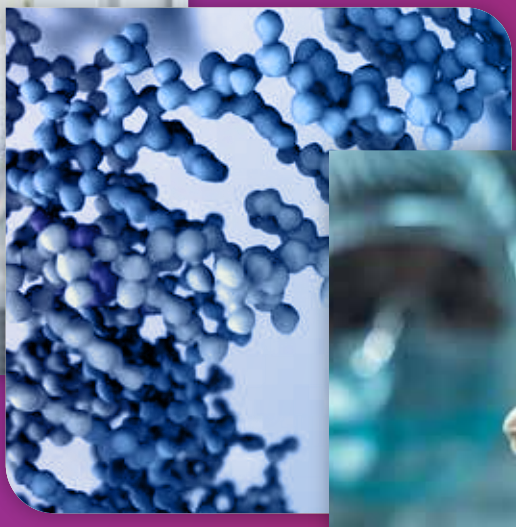
FROM **BENCH** TO **BIOREACTOR** TO **BREAKTHROUGH**





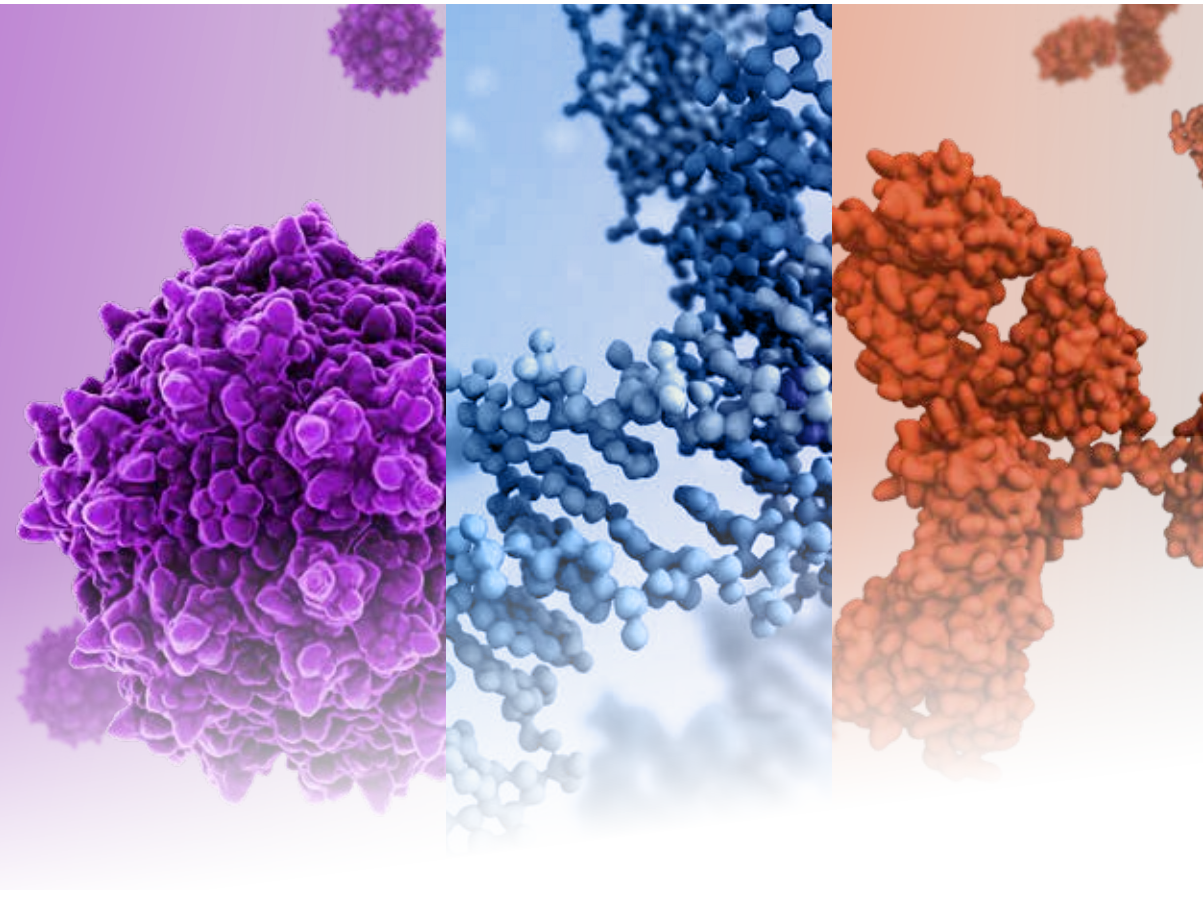


mirus bio®

Transfection Solutions for Applications from Bench to Bioreactor & Beyond

Whether you're producing viral vectors at clinical and commercial scales, manufacturing recombinant proteins and monoclonal antibodies, performing knock-out studies with siRNA, or editing genes using CRISPR, we have the nucleic acid delivery system you need.

DISCOVERY RESEARCH | VIRUS PRODUCTION | PROTEIN PRODUCTION



Contact us!

mirus bio®

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Lentivirus Production

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*Transduce*IT® Transduction Reagent

*Trans*IT®-Lentivirus System

Lentivirus Packaging Mix Powered by MISSION®

Nucleic Acid Delivery for Large-scale AAV and LV Production

GMP Products

The *Trans*IT-VirusGEN® GMP Transfection Reagent is designed to enhance delivery of packaging and transfer vector DNA to suspension and adherent HEK 293 cell types in order to increase production of recombinant lentivirus (LV) and adeno-associated virus (AAV).

Titers can be further increased with optimized enhancers included in the VirusGEN® GMP AAV and VirusGEN® GMP LV Transfection Kits.

*Trans*IT-VirusGEN® GMP is intended to be used as an ancillary material for cell, gene and tissue-engineered products, per USP <1043>

Key benefits of *Trans*IT-VirusGEN® GMP Transfection Reagent include:

Performance—Efficient DNA delivery for large-scale production of high-titer viral vectors

Quality—GMP manufactured and tested for appearance, identity, sterility, endotoxin and mycoplasma

Flexibility—Compatible with different virus production platforms and repeat filtration

Animal Origin Free—Fully synthetic transfection reagent formulation

PRODUCT NO.	QUANTITY
VirusGEN® GMP AAV Transfection Kit	
MIR 6815-GMP	Customizable
VirusGEN® GMP LV Transfection Kit	
MIR 6825-GMP	Customizable
***Trans*IT-VirusGEN® GMP Transfection Reagent**	
MIR 6845-GMP	150 mL

Mirus Bio Regulatory, Quality and Supply Chain Capabilities

		Process Dev & Scale-up	Clinical Trials	Commercial MFG
		VirusGEN (RUO) *Rev*IT (RUO)	VirusGEN GMP *Rev*IT GMP (coming soon)	
Characteristics		**RUO**	**GMP (VirusGEN GMP only)**	
Manufacturing standards	Manufactured in the USA	✓ Mirus quality management system	✓ cGMP standards ✓ ISO 13485	
Product documentation	COA CoO Shelf-life stability report	✓ ✓ ✓	✓ ✓ ✓	
Regulatory Docs & Assays	Animal origin free, BSE/TSE free Regulatory support package Identity testing (raw materials) Residual reagent assay Drug master file	✓ N/A N/A N/A N/A	✓ ✓ ✓ ✓ Coming soon	
Quality documentation	Supplier qualification packet Quality agreement template	Coming soon N/A	Coming soon ✓	
Support	Audit ready Onsite end user scale-up support	N/A ✓	✓ (Madison, WI, USA) ✓	
Supply chain	Annual VirusGEN capacity	✓ Supports 10,000+ liters of cell culture	✓ Supports ~400,000L liters of cell culture ✓ Multiple storage locations	

The Mirus Bio QMS is ISO 13485 certified for technology transfer and distribution of GMP grade ancillary materials.

Please read more about the quality standards for our GMP products here: https://www.mirusbio.com/quality

High Titer Virus Production

*Trans*IT-VirusGEN® Transfection Reagent

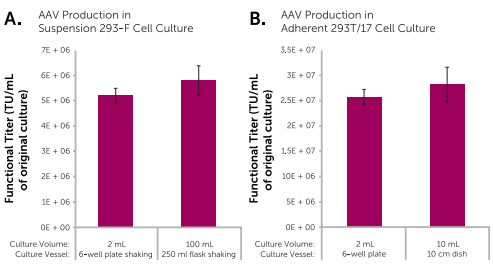
*Trans*IT-VirusGEN® Transfection Reagent is designed to enhance delivery of packaging and transfer vectors to adherent and suspension HEK 293 cell types to increase recombinant adeno-associated virus (AAV) and lentivirus (LV) production.

Reliable—Consistent high titer virus production

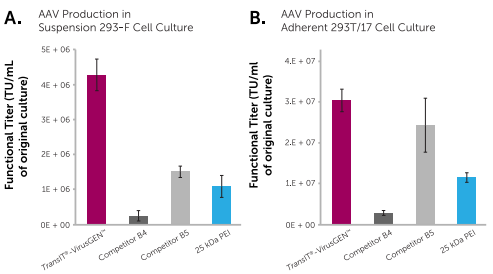
Scalable—Efficient across different formats

Flexible—Address different virus and cell culture system

*Trans*IT-VirusGEN® Transfection Reagent Outperforms Competitor Reagents in AAV Production



***Trans*IT-VirusGEN® Enables Broad Scalability (AAV).** Suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium (A) or adherent 293T/17 cells (B) were transfected with pAAV-hrGFP, pAAV-RC and pAAVHelper (1:1:1 ratio, 1.5 total µg/mL, Agilent Technologies) using the *Trans*ITVirusGEN® Transfection Reagent (2:1 reagent-to-DNA ratio (vol:wt), Mirus) at the indicated volumes per culture vessel. For experimental details, please visit https://www.mirusbio.com/product/transit-virusgen-transfection-reagent-and-kits-2/



***Trans*IT-VirusGEN® Outperforms Competitor Reagents in Suspension and Adherent AAV Cell Cultures.**
For experimental details, please visit https://www.mirusbio.com/product/transit-virusgen-transfection-reagent-and-kits-2/

*Trans*IT-VirusGEN® Transfection Reagent

PRODUCT NO.	QUANTITY
MIR 6703	0.3 mL
MIR 6704	0.75 mL
MIR 6700	1.5 mL
MIR 6705	5 x 1.5 mL
MIR 6706	10 x 1.5 mL
MIR 6720	30 mL
MIR 6740	150 mL

VirusGEN® AAV Transfection Kit

PRODUCT NO.	QUANTITY
MIR 6745	1 Kit for 10 L of cell culture
MIR 6750	1 Kit for 1 L of cell culture
MIR 6755	1 Kit for 50 L of cell culture

VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer

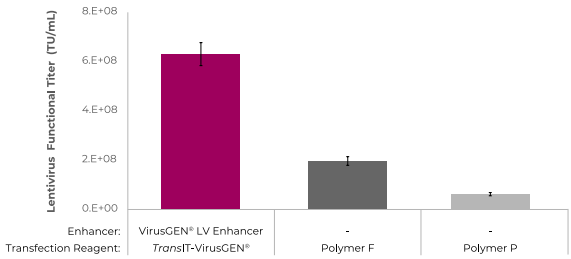
PRODUCT NO.	QUANTITY
MIR 8007	1 Kit for 1 L of cell culture
MIR 8008	1 Kit for 10 L of cell culture

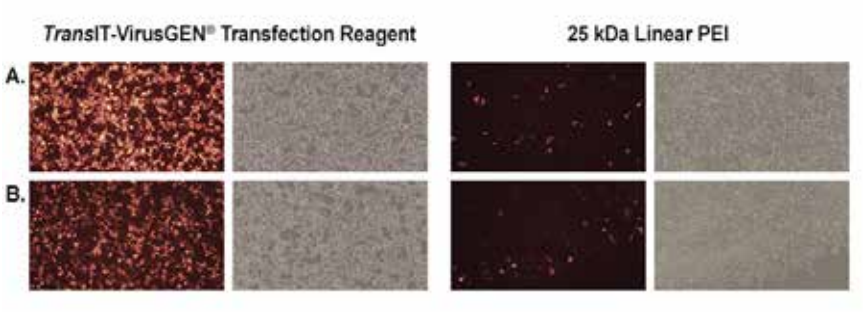
VirusGEN® LV Transfection Kit

PRODUCT NO.	QUANTITY
MIR 6760	1 Kit for 1 L of cell culture
MIR 6765	30 mL for 10 L of cell culture

*Trans*IT-VirusGEN® Transfection Reagent Also Outperforms Competitor Reagents in Lentivirus Production

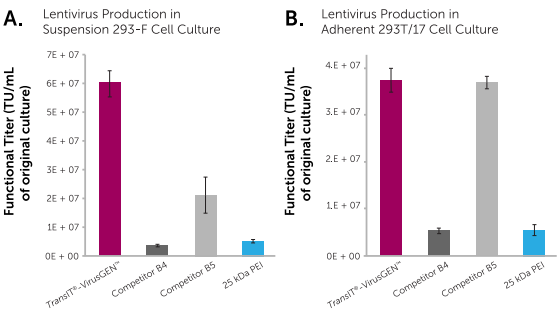
Higher Titers than Other Reagents.
The VirusGEN® LV Transfection Kit is superior to competitor reagents in suspension lentivirus cell cultures.





Higher Transfection Efficiency with *Trans*IT-VirusGEN® Over PEI for Lentivirus Generation.
HEK293T/17 cells were transfected with lentiviral packaging plasmids and either (a) control RFP transfer plasmid or (b) targeting SPdCas9-RFP transfer plasmids using either *Trans*IT-VirusGEN® Transfection Reagent (3:1 reagent to DNA ratio; vol:wt) or 25 kDa linear PEI (3:1 reagent to DNA ratio; wt:wt). Brightfield and RFP Images were taken 17-22 hours post-transfection.

