protein Expression)
- Huh-7
- Jurkat
- K562
- Mesenchymal Stem Cells
- NIH 3T3
- Primary Fibroblasts
- Renca
- Vero
- PC12
- Hep G2
- CV-1
- THP-1
- Min-6
- Panc-1
- L5278Y
- U2OS
- SH-SY5Y
- COS-1
- A549
- PC-3
- BHK-21
- RBL
- Neuro2a
- NS0
- C6
- CaCo-2
- RLE
- COS-7
- LNCaP
- DLD-1
- C2C12

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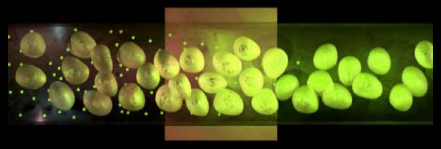

Developing a Cell Based Assay with the MaxCyte® STX™ Scalable Transfection System

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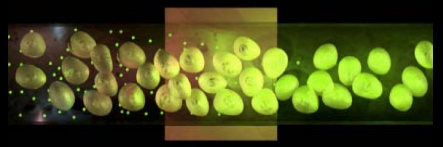
How to Use the MaxCyte STX: Assay Development Steps



1. DNA Preparation
2. Cell Culture
3. Electroporation and DNA Titration
4. Cell Handling Post-EP
5. Plating and Analysis
6. Scale-Up
7. Cryopreservation

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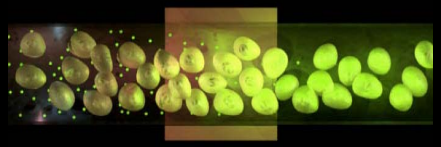
1) DNA Preparation: Specifications



- Prepare the DNA from commercially available ion exchange columns, preferably endotoxin-free
- Suspend the DNA in water, not TE
- $OD_{260/280} > 1.8$
- >85% covalently closed, circular conformation
- Stock solution at >5 mg/mL

High quality DNA is mandatory.

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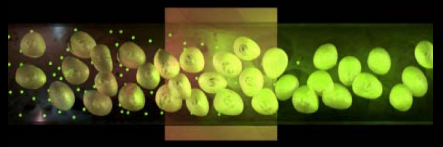
2) Cell Culture: Specifications



- Cells must be in log phase growth
- Typically split the cells one day prior to EP
- Adherent cells must be subconfluent
- Cells should be passaged several times post-thaw
- Late passage cells give poor results
- Cells must be dissociated – no clumps

Healthy cells are required.

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4) Cell Handling Post-EP:

- Immediately remove the cells from the PAs

DO NOT rinse the PAs! DO NOT add media!

- Cell recovery at 37°C for 20 minutes

Spread cells out for oxygen transfer + better viability.

- Remove the cells and plate as desired.

Note: Higher expression levels for ion channels may be obtained by subsequent incubation at 28°C.

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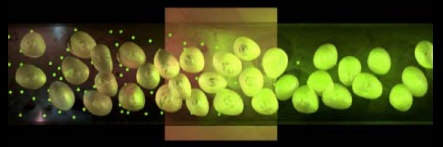
5) Plating and Analysis:



- Handle cells per standard procedures:
  Plate in multi-well plates or assay chambers

- Assay responsiveness
  - DNA concentration (MaxCyte STX)
  - Number of cells/well
  - Length of time between transfection and analysis

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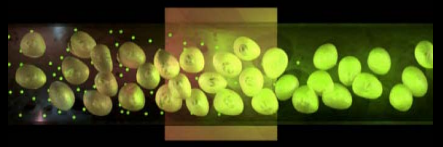
Seamless Scale-Up: Flow (Large Scale) Electroporation



- Choose DNA concentration from the static EP (small scale) experiments
- Prepare appropriate quantity of DNA and cells

- Choose CL-2 from the dropdown menu
- Press start

- < 30 minutes later, recover the cells, plate/cryopreserve, run the assay

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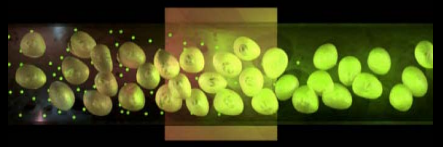
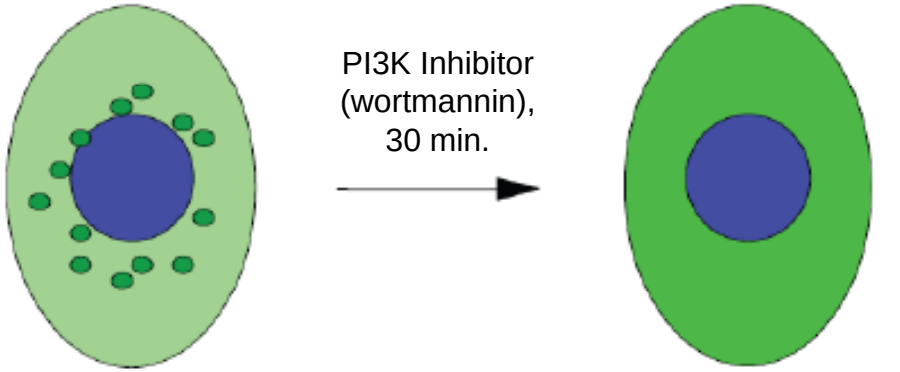
Case Study: Using the MaxCyte STX for Assay Development