Data courtesy of Zuzana Drobna, PhD (Dr. Keung's Lab), Department of Chemical and Biomolecular Engineering, North Carolina State University



***Trans*IT-VirusGEN® Outperforms Competitor Reagents in Suspension and Adherent Lentivirus Cell Cultures.**
For experimental details, please visit https://www.mirusbio.com/product/transit-virusgen-transfection-reagent-and-kits-4/

Paired with Any Transfection Reagent, the *Rev*IT™ AAV Enhancer Delivers Higher Titers for Recombinant AAV Production

*Rev*IT™ AAV Enhancer

This novel enhancer is easily integrated into existing workflows, and it produces 2-4x higher titers across a range of serotypes, cellular growth media formulations, and transfection platforms. *Rev*IT™ AAV Enhancer can further drive down upstream manufacturing costs by lowering plasmid DNA usage while maintaining high-quality titers.

Designed to deliver higher titer for any*:

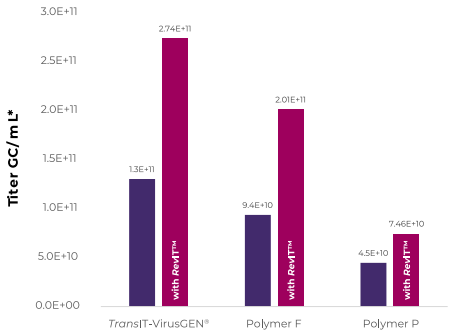
- **Serotype**
- **Reagent**
- **Media**
- **HEK 293 Cell Line**

PRODUCT NO.	QUANTITY
MIR 8000	1.5 mL for 1 L of cell culture
MIR 8006	10 x 1.5 mL for 10 L of cell culture
MIR 8080	75 mL for 50 L of cell culture

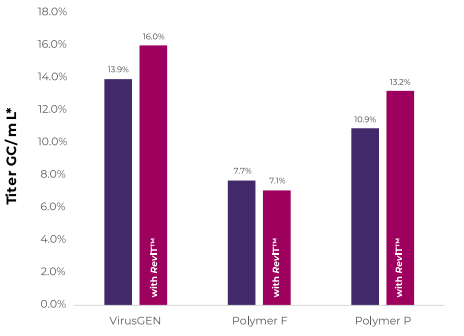
2-4X
increase in AAV titer

>50%
reduction in cost per dose

2X
doses per bioreactor run



6.1X Increase
in titer over Polymer P

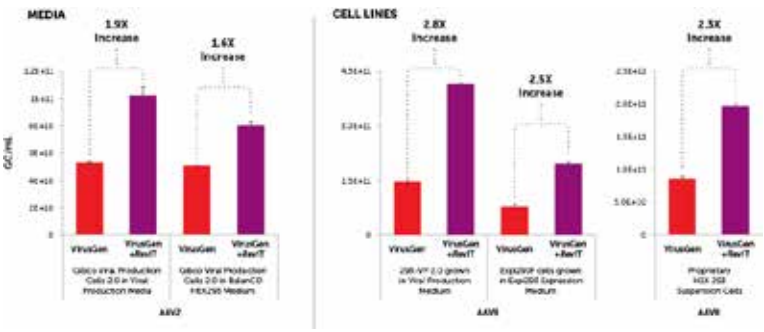


~2X Increase
in full capsids over Polymer F

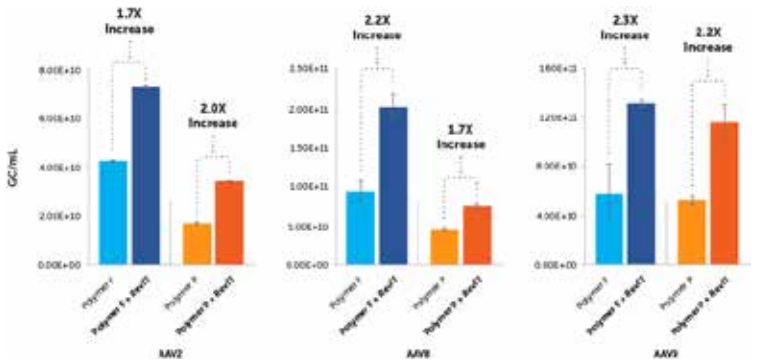
***Trans*IT-VirusGEN® plus *Rev*IT™ AAV Enhancer outperformed polymer-only reagents.**

** In all conditions tested to date.*
Up to 4x increase in titer when used in combination with the VirusGEN® Transfection Platform.
Up to an average increase of 2x when used with polymer-only transfection reagents.

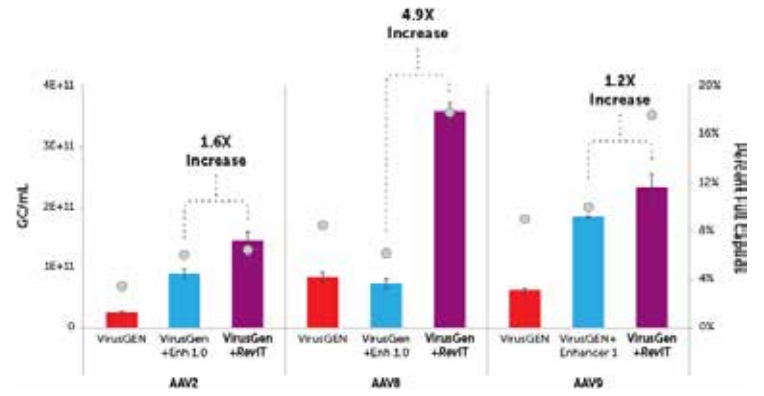
*Rev*IT™ AAV Enhancer, con't



*Rev*IT™ AAV Enhancer can be used with any media or cell line to increase titer.



*Rev*IT™ AAV Enhancer improves titer across serotypes when used with polymer-only transfection reagents.



*Rev*IT™ AAV Enhancer universally reduces cost per dose and doubles the doses from a single bioreactor run.

Deliver Multiple Nucleic Acid Types Into a Broad Range of Cell Types

*Trans*IT-X2® Dynamic Delivery System

Achieve superior transfections with an innovative lipopolymeric system that efficiently delivers both DNA and siRNA/miRNA out of the endosome and into the cytoplasm, overcoming a critical barrier to nucleic acid delivery.

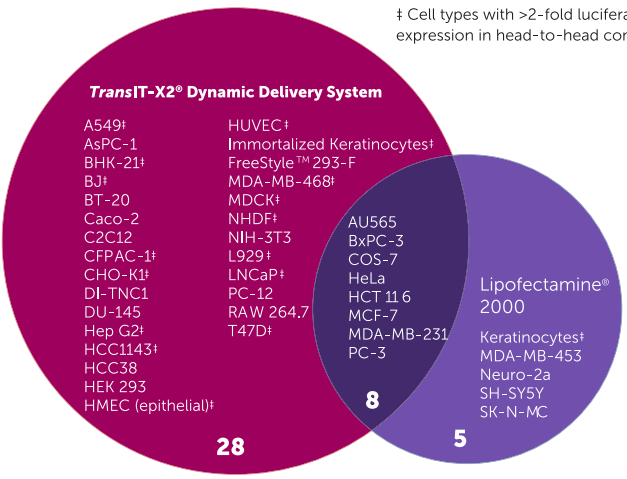
High Efficiency—Exceptional broad spectrum transfection

Versatile—Cutting edge delivery of plasmid DNA, siRNA/miRNA or ribonucleoprotein (RNP) complexes

Technology—Novel, non-liposomal, polymeric delivery

PRODUCT NO.	QUANTITY
MIR 6003	0.3 mL
MIR 6004	0.75 mL
MIR 6000	1.5 mL
MIR 6005	5 x 1.5 mL
MIR 6006	10 x 1.5 mL

‡ Cell types with >2-fold luciferase expression in head-to-head comparisons.

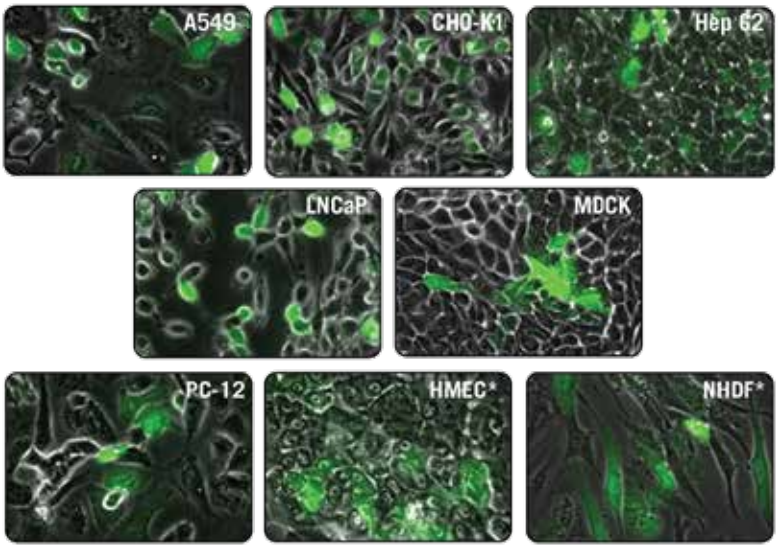


The *Trans*IT-X2® Dynamic Delivery System Enables Superior Gene Expression in a Variety of Cell Types.
The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect plasmid DNA encoding luciferase into 41 different cell types at three reagent-to-DNA ratios. Luciferase expression was compared at 24 hours post-transfection using a standard luciferase assay. Head-to-head comparisons at optimized ratios illustrate superior or equal luciferase expression using *Trans*IT-X2® (Mirus Bio) in 36 of 41 cell types; 17 cell types that had expression levels 2-fold higher are denoted with ‡.

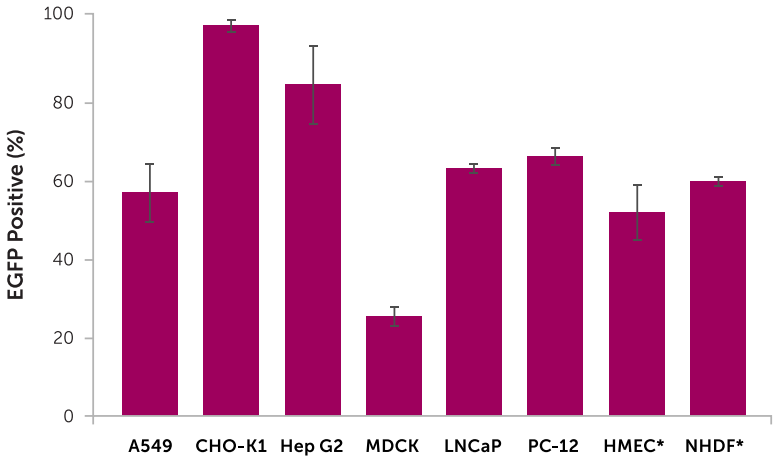
We recently tested the *Trans*IT-X2® Dynamic Delivery System head-to-head against Lipofectamine® 2000 for DNA transfection of NIH-3T3 fibroblasts and the breast cancer cell line ZR-75-1. We observed higher efficiency and less toxicity when using *Trans*IT-X2®.

Dr. Edwin Li, Associate Professor
Saint Joseph's University

*Trans*IT-X2® Dynamic Delivery System, *con't*

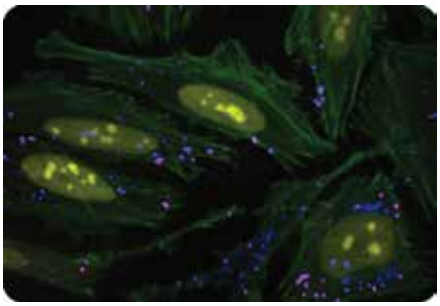


Visualization of High GFP Expression Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, LNCaP, MDCK, PC-12, primary human mammary epithelial cells (HMEC) and primary normal human dermal fibroblasts (NHDF). Transfections were performed in 35 mm dishes (MatTek) using 4-8 µl of *Trans*IT-X2® (Mirus Bio) to deliver 2 µg of DNA. Images (32X) were captured at 48 hours post-transfection using a Zeiss Axiovert S100 inverted fluorescence microscope. *Indicates primary cell types.



High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, MDCK, LNCaP, PC-12, primary human mammary epithelial cells (HMEC) and primary normal human dermal fibroblasts (NHDF). Transfections were performed in 96-well plates using 0.2-0.4 µl of *Trans*IT-X2® (Mirus Bio) to deliver 0.1 µg of DNA (2:1, 3:1 or 4:1 reagent:DNA ratio). Triplicate wells were assayed 48 hours post-transfection on a guava® easyCyte™ 5HT Flow Cytometer (MilliporeSigma). *Indicates primary cell types.

*Trans*IT-X2® Dynamic Delivery System, *con't*



Functional Co-delivery of Plasmid DNA and siRNA Using the *Trans*IT-X2® Dynamic Delivery System.
The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA Cy®5 labeled encoding nuclear YFP and Cy®3 labeled siRNA into HeLa cells. Transfection was performed in a 6-well plate with Poly-L-Lysine (PLL) coated coverslips using 4 µl of *Trans*IT-X2® (Mirus Bio) to deliver 2 µg of DNA (2:1 reagent:DNA ratio) and 25 nM siRNA. Actin cytoskeleton was stained using Alexa Fluor® 350 Phalloidin (Thermo Fisher Scientific). Image (63X) was captured at 24 hours post-transfection using a Nikon A1R confocal microscope. Image key: yellow (nuclear YFP), blue (Cy®5 labeled DNA), red (Cy®3 labeled siRNA), green (actin cytoskeleton).

We work on non-small cell lung cancer (NSCLC) which is an adherent cell culture line. Previously, we have tested many transfection products from several companies without much success, but the *Trans*IT-X2® Dynamic Delivery System works very well with NSCLC using my protocol.

Dr. Luo Wang
Comprehensive Cancer Center
University of Michigan

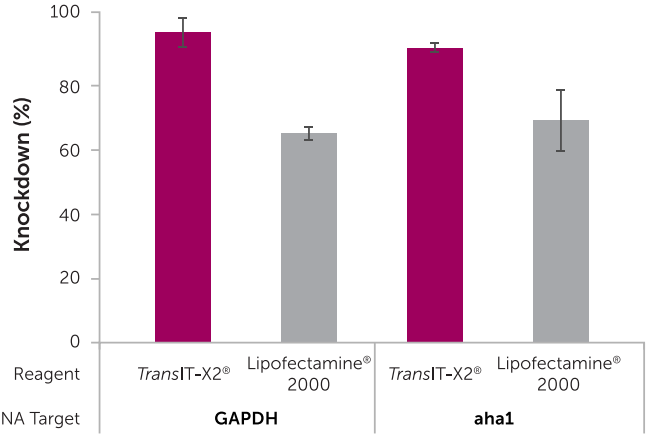
The *Trans*IT-X2® Dynamic Delivery System outperformed all other transfection reagents we have tested for DNA transfection of our C2C12 mouse myoblast cell line. In addition, *Trans*IT-X2® was also less toxic.

Dr. G. Du, Professor
Texas Medical Center

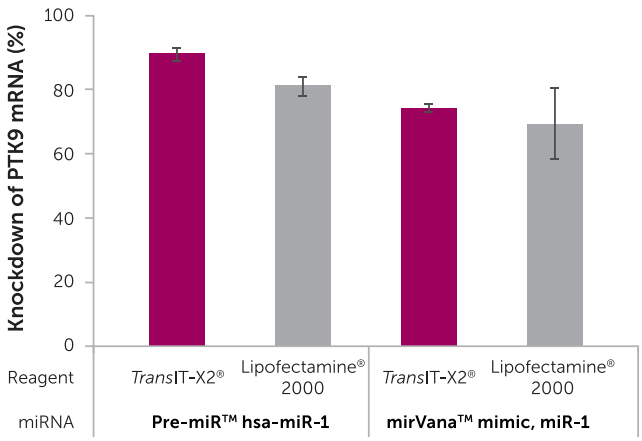
We are pleased with the performance of the *Trans*IT-X2® Dynamic Delivery System when transfecting our renal carcinoma cell line 786-O.

Sathish Padi
North Dakota State University

*Trans*IT-X2® Dynamic Delivery System, *con't*



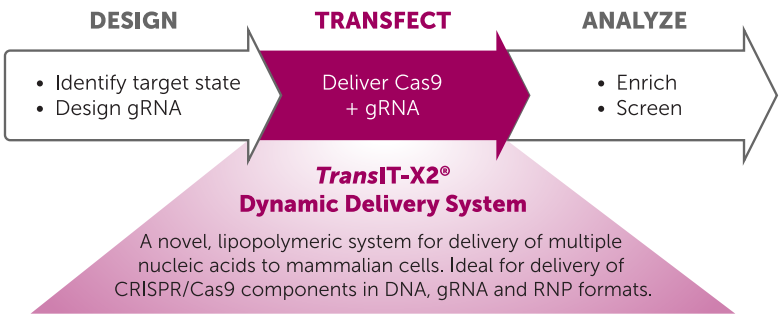
The *Trans*IT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect siRNA targeting endogenous proteins, GAPDH and aha1, or to deliver a non-targeting control in primary normal human dermal fibroblasts (NHDF). Cells were transfected in a 6-well plate using 4 µl of *Trans*IT-X2® (Mirus Bio) or 6 µl of Lipofectamine® 2000 (Thermo Fisher Scientific) and 25 nM siRNA according to each manufacturer's protocol. The amount of GAPDH or aha1 mRNA was measured relative to 18S rRNA levels using qRT-PCR and then scaled to the mRNA levels of the negative control, 48 hours post-transfection. Error bars represent the standard deviation of triplicate wells.



Effective miRNA Delivery Using the *Trans*IT-X2® Dynamic Delivery System Yields Decreased Levels of PTK9 mRNA. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect T47D cells with Pre-miR™ hsa-miR-1 miRNA Precursor (Thermo Fisher Scientific) or mirVana™ miRNA mimic (Thermo Fisher Scientific), miR-1, both known to decrease PTK9 mRNA levels. A Pre-miR negative control was transfected to assess baseline mRNA levels. Cells were transfected in a 12-well plate using 3 µl of *Trans*IT-X2® (Mirus Bio) or Lipofectamine® 2000 (Thermo Fisher Scientific) and 50 nM miRNA according to each manufacturer's protocol. The amount of PTK9 mRNA was measured relative to 18S rRNA levels using qRT-PCR and then scaled to the mRNA levels of the negative control, 48-hours post-transfection. Error bars represent the standard deviation of triplicate wells.

*Trans*IT-X2® Dynamic Delivery System, *con't*

CRISPR Gene Editing Workflow Using *Trans*IT-X2®

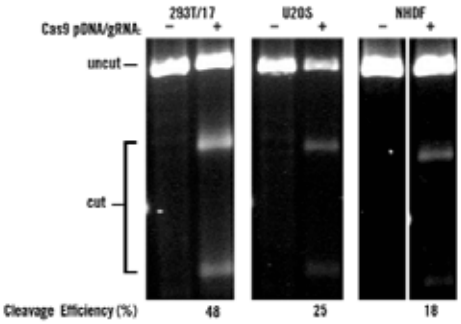


Plasmid DNA and Guide RNA Oligonucleotide Transfection

Cas9 protein and guide RNA can both be encoded by plasmid DNA for transfection. Alternatively, Cas9 can be delivered as plasmid DNA, and guide RNA can be supplied as an RNA oligonucleotide.

Benefits of these approaches include:

- **Low Cost**—Plasmid DNA is a renewable, cost-effective format
- **Flexibility**—Cas9 and guide RNA plasmids are suitable for stable or transient transfection
- **Ease-of-use**—Guide RNA oligonucleotide format enables simple retargeting of Cas9 to different loci



Efficient Genome Editing with Cas9 Plasmid DNA and Guide RNA Oligonucleotides. HEK 293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding pDNA (MilliporeSigma) and 50 nM PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using the *Trans*IT-X2® Dynamic Delivery System (2 µl/well of a 24-well plate, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.

I was recently tasked with developing a CRISPR protocol for primary and bone-derived cell lines. *Trans*IT-X2® was simple to use, 2-3 times better for transfection and much gentler on my cells than other products! I feel I have hit the jackpot and have already passed this exciting information on to my colleagues.

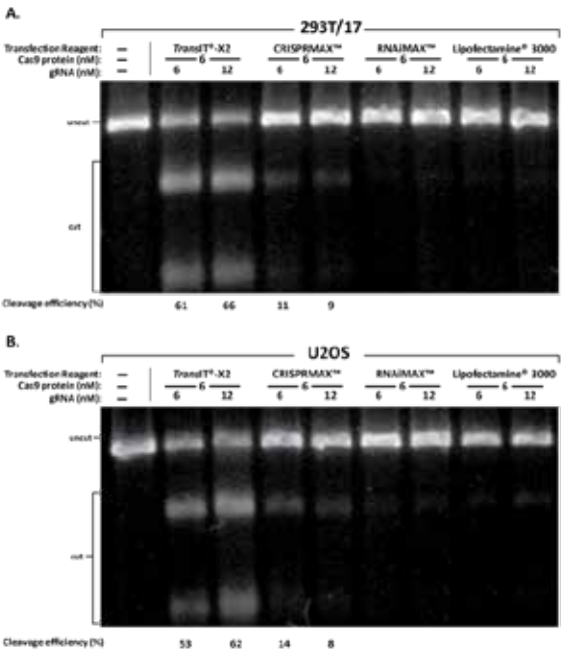
Joshua Chou, PhD
Harvard School of Dental Medicine

*Trans*IT-X2® Dynamic Delivery System, *con't*

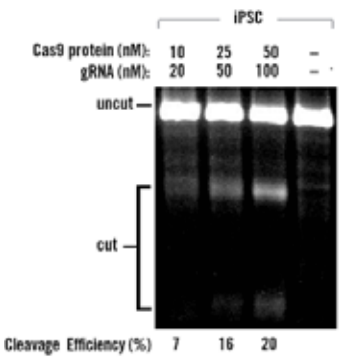
Cas9/gRNA Ribonucleoprotein (RNP) Transfection

Purified Cas9 protein can be combined with guide RNA to form an RNP complex to be delivered to cells for rapid and highly efficient genome editing. Benefits of RNP-based genome editing include:

- **High Efficiency Delivery**—Deliver Cas9/gRNA complexes to multiple cell types, including hard to transfect cells such as immune and stem cells
- **High Specificity**—Pre-formed RNP complexes provide a rapid pulse of genome editing activity
- **DNA Free**—Less risk of insertional mutagenesis



***Trans*IT-X2® Outperforms Other Reagents for RNP Delivery.** Ribonucleoprotein (RNP) complexes composed of PPIB (cyclophilin B) targeting 2-part gRNA (IDT) and Cas9 protein (PNA Bio) were delivered into (A) HEK 293T/17 and (B) U2OS cells using *Trans*IT-X2® Dynamic Delivery System (1 µl/well, Mirus Bio) or Lipofectamine® CRISPRMAX™ (1.5 µl/well and 1 µl/well of Lipofectamine® Cas9 Plus™ Reagent, Thermo Fisher) or Lipofectamine® RNAiMAX (1.5 µl/well, Thermo Fisher) or Lipofectamine® 3000 (1.5 µl/well and 1 µl/well of P3000™ Reagent, Thermo Fisher) in a 24-well format according to the manufacturers' protocol. Varying levels of gRNA (6 nM or 12 nM) were tested with 6 nM Cas9 protein (PNA Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.



Genome Editing in IPS Cells with Cas9 and Guide RNA Ribonucleoprotein Complexes. The *Trans*IT-X2® Dynamic Delivery System was used to deliver Cas9 protein/guide RNA ribonucleoprotein (RNP) complexes in human induced pluripotent stem cells (iPSCs). A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection.

Low Toxicity DNA Transfection

*Trans*IT®-LT1 Transfection Reagent

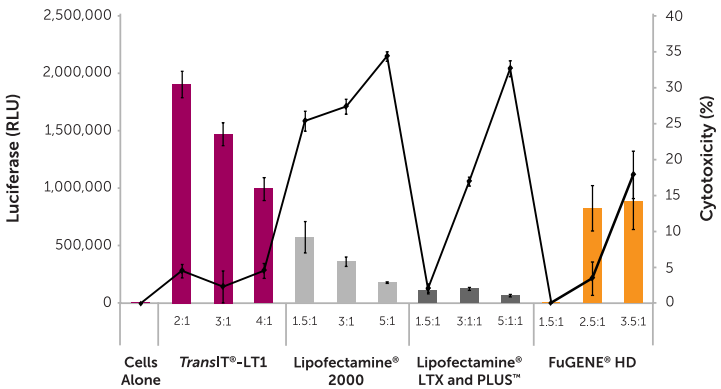
The *Trans*IT®-LT1 (Low Toxicity) Reagent is a broad spectrum, high efficiency DNA transfection reagent that is easy-to-use and exhibits minimal cellular toxicity. This reagent is a proprietary formulation of polyamines and cationic lipids that efficiently transfects cells in the presence of serum.

Broad Spectrum DNA Delivery—Utilize one transfection reagent and protocol for a variety of cells

Low Cellular Toxicity—Maintain cell density and reduce experimental biases

Deliver Single or Multiple Plasmids—Suitable for many applications such as gene expression, shRNA expression, virus production and promoter analysis

PRODUCT NO.	QUANTITY
MIR 2304	0.4 mL
MIR 2300	1.0 mL
MIR 2305	5 x 1.0 mL
MIR 2306	10 x 1.0 mL

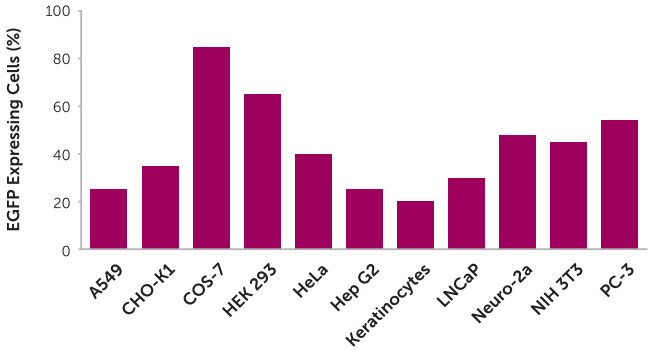


***Trans*IT®-LT1 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents.** Hep G2 cells were transfected with a luciferase expression plasmid using the designated reagents at the manufacturers' recommended reagent-to-DNA ratio indicated beneath each bar. Luciferase expression (bar graph) and lactate dehydrogenase (LDH) levels (line graph) were measured at 24-hours post-transfection. LDH levels are reported as percent cytotoxicity compared to cells alone. Experiments were performed as per industry accepted testing protocols. FuGENE is a registered trademark of Fugent LLC.