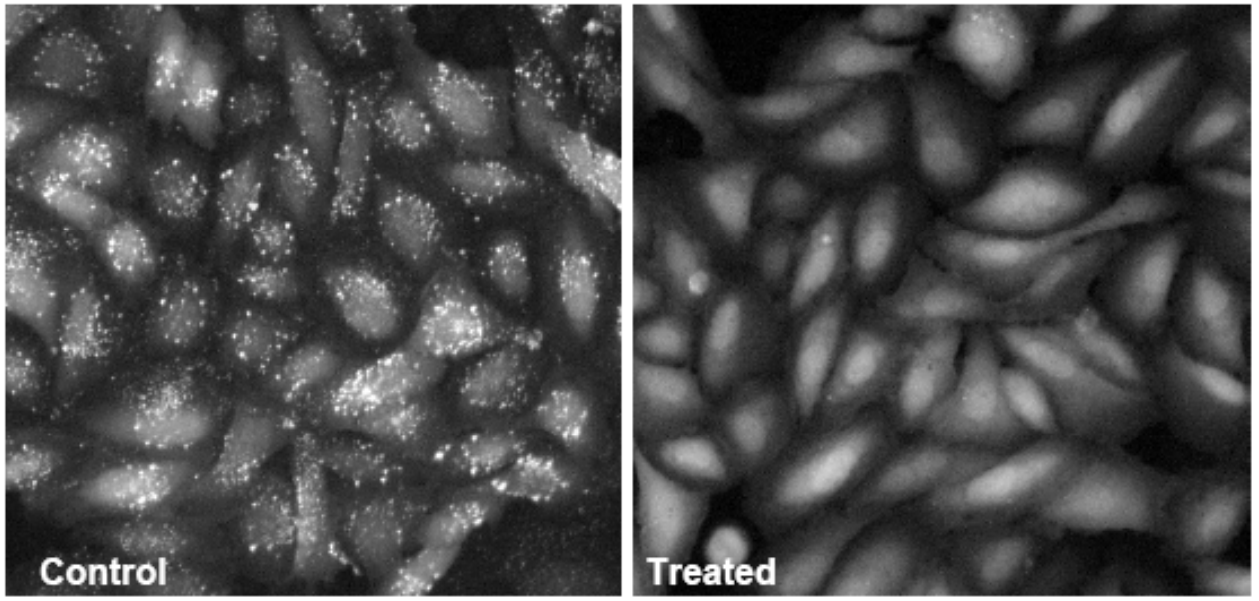
2XFYVE-eGFP Redistribution Assay in Tumor Cells: Measuring PI3K Activity