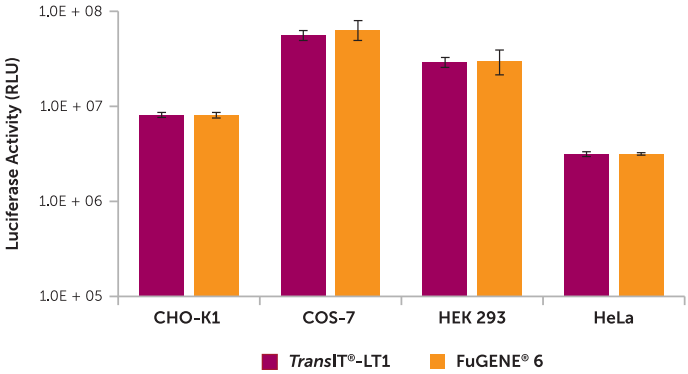
We routinely use Mirus *Trans*IT®-LT1 Transfection Reagent for the delivery of plasmid DNA to carry out immunoprecipitation experiments. Our lab recently published using *Trans*IT®-LT1 for this application to reveal a crucial regulator (MCUR1) for calcium uptake in the mitochondria to regulate cellular metabolism. (Mallilankaraman, K *et al*. *Nature Cell Biology*. December 2012)

Dr. Karthik Mallilankaraman,
Madesh Laboratory, Center for Translational Medicine, Temple University

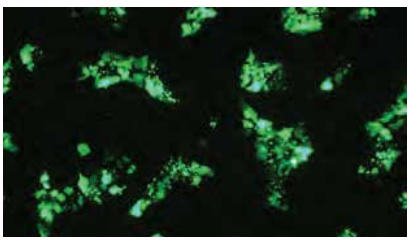
*Trans*IT®-LT1 Transfection Reagent, *con't*



The *Trans*IT®-LT1 Reagent Efficiently Delivers DNA to a Wide Variety of Cell Lines. Using the *Trans*IT®-LT1 Transfection Reagent (Mirus Bio), cells were transfected with the pEGFP-C1 expression vector, and the percentage of EGFP expressing cells was determined 24-48 hours post-transfection by flow cytometry.



Comparable Luciferase Expression With *Trans*IT®-LT1 Reagent and FuGENE® 6 in Multiple Cell Types. The indicated cell lines were transfected in duplicate with 1 µg of a luciferase expression vector per well of a 12-well plate using either 3 µl of *Trans*IT®-LT1 (Mirus Bio) or FuGENE® 6 Reagents (Fugent LLC) according to industry accepted testing protocols. Cells were harvested 24 hours post-transfection and assayed for luciferase activity. FuGENE is a registered trademark of Fugent LLC.



Exceptional Transfection Efficiency in Human Induced Pluripotent Stem Cells (iPSCs) via Reverse Transfection with the *Trans*IT®-LT1 Transfection Reagent. The *Trans*IT®-LT1 Transfection Reagent (Mirus Bio) was used to reverse transfect 1.3 x 10^6 iPSCs with a ZsGreen expressing plasmid (Clontech). Cells were visualized 48 hours post-transfection.

Data courtesy of Cellular Dynamics International (CDI), a FUJIFILM Company.

Plasmid DNA Transfection

*Trans*IT®-2020 Transfection Reagent

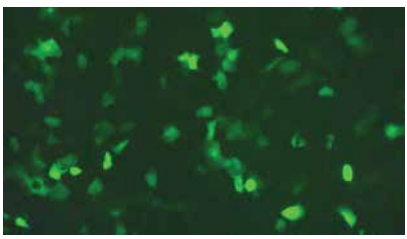
The *Trans*IT®-2020 Reagent is a versatile transfection solution for braod spectrum DNA delivery into mammalian cells. This reagent is animal component free allowing maximum compatibility for all downstream applications while outperforming other reagents in many cell types.

Broad Spectrum DNA Delivery—Achieve high expression in many cell types, including hard-to-transfect and primary cells

Outperforms Competitor Reagents—*Trans*IT®-2020 demonstrates higher transfection efficiency and less toxicity when compared to other transfection reagents

Animal Origin Free—provides high performance with maximum compatibility

PRODUCT NO.	QUANTITY
MIR 5404	0.4 mL
MIR 5400	1.0 mL
MIR 5405	5 x 1.0 mL
MIR 5406	10 x 1.0 mL



High Performance Plasmid Transfection. Primary Human Small Airway Epithelial cells (HSAEpC) were transfected using *Trans*IT®-2020 (Mirus Bio) and an EGFP expression plasmid (4:1 reagent-to-DNA ratio). Images were taken 24 hours post-transfection using an inverted fluorescence microscope (Zeiss Axiovert).

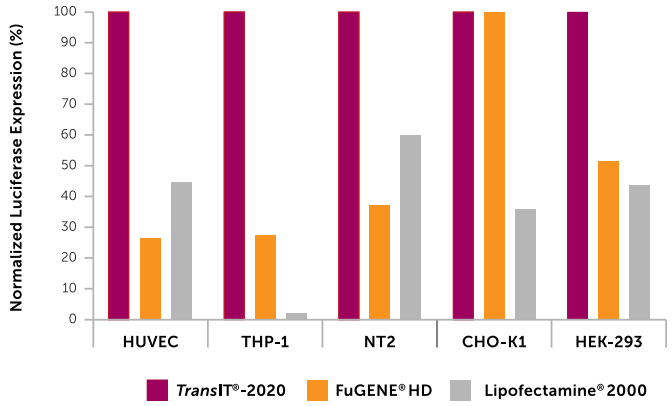
Using *Trans*IT®-2020, we transfected HeLa cells in 6-well plates with 1.25 µg of the Zhang lab construct (pX330) from Addgene that harbors both a specific guide RNA against a recognition sequence in our gene of choice, and 1.25 µg of a donor plasmid with 1 kb of 5′ and 3′ homology sequence. We then selected the cells using puromycin and came across a population that harbored the modification we were interested in. Thank you so much for the sample of *Trans*IT®-2020. Mirus has always been without exception the gold standard for me and why anyone would want to use anything else is just beyond me.

Aviva Joseph
University of Massachusetts Medical School

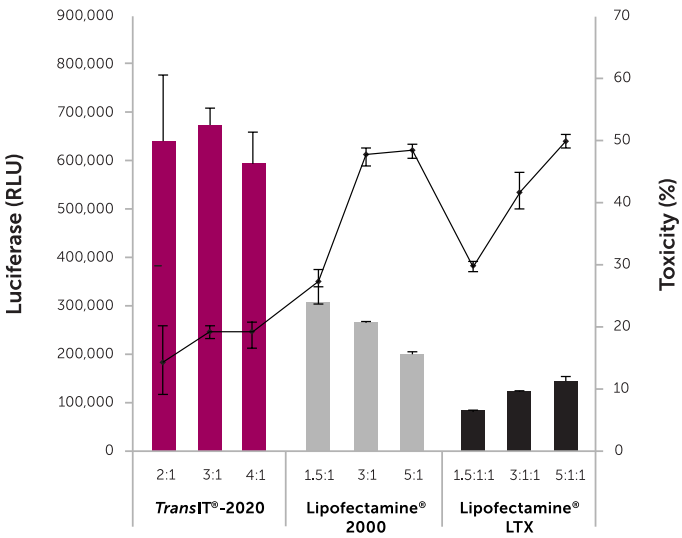
In our tests, transfection efficiencies with *Trans*IT®-2020 and *Trans*IT®-LT1 were clearly higher than with Lipofectamine® 3000 at transfecting human-derived iPS cells with CRISPR constructs and a fluorescent protein reporter.

Fedir Kiskin
University of Cambridge

*Trans*IT®-2020 Transfection Reagent, *con't*



Superior Gene Expression in a Broad Spectrum of Cell Types. The indicated cell types were transfected in 96-well plates with a luciferase expression plasmid (0.1 µg/well) according to industry accepted testing protocols. Reagent-to-DNA ratios were optimized for each cell type: *Trans*IT®-2020 (Mirus Bio, 2:1 or 3:1), FuGENE® HD (Promega, 3.5:1), Lipofectamine® 2000 (Thermo Fisher Scientific, 1.5:1, 3:1 or 5:1). Luciferase activity was measured 24 hours post-transfection. Values were normalized to TransIT®-2020 and presented as a percentage of luciferase expression. FuGENE is a registered trademark of Fugent LLC.



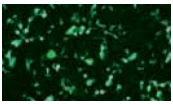
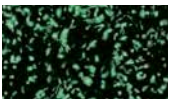
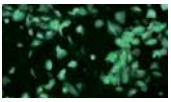
***Trans*IT®-2020 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents.** Human Umbilical Vein Endothelial Cells (HUVEC) were transfected with a luciferase expression plasmid using the designated reagents at the indicated reagent-to-DNA ratios. Transfections were performed in 96-well plates. Luciferase expression (bar graph) and lactate dehydrogenase (LDH) levels (line graph) were measured at 24 hours post-transfection. LDH levels are reported as percent cytotoxicity compared to cells alone. Error bars represent the standard deviation of triplicate wells.

*Trans*IT® Cell Type Specific Transfection Reagents

*Trans*IT® Cell Type Specific DNA Transfection Reagents are formulated to maximize transfection efficiency while maintaining cellular health in many popular or hard-to-transfect cell types.

All of these reagents offer:

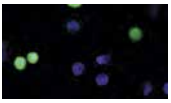
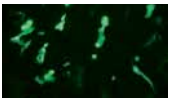
- **Optimized Formulations**—Designed for each cell type
- **Low Cellular Toxicity**—Maintain cell density and reduce experimental biases due to toxicity-induced cellular changes
- **Serum Compatible**—No media changes necessary or extensive optimization required, saving valuable research time

PRODUCT*	APPLICABLE CELL LINES/TYPES	EFFICIENCY**	PRODUCT NO.	QUANTITY
***Trans*IT®-293 Transfection Reagent**				
	Adherent HEK 293 and related	75-85%	MIR 2704	0.4 mL
			MIR 2700	1.0 mL
			MIR 2705	5 x 1.0 mL
			MIR 2706	10 x 1.0 mL
***Trans*IT®-BrCa Transfection Reagent**				
	MCF-7, MDA-MB-453, MD-AMB-453, MDA-MB-468, T47D	40-80%	MIR 5504	0.4 mL
			MIR 5500	1.0 mL
			MIR 5505	5 x 1.0 mL
			MIR 5506	10 x 1.0 mL
***Trans*IT®-CHO Transfection Kit (*Trans*IT®-CHO Reagent & CHO Mojo Reagent)**				
	Adherent CHO-K1 and related	50-60%	MIR 2174	0.4 mL
			MIR 2170	1.0 mL
			MIR 2175	5 x 1.0 mL
			MIR 2176	10 x 1.0 mL
***Trans*IT-HeLaMONSTER® Transfection Kit (*Trans*IT®-HeLa Reagent and MONSTER Reagent)**				
	HeLa and related	50-60%	MIR 2904	0.4 mL
			MIR 2900	1.0 mL
			MIR 2905	5 x 1.0 mL
			MIR 2906	10 x 1.0 mL

Transfections exhibit high target protein expression with very little cell toxicity. Cells remain viable post-transfection and can be readily infected with virus without any problems.

Dr. Corine St. Gelais
Center for Retrovirus Research
The Ohio State University

*Trans*IT® Cell Type Specific Transfection Reagents, *con't*

PRODUCT*	APPLICABLE CELL LINES/TYPES	EFFICIENCY**	PRODUCT NO.	QUANTITY
***Trans*IT®-Jurkat Transfection Reagent**				
	Jurkat and related	5-10%	MIR 2124	0.4 mL
			MIR 2120	1.0 mL
			MIR 2125	5 x 1.0 mL
			MIR 2126	10 x 1.0 mL
***Trans*IT®-Keratinocyte Transfection Reagent**				
	Keratinocyte	20-30%	MIR 2804	0.4 mL
			MIR 2800	1.0 mL
			MIR 2805	5 x 1.0 mL
			MIR 2806	10 x 1.0 mL

* Single tube reagents contain the indicated transfection reagent. Transfection reagents with two components are named "Kits" and both components are listed following the product name.

** Transfection efficiency determined by transfection of an EGFP expression vector followed by visual quantification of the percentage of cells expressing EGFP or via flow cytometry.

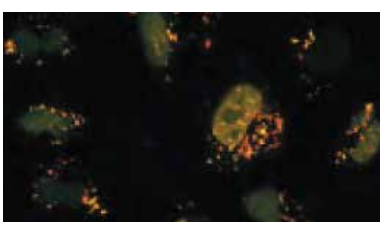
Oligonucleotide Transfection

*Trans*IT®-Oligo Transfection Reagent

Unique Formulation—Maximize DNA and RNA oligonucleotide transfection efficiency in a wide range of cells

Oligonucleotides Tested—Phosphorothioate DNA and RNA, 2' O-methyl oligos, siRNA, miRNA, ASO, shRNA, and CRISPR gRNA

PRODUCT NO.	QUANTITY
MIR 2164	0.4 mL
MIR 2160	1.0 mL
MIR 2165	5 x 1.0 mL
MIR 2166	10 x 1.0 mL



***Trans*IT®-Oligo Reagent Achieves High Transfection Efficiency.** HeLa cells were transfected with Cy®3 and fluorescein labeled phosphorothioate DNA oligos using *Trans*IT®-Oligo Reagent and observed 24 hours post-transfection. The yellow punctate fluorescence is the result of co-localization of the fluorescein and Cy®3 labeled oligonucleotides after transfection. The image was acquired using a confocal microscope.

siRNA/miRNA Transfection

*Trans*IT-TKO® & *Trans*IT-siQUEST® Transfection Reagents

*Trans*IT-TKO® and *Trans*IT-siQUEST® small interfering RNA (siRNA and miRNA) Transfection Reagents are broad spectrum reagents that are easy to use and exhibit minimal cellular toxicity. Each reagent is uniquely formulated and exhibits distinct siRNA/miRNA transfection profiles. Testing these two reagents allows users to identify a formulation which is optimal for achieving knockdown in their particular cell line.

High Knockdown Efficiency—Achieve optimal gene silencing in a large percentage of cells to ensure experimental success

Low Cellular Toxicity—Maintain cell density and reduce experimental biases due to alterations in cellular health

Flexible Protocol—use with either standard or reverse transfections

*Trans*IT-TKO® Transfection Reagent

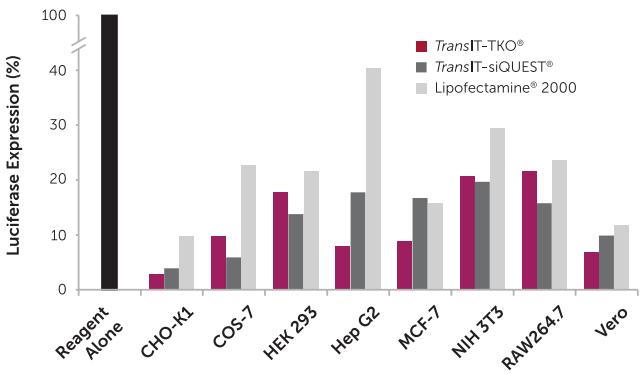
PRODUCT NO.	QUANTITY
MIR 2154	0.4 mL
MIR 2150	1.5 mL
MIR 2155	5 x 1.5 mL
MIR 2156	10 x 1.5 mL

We have tried other transfection reagents, but only the *Trans*IT-TKO® reagent gives us a 100% transfection rate and gene knockdown without toxicity in these cells (RAW 264.7).

Nature Protocols, 1: 508 - 517 (2006)

*Trans*IT-siQUEST® Transfection Reagent

PRODUCT NO.	QUANTITY
MIR 2114	0.4 mL
MIR 2110	1.5 mL
MIR 2115	5 x 1.5 mL
MIR 2116	10 x 1.5 mL



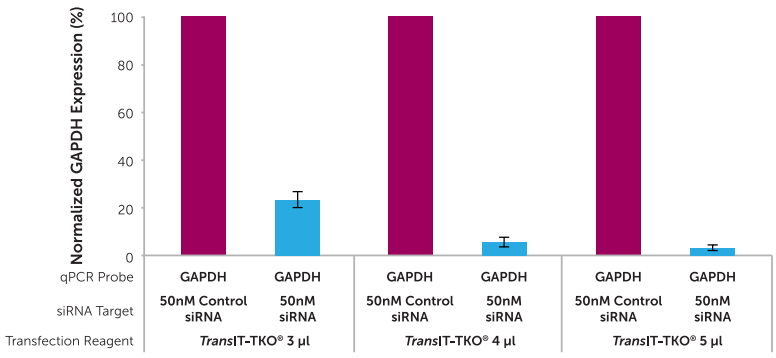
Knockdown Efficiencies Using *Trans*IT-siQUEST®, *Trans*IT-TKO® Reagents and Lipofectamine® 2000. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines using the *Trans*IT®-LT1 Reagent (Mirus Bio). Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either *Trans*IT-siQUEST® (maroon, (Mirus Bio)), *Trans*IT-TKO® (dark gray, (Mirus Bio)) or Lipofectamine® 2000 (light gray, Thermo Fisher Scientific) Reagents. Bars indicate the percent of normalized firefly luciferase expression as compared to each reagent alone control 24 hours post-transfection.

*Trans*IT-TKO® & *Trans*IT-siQUEST®, *con't*

Knockdown of Genes Using *Trans*IT-TKO® or *Trans*IT-siQUEST® Transfection Reagents. Cells were transfected with siRNAs targeting the indicated genes using the *Trans*IT-TKO® or *Trans*IT-siQUEST® Reagents (Mirus Bio), and the knockdown percentage was determined using quantitative RT-PCR or luciferase assays.

CELL LINE (SOURCE)	ENDOGENOUS TRANSCRIPT	*Trans*IT-TKO® KNOCKDOWN EFFICIENCY	*Trans*IT-siQUEST® KNOCKDOWN EFFICIENCY
A549-luc (human lung)	Luciferase*	77%	82%
BNL CL.2 (mouse liver)	MAPK1	80%	—
	MAPK3	83%	—
CHO-luc (hamster ovary)	Luciferase*	86%	91%
HEK 293-luc (human kidney)	Luciferase*	83%	77%
HeLa (human cervix)	Lamin A/C	80%	—
	GAPDH	80%	—
HeLa-luc (human cervix)	Luciferase*	84%	82%
Hepa-luc (mouse liver)	Luciferase*	—	92%
Hep G2 (human liver)	MAPK1	80%	—
NIH 3T3-luc (mouse fibroblast)	Luciferase*	85%	89%
NIH 3T3-L1 (mouse fibroblast)	MAPK1	70%	—
	MAPK3	70%	—
Secondary Human Astrocytes	Lamin A/C	80%	—
Primary Mouse Hepatocytes	ABC A1	70%	—
	Lamin A/C	81%	—
	PPAR-alpha	—	82%

*Firefly luciferase expression vectors were stably integrated into the parent cell lines and clonal lines constitutively expressing firefly luciferase were used.



High Efficiency Endogenous Knockdown in iCell® Cardiomyocytes. The *Trans*IT-TKO® Transfection Reagent (Mirus Bio) was used to transfect iCell® Cardiomyocytes (Cellular Dynamics International (CDI), a FUJIFILM Company) plated at a density of 136,500 cells per well of a 12-well plate pre-coated with fibronectin. Seven days post-plating, triplicate wells were transfected with *Trans*IT-TKO® (3-5 µl per well, Mirus Bio) and non-targeting control siRNA or GAPDH targeting siRNA (50 nM per well). Seventy-two hours post-transfection, the amount of GAPDH mRNA was measured relative to 18S rRNA levels using qRT-PCR and then scaled to the expression level of the non-targeting control siRNA. Error bars represent the standard error of the mean (SEM) of three independent complexes.

mRNA & Viral RNA

*Trans*IT®-mRNA Transfection Kit

The *Trans*IT®-mRNA Transfection Kit provides high efficiency transfection of large RNA molecules such as mRNA or viral RNA, is easy to use and minimizes cellular toxicity due to its ability to transfect RNA in the presence of serum. The kit consists of the *Trans*IT-mRNA Reagent - a novel, non-liposomal cationic polymer/lipid formulation and the mRNA Boost Reagent - a proprietary compound. is easy to use and minimizes cellular toxicity due to its ability to transfect RNA in the presence of serum.

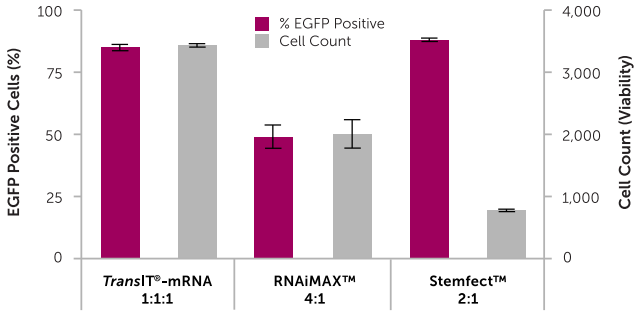
High Efficiency Delivery—Ensures experimental success by effectively transfecting RNA into a large percentage of the cell population

Low Cellular Toxicity—Maintain cell density and reduce transfection induced toxicity

Serum Compatible—Perform transfections in the presence of serum which eliminates the need for a media change and maintains cellular health

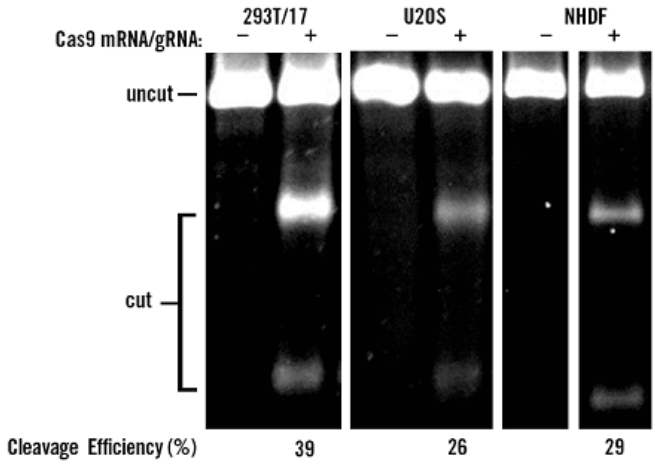
Deliver Various Sizes of RNA—Ideal for specialized applications, such as viral production, protein expression from mRNA and stem cell reprogramming

PRODUCT NO.	QUANTITY
MIR 2225	0.4 mL
MIR 2250	1.0 mL
MIR 2255	5 x 1.0 mL
MIR 2256	10 x 1.0 mL

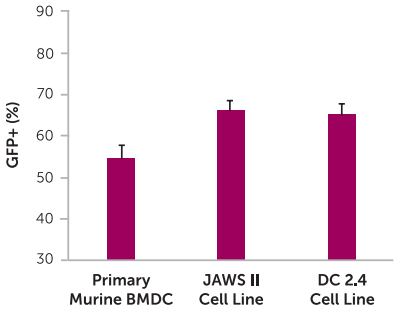


High Efficiency and Low Toxicity Transfection Following 14 Consecutive Transfections With the *Trans*IT®-mRNA Transfection Kit. Repeated daily transfections were performed in the same population of BJ fibroblasts using three commercially available transfection reagents – the *Trans*IT®-mRNA Transfection Kit (Mirus Bio), Lipofectamine® RNAiMAX (Thermo Fisher Scientific) and Stemfect™ RNA Transfection Kit (Stemgent) - with a capped and polyadenylated EGFP mRNA incorporating pseudouridine and 5mC modified bases (Trilink Biotechnologies). Multiple reagent-to-RNA ratios were tested and the optimal ratio is represented. Transfections were performed in 12-well plates using the indicated reagent-to-RNA ratios to deliver 1 µg of RNA. Transfection efficiency was measured by flow cytometry on a guava® easyCyte™ 5HT Flow Cytometer (MilliporeSigma) following 14 consecutive daily transfections (maroon bars). Cell viability was determined using cell counts measured during flow cytometry (light grey bars). Error bars represent the standard deviation of triplicate wells.

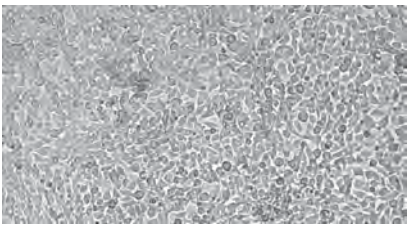
*Trans*IT®-mRNA Transfection Kit, *con't*



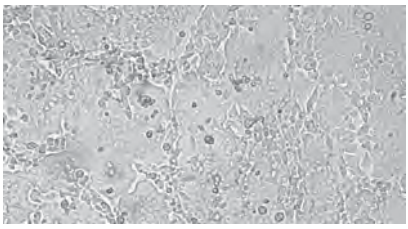
Efficient Genome Editing with Cas9 mRNA and Guide RNA Oligonucleotides. HEK 293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding mRNA, 5mC, (Trilink Biotechnologies) and 25 nM of PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using *Trans*IT®-mRNA Transfection Kit (0.5 µl/well of 24-well plate of both *Trans*IT®-mRNA Transfection and mRNA Boost Reagent, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.



Multiple Dendritic Cell Types Express GFP from mRNA Transfected Using the *Trans*IT®-mRNA Transfection Kit. Murine primary bone marrow derived dendritic cells (BMDC) and murine dendritic cells (JAWS II and DC 2.4) were transfected with 1 µg of capped and polyadenylated mRNA encoding GFP using a *Trans*IT®-mRNA Reagent (Mirus Bio): Boost: mRNA ratio of 1:1:1 (µl:µl:µg). All cells were seeded (80,000 cells/well) overnight in 24-well plates. Cells were assayed via flow cytometry 8 hours post-transfection. Error bars represent the standard deviation of at least 3 separate experiments.[1]



No RNA Control



MHV RNA Transfected

The *Trans*IT®-mRNA Transfection Kit Successfully Delivers Viral RNAs 32 kb Long. A 32 kb *in vitro* transcribed RNA of the murine coronavirus, MHV, was transfected into DBT cells using the *Trans*IT®-mRNA Transfection Kit (Mirus Bio). Successful transfection assessed by the formation of syncytia 24-48 hours post-transfection. Syncytia were visualized by phase contrast microscopy.[2]

[1] *Data courtesy of Kyle Phua (Principal Investigator: Kam W. Leong), Duke University.*

[2] *Data courtesy of Mark Clemenz, Loyola University of Chicago.*

Insect Cell Transfection & Baculovirus Production

*Trans*IT®-Insect Transfection Reagent

Transient transfection and recombinant baculovirus production are commonly used methods for insect cell expression. The *Trans*IT®-Insect Transfection Reagent is an animal origin free transfection reagent specifically optimized for high gene expression in a variety of insect cell types.