FYVE=
PI3P binding
domain

Untreated cell: eGFP-2XFYVE is concentrated in endosomes

Treated cell: eGFP-2XFYVE is redistributed to the cytoplasm



96 Well Assay Format

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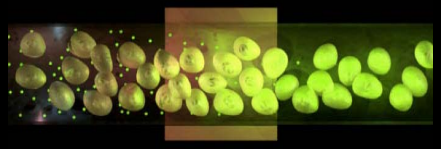
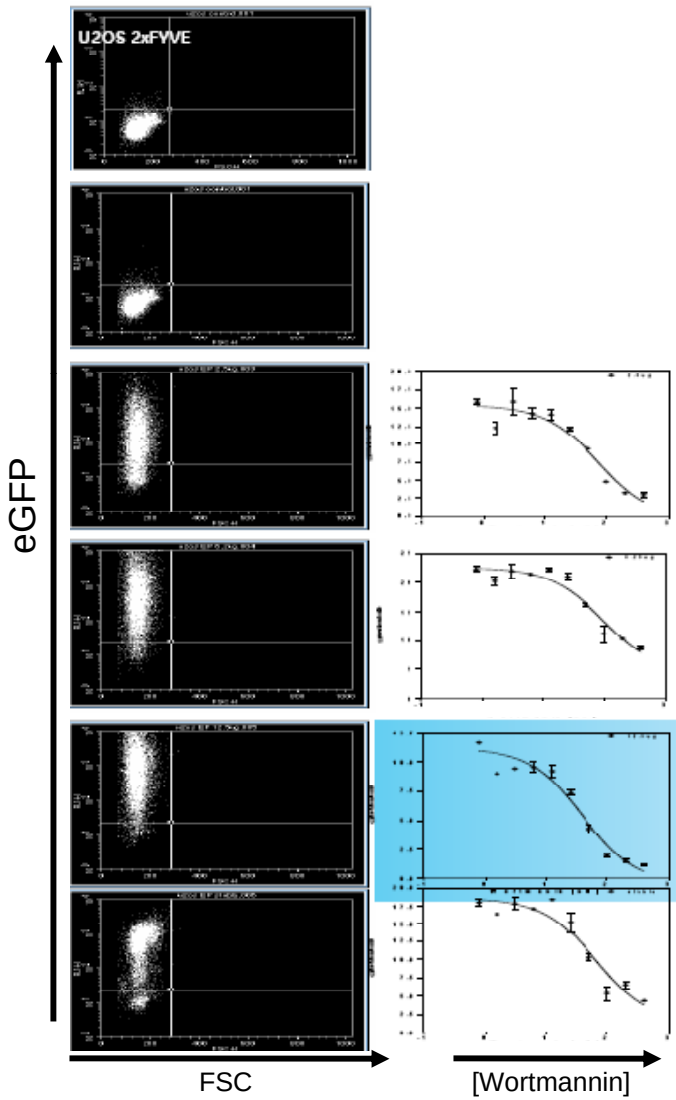
Optimizing Plasmid DNA Concentrations in Small Scale Transfections





	% positive cells	Geo Mean	EC50 (nM)	S:B	
control	0.72	34.5			No EP
control	0.98	32.02			EP, No DNA
2.5µg pDNA	84.58	166.24	73.58	4.67	EP + DNA
6.25µg pDNA	95.04	465.11	99.2	2.8	
12.5µg pDNA	97.12	717.51	40.29	10.4	
2xFYVE stable	93.34	544.5	43.36	4.64	Stable cells

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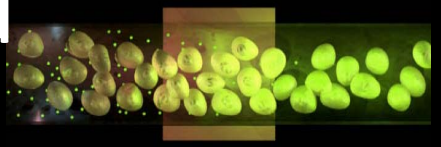
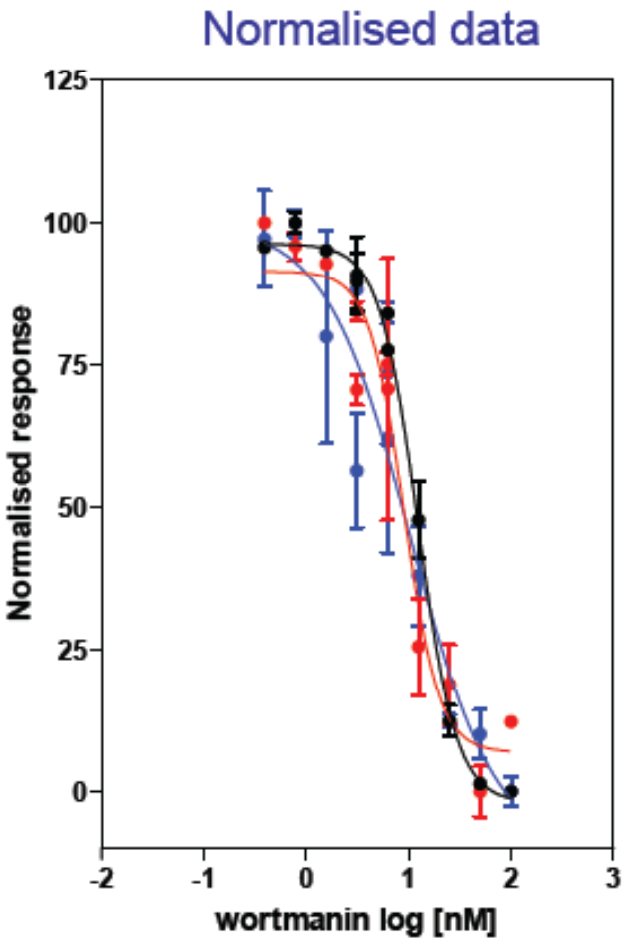
Seamless Scale-Up: MaxCyte STX-Transfected Cells Comparable to Stable Cell Line