Exceptional DNA Delivery—In insect cell types including Sf9, High Five™ and S2

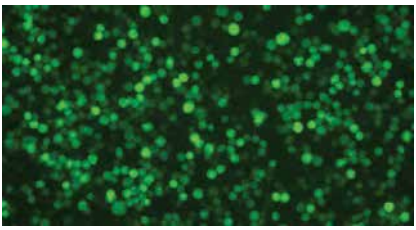
High Baculovirus Production—Ideal for baculovirus expression in insect cells

Serum Compatibility—Non-liposomal, animal origin free formulation that eliminates the need for media change

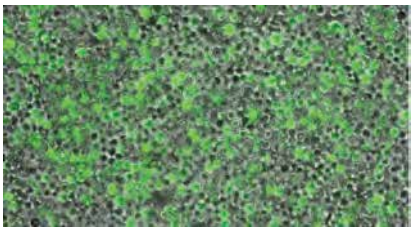
Better Value—Less reagent required per transfection

PRODUCT NO.	QUANTITY
MIR 6104	0.4 mL
MIR 6100	1.0 mL
MIR 6105	5 x 1.0 mL
MIR 6106	10 x 1.0 mL

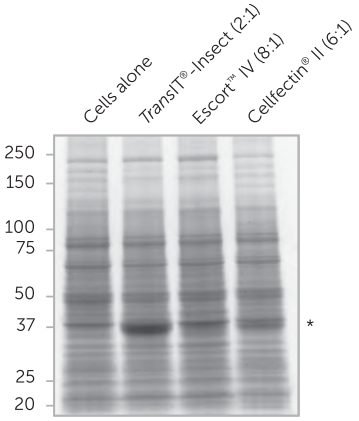
A.



B.

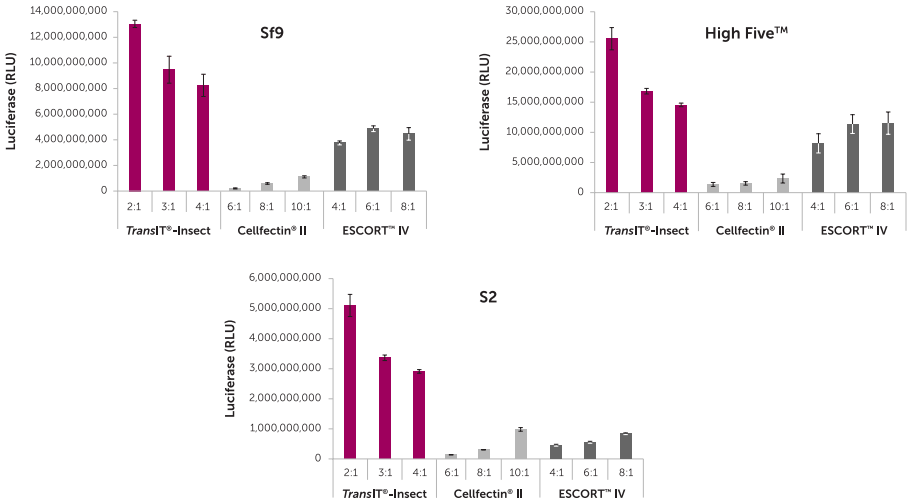


Efficient Transfection of Baculovirus Genomic DNA Using the *Trans*IT®-Insect Reagent. Transfections were performed in 6-well plates with 5 x 10^5 Sf9 cells per well using the *Trans*IT®-Insect Transfection Reagent (Mirus Bio) at the reagent-to-total DNA ratio of 3:1 (µl:µg). Cells were co-transfected with 0.5 µg of ProGreen™ (AB Vector) baculovirus genomic vector DNA (AB Vector) encoding green-fluorescent protein (GFP) and 0.1 µg of pVL1393 transfer vector (AB Vector). (A) Fluorescence and phase contrast images were taken at 6 days post- transfection using a Zeiss S100 fluorescent microscope. Merge shown in (B).

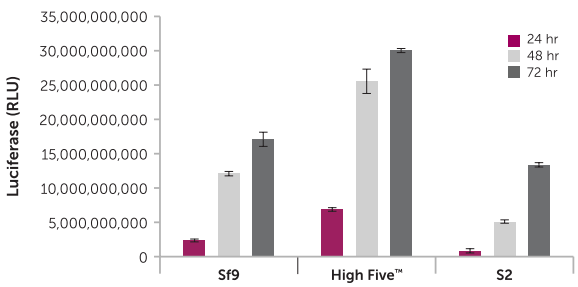


Superior Recombinant Protein Expression in High Five™ Cells Using *Trans*IT®-Insect. High Five™ cells (Thermo Fisher Scientific) were transfected in 6-well plates with 2.5 µg of a GFP expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated reagent at the indicated reagent-to-DNA ratios (µl:µg). Total soluble cell lysates were prepared from cells 72 hours post-transfection. Lysates from 100 µl cultures were analyzed by SDS-PAGE and Coomassie blue staining; cells alone (untransfected) is shown as control. The highest level of expression of GFP-6XHis-S-HSV (*, ~38 kDa) was detected in lysate from the cells that were transfected with *Trans*IT®-Insect (Mirus Bio).

*Trans*IT®-Insect Transfection Reagent, *con't*



***Trans*IT®-Insect Outperforms Competitor Transfection Reagents.** Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific) and Drosophila S2 cells were transfected in 96-well plates with 0.1 µg of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated reagent at the indicated reagent-to-DNA ratios (µl:µg). Luciferase expression was measured at 48 hours post-transfection. Sf9 and High Five™ (Thermo Fisher Scientific) cells were cultured and transfected in serum-free media formulations; S2 cells were in serum-containing medium. Error bars represent the standard error of the mean for triplicate wells.



***Trans*IT®-Insect Yields Increased Protein Expression Over Time.** Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific) and Drosophila S2 were transfected in a 96-well plate with 0.1 µg of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the *Trans*IT®-Insect Transfection Reagent (Mirus Bio) at a reagent-to-DNA ratio of 2:1 (µl:µg). Luciferase expression was measured at three time points: 24, 48 and 72 hours post-transfection. Sf9 and High Five™ (Thermo Fisher Scientific) cells were cultured and transfected in serum-free media formulations; S2 cells were in serum-containing medium. Error bars represent the standard error of the mean for triplicate wells.

Our lab successfully tested *Trans*IT®-Insect Transfection Reagent for generating recombinant baculovirus in insect cells. Using *Trans*IT®-Insect with multiple BEVS we were able to generate high-titer baculovirus that resulted in consistently higher protein expression in High Five™ and Sf9 cells compared to Cellfectin® II (Thermo Fisher Scientific). (Kuo *et al*, *Protein Eng Des Sel.* Oct 2012)

Dr. Linda Lua, Director
Protein Expression Facility
The University of Queensland

Nucleic Acid & Complex Molecules

Ingenio® EZporator® Device and Electroporation Kits & Solutions

Ingenio® Electroporation Solution facilitates efficient and reliable delivery of nucleic acids to eukaryotic cells refractory to chemical transfection. Ingenio® is a broad spectrum solution that supports high efficiency electroporation with minimal toxicity and replaces standard electroporation solutions including phosphate buffered saline and serum-free media.

Ingenio® Kits (include solution, cuvettes and cell droppers) are compatible with multiple instruments. All Ingenio® products can be purchased separately.

High Efficiency Electroporation—Deliver DNA or RNA to hard-to-transfect, stem and primary cells

Compatible with Most Conventional Electroporation Devices—Use your existing system including Lonza-Amaxa®, Bio-Rad® or Harvard BTX®

Save Money and Reduce Research Costs Without Sacrificing Performance—Ingenio® Electroporation Solution is available as a stand-alone solution or as part of a complete kit with cuvettes and cell droppers



The Ingenio® EZporator® Electroporation System is designed to be used with the Ingenio® Electroporation Solution which is ideal for mammalian and insect cell transfection. Electroporation is the method of choice for many hard-to-transfect cell types, and the Ingenio® EZporator® Electroporation System is a cost effective, straight-forward, open system that is perfect for any lab seeking performance without breaking the bank.

Ingenio® EZporator® Electroporation System

PRODUCT NO.	QUANTITY
MIR 51000	1 System

Ingenio® Electroporation Kits for Amaxa® Nucleofector® II/2b Nucleofector Devices

(solution, 0.2 cm cuvettes, cell droppers)

MIR 50112	25 RXN
MIR 50115	50 RXN
MIR 50118	100 RXN

Ingenio® Electroporation Kits for all other Electroporators, such as Bio-Rad® and Harvard BTX®

(solution, 0.4 cm cuvettes, cell droppers)

MIR 50113	25 RXN (6.25 mL)
MIR 50116	50 RXN (12.5 mL)
MIR 50119	100 RXN (25 mL)

Ingenio® Electroporation Solution

MIR 50111	25 RXN (6.25 mL)
MIR 50114	50 RXN (12.5 mL)
MIR 50117	100 RXN (25 mL)

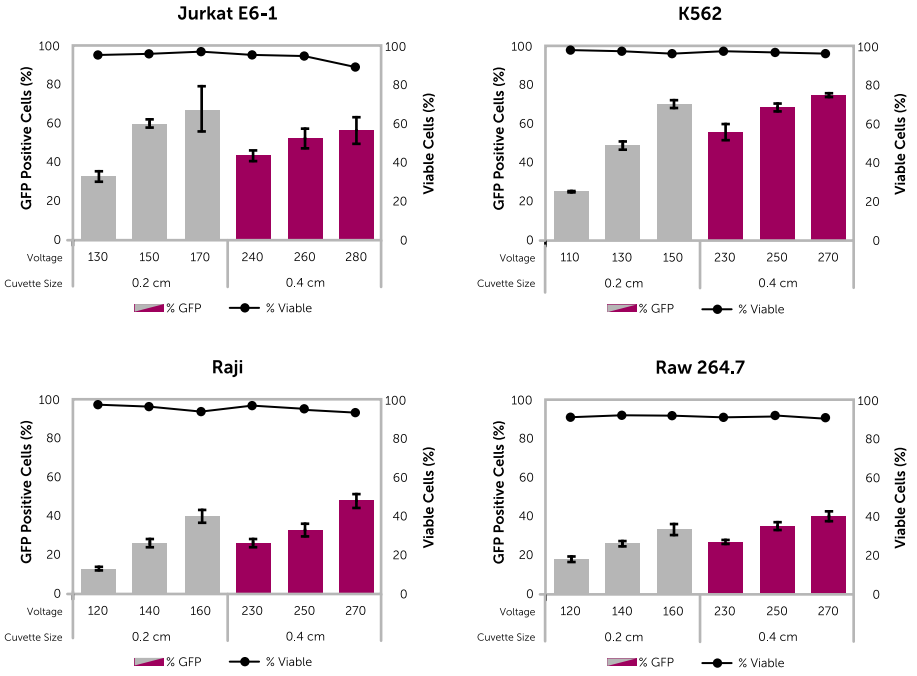
Cuvettes

PRODUCT NO.	SIZE	QTY
MIR 50121	0.2 cm	50 PK
MIR 50123	0.4 cm	50 PK

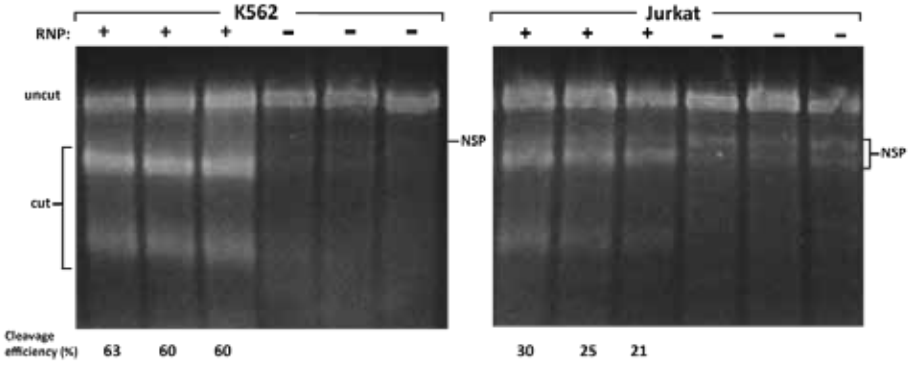
Cell Droppers

PRODUCT NO.	QUANTITY
MIR 50125	50 PK

Ingenio® Electroporation Kits & Solutions, *con't*



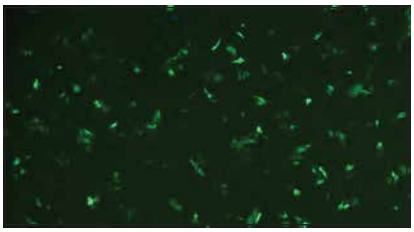
High Efficiency Electroporation and Viability with the Ingenio® EZporator® Electroporation System. Jurkat E6-1, K562, Raji and Raw 264.7 cells were electroporated with an EGFP reporter plasmid at various voltages using the Ingenio® Electroporation Solution (Mirus Bio) and the Ingenio® EZporator® Electroporation system (Mirus Bio) using either 0.2 cm or 0.4 cm electroporation cuvettes. The percentage of GFP positive cells (bar) and viability (dot) using propidium iodide was determined by flow cytometry at 48-hours post-electroporation.



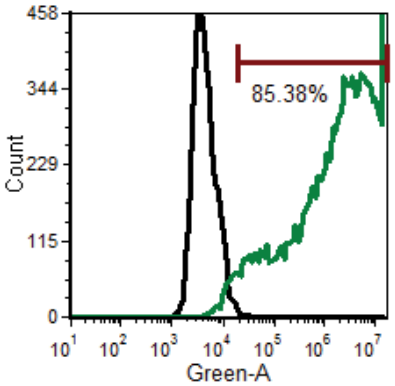
Efficient RNP Delivery with Electroporation Ingenio® Solution. K562 and Jurkat cells were electroporated with a Cas9 protein/gRNA, ribonucleoprotein (RNP) complex, comprised of 750 nM Cas9 protein (EnGen® Cas9 NLS, NEB) and 1,500 nM pre-complexed two-part gRNA (IDT) targeting PPIB using the Ingenio® Electroporation Solution (Mirus Bio) and a Gene Pulser® Xcell™ Eukaryotic System (Bio-Rad Laboratories, Inc.). Exponential pulse conditions of 130V, 950 µF for K562 and 150 V, 950 µF for Jurkat cells were applied to triplicate 0.2 cm cuvettes, 100 µl volume, 10 x 10^6 cells/ml +/- RNP complex. A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection. Non-specific bands (NSP) were observed in the negative control of both cell lines. Cleavage efficiency was calculated based on the ratio of cleaved band intensities to the sum of cleaved and uncleaved band intensities minus the average signal of the non-specific band(s) in negative control lanes.

Ingenio® Electroporation Kits & Solutions, *con't*

A.

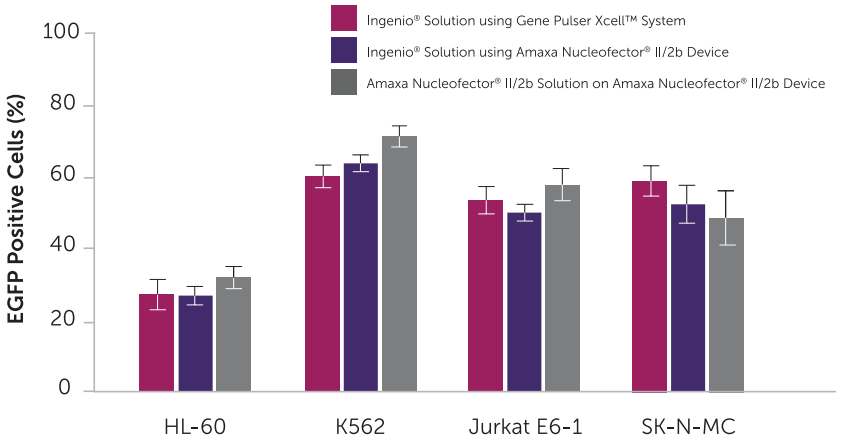


B.



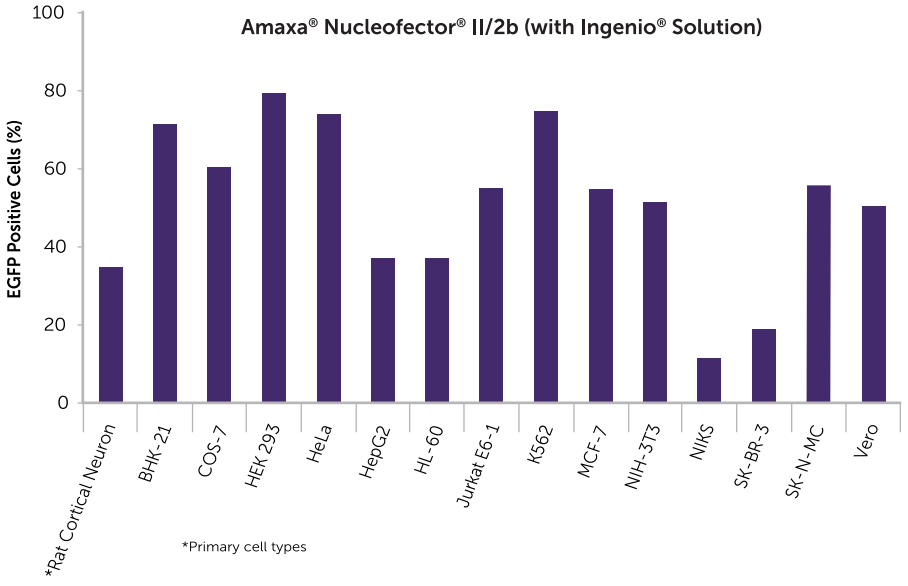
High Efficiency Plasmid DNA Electroporation of Human Induced Pluripotent Stem (iPS) Cells using Ingenio®. The Ingenio® Electroporation Kit (Mirus Bio) was used to transfect 2 x 10^6 iPS cells on the Amaxa® Nucleofector® II/2b Device (Lonza Group Ltd). Cells were electroporated with 8 µg ZsGreen expressing plasmid (Clontech) in 100 µl and plated in 6-well plates at 0.33 x 10^6 cells/well. Cells were visualized 24 hours post-transfection and imaged under 4X objective with an Olympus IX71® Inverted Microscope (Olympus Corporation). Image is (A) green fluorescence. Cells were also assayed 24 hours post-transfection on an Accuri® Cytometer (Becton Dickenson and Company). The histogram (B) shows unelectroporated cells (black line) compared to cells electroporated with plasmid using the Ingenio® Electroporation Kit (green line, Mirus Bio).

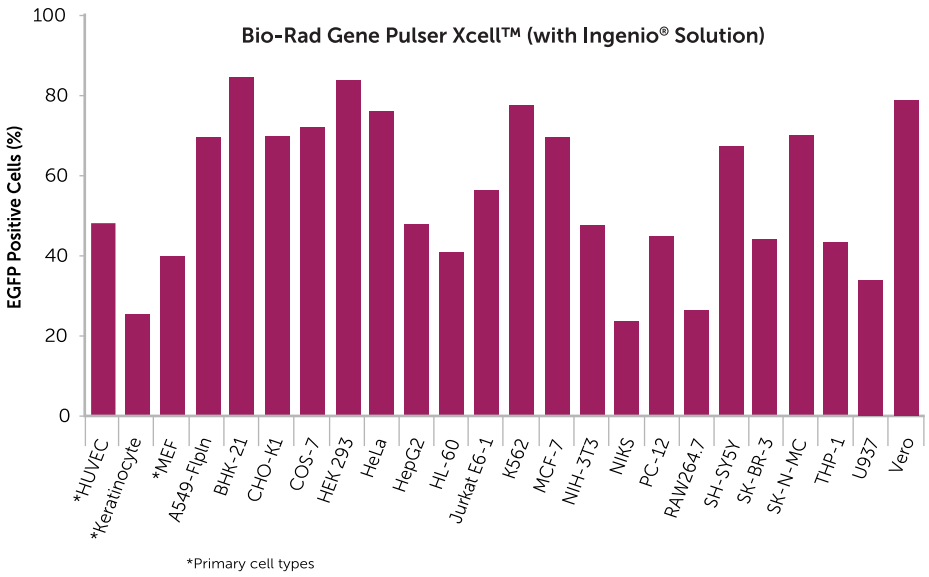
Data courtesy of Cellular Dynamics International (CDI), a FUJIFILM Company.



The Ingenio® Solution is Compatible with Multiple Devices. Cells were electroporated in parallel with an EGFP reporter vector. Two electroporators were used with different electroporation solutions: the Ingenio® Electroporation Kit (Mirus Bio) was used in the Gene Pulser Xcell™ Eukaryotic System (Bio-Rad) and the Nucleofector® II/2b Device (Lonza); the Nucleofector® Kit V was used in the Nuclefector® II/2b Device, all according to manufacturers' recommendations.

Ingenio® Electroporation Kits & Solutions





Efficient Plasmid DNA Delivery in Many Cell types Using the Lonza Nucleofector II/2b and the Bio-Rad® GenePulser Xcell™ Systems with the Ingenio® Electroporation System. Cells were assayed at 24 hours post-electroporation by flow cytometry and reported as percentage of live cell population.

For ideal pulse conditions, visit: https://hub.mirusbio.com/m/0da44c66cbbf80f3/original/Ingenio-Pulse-Conditions.pdf

Lentivirus Production

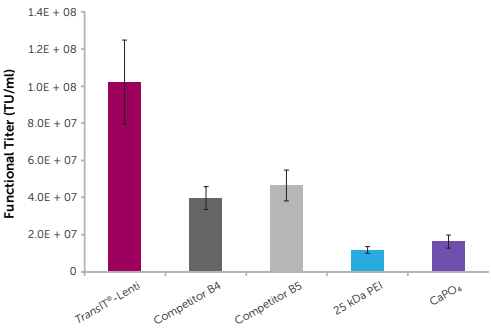
*Trans*IT®-Lenti Transfection Reagent

*Trans*IT®-Lenti Transfection Reagent is designed to enhance delivery of packaging and transfer vectors to adherent HEK 293T cell types and increase recombinant lentivirus production. The *Transduce*IT™ Transduction Reagent enhances recombinant lentivirus infection of target cells.

High Performance—Provide up to eight-fold higher functional titers

Simple Protocol—No media change required, single harvest

Animal Origin Free—Regulatory friendly



High Functional Titers with the *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate with pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma-Aldrich, Inc. LLC) and the Lentivirus Packaging Mix Powered by MISSION® (1:1 ratio, 2 µg/well) with the following reagents: *Trans*IT®-Lenti (3:1, vol:wt; Mirus Bio), Competitor B4 (3:1), Competitor B5 (3:1:1), 25 kDa PEI (6:1) or $CaPO_4$ precipitation (4 µg pDNA/well). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ (Mirus Bio) and GFP expression was measured 72 hours post-transduction using guava® easyCyte™ 5HT Flow Cytometer (MilliporeSigma). Error bars represent triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

*Trans*IT®-Lenti Transfection Reagent

PRODUCT NO.	QUANTITY
MIR 6603	0.3 mL
MIR 6604	0.75 mL
MIR 6600	1.5 mL
MIR 6605	5 x 1.5 mL
MIR 6606	10 x 1.5 mL

*Transduce*IT™ Transduction Reagent

PRODUCT NO.	QUANTITY
MIR 6620	1 mL

*Trans*IT® Lentivirus System

(System includes: *Trans*IT®-Lenti Transfection Reagent, *Transduce*IT™ Transduction Reagent, Lentivirus Packaging Mix Powered by MISSION® Genomics)

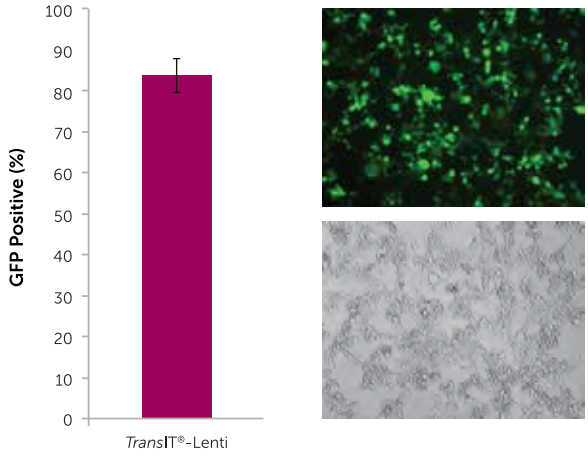
PRODUCT NO.	QUANTITY
MIR 6650	5 RXN*
MIR 6655	34 RXN*

Lentivirus Packaging Mix Powered by MISSION® Genomics

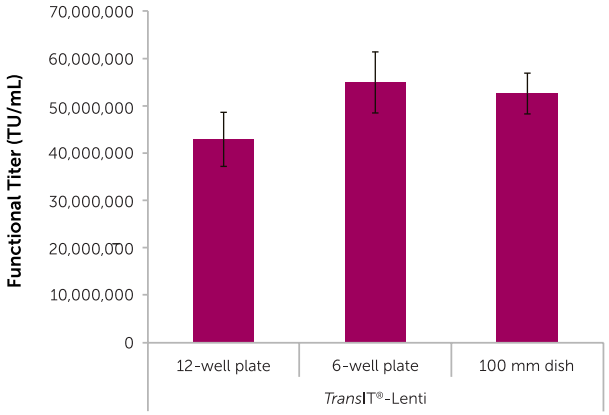
PRODUCT NO.	QUANTITY
MIR 6630	5 RXN*
MIR 6640	34 RXN*

* The number of reactions per kit is determined by the amount of *Trans*IT®-Lenti Reagent and Lentivirus Packaging Mix required for one 10-cm dish transfection.

*Trans*IT®-Lenti Transfection Reagent, *con't*

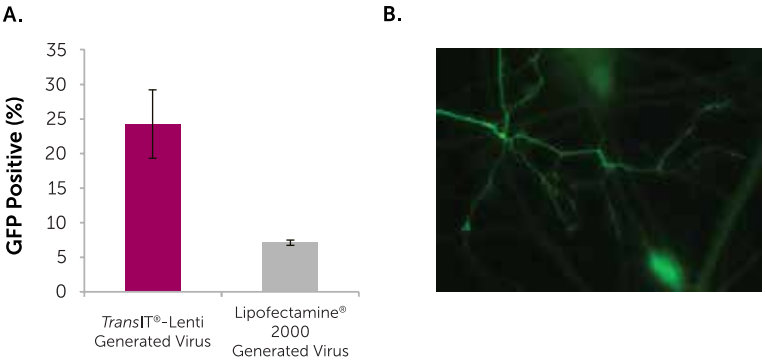


High Efficiency Transfection with the *Trans* IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate format using MISSION® pLKO.1-puro-CMV-TurboGFP™ transfer vector and Lentivirus Packaging Mix Powered by MISSION® (Sigma-Aldrich, Inc. LLC) using the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt; Mirus Bio). GFP efficiency was measured at 48 hours post-transfection. Error bars represent five transfection complexes. Images were captured at 48 hours post-transfection. The observed cell rounding and cell-cell fusion is due to high expression of the vesicular stomatitis virus G protein (VSV-G) for pseudotyping the recombinant lentivirus.