Sample	EC50 (nM)
Stable cell line	12.52
SCEP	10.60
LSEP	9.0

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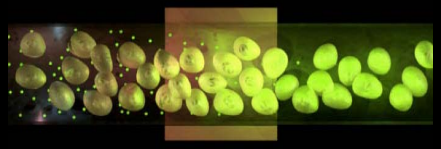
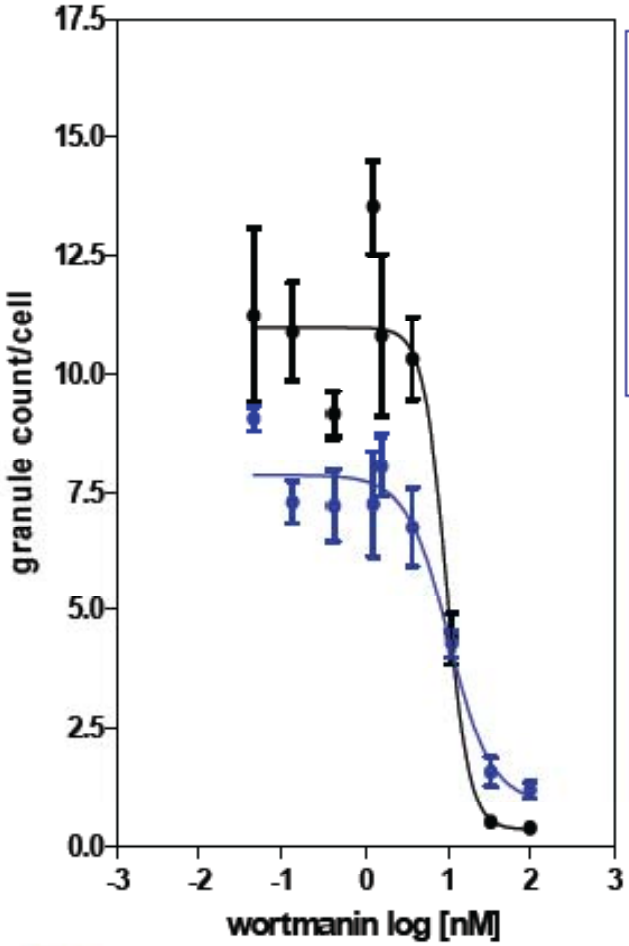
Assay Results: Cryopreserved Cells Comparable to Stable Cell Line

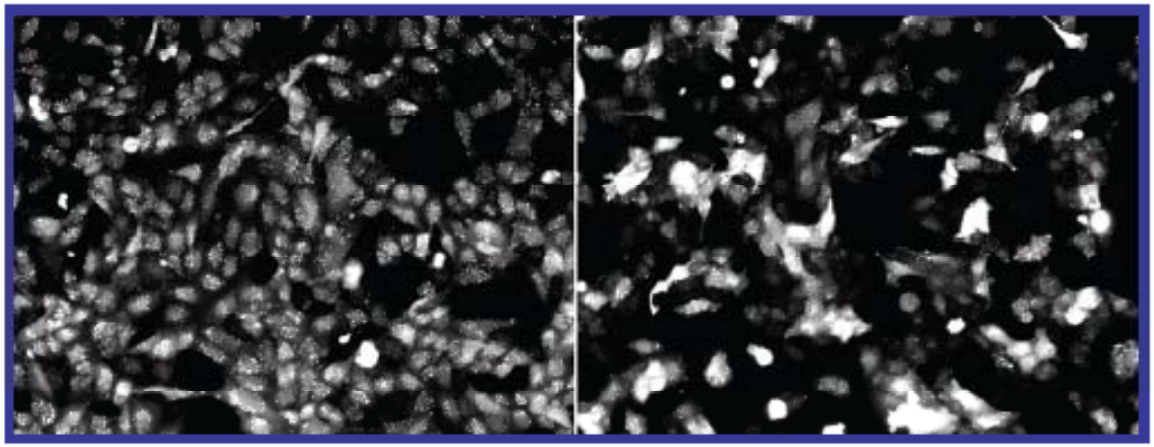




EP'ed cells seeded into 4xT162 for 24h
Cells harvested and 12 x vials cryopreserved @ 2E06/ml in Medium/FBS/DMSO

T=1 week at -140°C

Sample	EC50 (nM)
Stable cell line	10.29
SCEP	9.50



Stable Control

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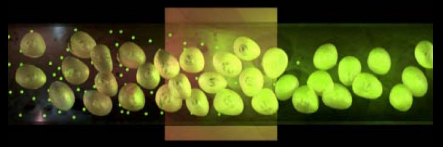
MaxCyte STX Advantages



- Small to large volume scalability, ~10 billion cells in < 30 minutes
- Consistent and reproducible transfections
- High cell viability and transfection efficiency
- Transfection of multiple agents simultaneously
- Minimal off-target effects

- More relevant assays
- Faster experimental turnaround
- Greater productivity

Better Drug Candidates

MaxCyte®