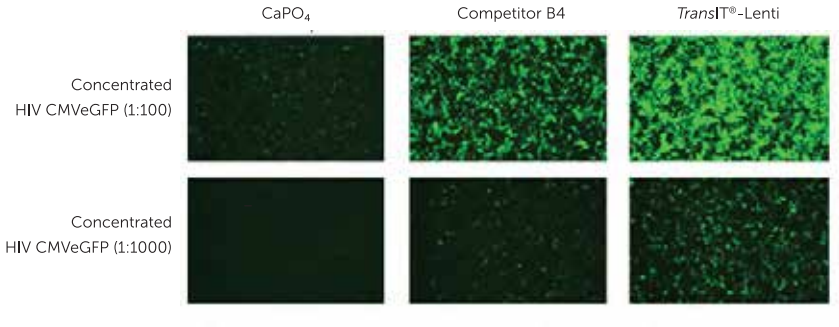


Lentivirus Production is Scalable. Adherent 293T/17 cells were transfected in a 12-well, 6-well or 100-mm plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix Powered by MISSION® at a 1:1 ratio; Sigma-Aldrich, Inc. LLC) and the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt; Mirus Bio). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ (Mirus Bio) and GFP expression was measured 72 hours post-transduction. Error bars represent triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

*Trans*IT®-Lenti Transfection Reagent, *con't*



High Transduction Efficiency with Unconcentrated Lentivirus Using *Trans* IT®-Lenti. (A) Lentivirus was produced with the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt; Mirus Bio) or Lipofectamine® 2000 (Thermo Fisher Scientific) using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix Powered by MISSION®, Sigma-Aldrich, Inc. LLC). The supernatant was harvested, filtered (0.45 µm) and frozen. Lentivirus transductions were performed 5 days post-plating with iCell® Motor Neurons (Cellular Dynamics International (CDI, a FUJIFILM Company)). For both *Trans*IT®-Lenti (Mirus Bio) and Lipofectamine® 2000 (Thermo Fisher Scientific), 1 µM of unconcentrated supernatant was added per well of a 96-well plate. GFP efficiency was measured 72 hours post-transduction using guava® easyCyte™ 5HT Flow Cytometer (MilliporeSigma). Error bars represent the SEM of duplicate wells. (B) iCell® Motor Neurons (Cellular Dynamics International (CDI, a FUJIFILM Company)) were plated in 35 mm dishes (Ibidi) and transduced with lentivirus produced using the *Trans*IT®-Lenti Transfection Reagent (Mirus Bio) and MISSION® vectors (Sigma-Aldrich, Inc. LLC). Images were captured at 72 hours post-transduction with a Zeiss Axiovert S100 inverted fluorescence microscope using a 63X objective under oil.



Comparing Functionality of $CaPO_4$, Competitor B4 or *Trans*IT®-Lenti Generated Lentivirus. HIV CMVeGFP virus was produced in HEK 293FT cells using either $CaPO_4$, Competitor B4 and *Trans*IT®-Lenti Transfection Reagent (Mirus Bio) per manufacturers' protocol. Lentivirus was collected 48 hours post-transfection and concentrated by ultracentrifugation. HEK 293FT cells were infected with a 1:100 or 1:1000 dilution of each concentrated lentivirus. Images were captured 48 hours post-transduction.

Data courtesy of Jeremy Coffin, University of Iowa Viral Vector Core

Recombinant Protein & mAb Production

CHOgro® High Yield Expression System

The CHOgro® High Yield Expression System is the most advanced and cost- effective transient transfection system for high-yield protein production in suspension CHO cells. This second-generation system features the CHOgro® Titer Enhancer, which provides rapid, industry leading protein yields.

High Yield—Reach higher antibody titers in seven days - faster than the ExpiCHO™ Expression System

Simple Workflow—Same-day transfection, enhancer addition and temperature shift

Worry Free—No commercial license required; animal origin free

Convenient—Quick adaptation for suspension CHO cell lines

Optimized—High density growth with minimal cell clumping post transfection



Complete CHOgro® High Yield Expression System

CHOgro® Expression System

Complete system includes CHOgro® Expression Media, *Trans*IT-PRO® Transfection Reagent, CHOgro® Complex Formation Solution, CHOgro® Enhancer and L-Glutamine Solution

PRODUCT NO.	QUANTITY
MIR 6270	1 System

Individual components available separately

CHOgro® Titer Enhancer Kit

Kit includes *Trans*IT-PRO® Transfection Reagent, 1 mL, CHOgro® Enhancer, 20 mL)

PRODUCT NO.	QUANTITY
MIR 6225	1 Kit

CHOgro® Expression Medium

MIR 6200	1 L

Liquid Polybag CHOgro® Expression Medium

MIR 6202	10 L

Dry Powder CHOgro® Expression Medium

MIR 6201	Makes 10 L

*Trans*IT-PRO® Transfection Reagent

MIR 5740	1 mL
MIR 5750	10 mL

CHOgro® Complex Formation Solution

MIR 6210	100 mL

Poloxamer 188 Solution

MIR 6230	100 mL

L-Glutamine Solution

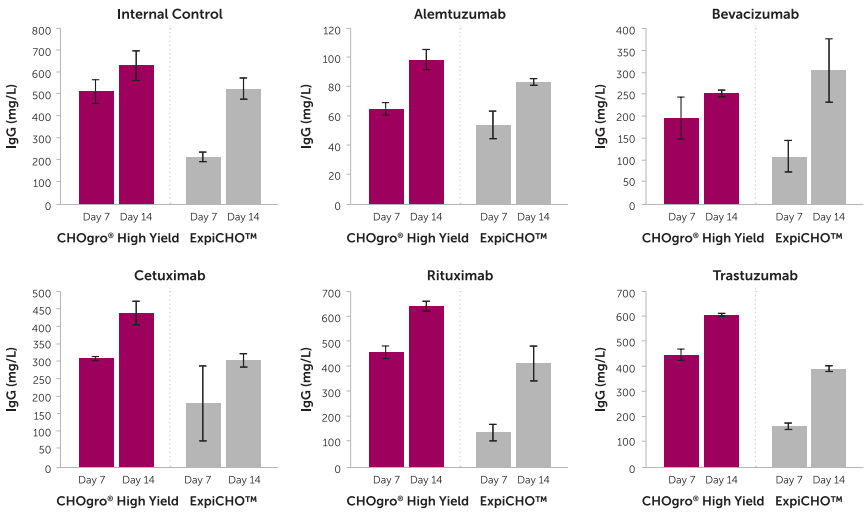
MIR 6240	100 mL

Human IgG1 Expression Control

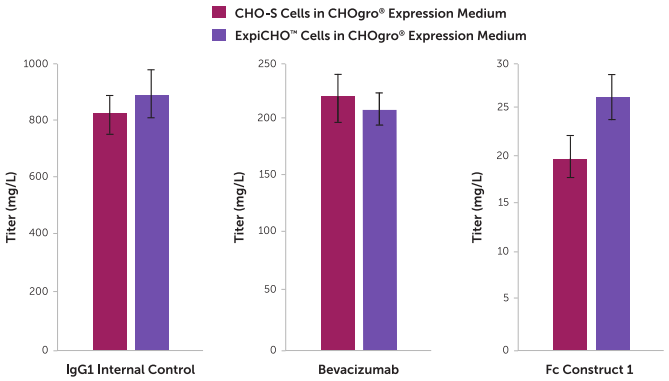
MIR 6250	100 µg

Accessory, sold separately, not included with kit

CHOgro® High Yield Expression System, *con't*

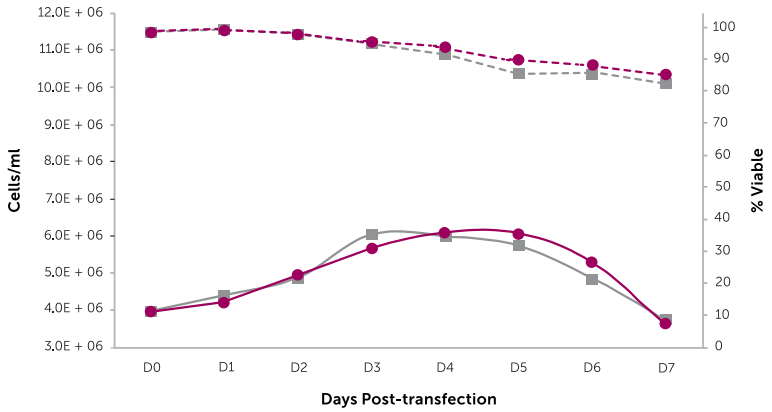


The CHOgro® High Yield Expression System Outperforms the ExpiCHO™ Expression System Using Multiple Antibody Constructs. Six different IgG1 antibody constructs, including five therapeutically relevant constructs synthesized in the same vector backbone were produced by transient transfection using either the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 reagent-to-DNA ratio (vol:wt) and 1 µg plasmid DNA per milliliter of culture in FreeStyle™ CHO-S cells (Thermo Fisher Scientific) cultured in CHOgro® Expression Medium (Mirus Bio) at a cell density of 4 x 10^6 cells/mL, or the Expifectamine CHO Transfection Kit (Thermo Fisher Scientific) at a 3.2:1 reagent-to-DNA (vol:wt) and 1 µg plasmid DNA per milliliter of culture in ExpiCHO™-S cells (Thermo Fisher Scientific) cultured in ExpiCHO™ Expression Medium (Thermo Fisher Scientific) at a cell density of 6 x 10^6 cells/mL. For complete experimental details, please visit: https://www.mirusbio.com/protein-production

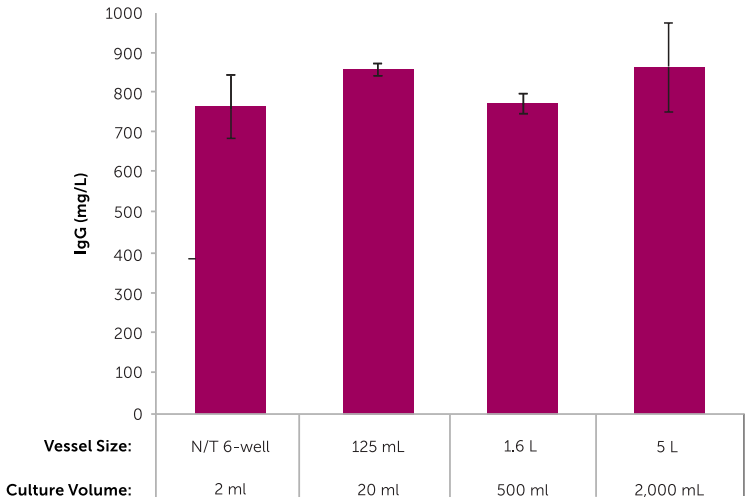


FreeStyle™ CHO-S and ExpiCHO™ Cell Grown in CHOgro® Expression Medium Yield Similar Titers. FreeStyle™ CHO-S cells (Thermo Fisher Scientific) or ExpiCHO-S cells (Thermo Fisher Scientific) were cultured in CHOgro® Expression Medium (Mirus Bio). Both cell lines were transiently transfected with plasmid DNA encoding a human IgG1 internal control antibody, Bevacizumab or Fc-fusion construct using the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 reagent-to-DNA ratio (vol:wt) and 1 µg plasmid DNA per milliliter of culture in a non-treated 6-well plate. All cultures were shifted to 32°C, shaking, immediately post-addition of the transfection complexes and the CHOgro® Titer Enhancer to the culture. Day 14 supernatants were analyzed using a standard sandwich human IgG ELISA. Error bars represent the standard deviation of triplicate technical replicates.

CHOgro® High Yield Expression System, *con't*



CHOgro® Titer Enhancer Does Not Adversely Affect Cell Growth and Viability Post-transfection. Triplicate flasks of FreeStyle™ CHO-S cells adapted to CHOgro® Expression Medium were transiently transfected with the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 reagent-to-DNA ratio (vol:wt) and 1 µg plasmid DNA per milliliter of culture at 4 x 10^6 cells/mL in 125 ml Optimum Growth flasks (25 ml/flask, Thomson Instrument Company). As indicated, CHOgro® Titer Enhancer was added and all of the cultures were shifted to 32°C immediately post-addition of the transfection complexes to the culture. Cell counts (solid line) and viability (propidium iodide staining, dotted line) were measured daily post-transfection using a Guava® easyCyte™ 5HT flow cytometer (EMD Millipore).



CHOgro® High Yield Expression System Enables 1,000-fold Scalability. Human IgG1 was produced by transient transfection with the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) and 1 µg plasmid DNA per milliliter of culture at a 1:1 reagent:DNA ratio. Freestyle CHO cells (Thermo Fisher Scientific) were transfected at a density of 4 x 10^6 cells/mL in CHOgro® Expression Medium (Mirus Bio) at the following volumes/culture vessels: 2 ml/non-tissue culture treated 6-well dish, 20 mL/125 mL Thomson flask, 500 ml/1.6 L Thomson flask, 2,000 mL/5 L Thomson flask. All cultures were shifted to 32°C, shaking, immediately post-addition of the transfection complexes and the CHOgro® Titer Enhancer to the culture. Cultures were maintained at the appropriate shake speed for the remainder of the experiment. Day 14 supernatants were analyzed using a standard sandwich human IgG ELISA. Error bars represent the standard deviation of triplicate technical replicates.

Large-Scale Protein Production

*Trans*IT-PRO® Transfection Kit, *con't*

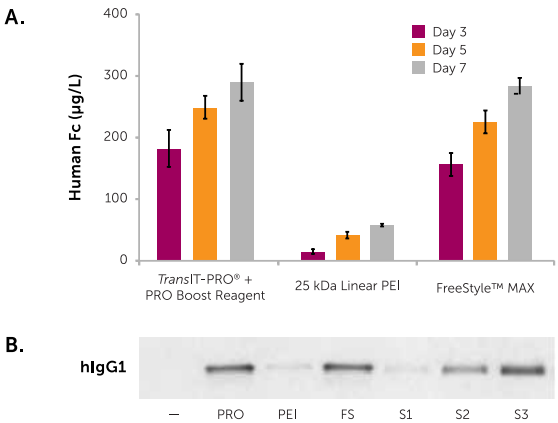
Decrease time to produce usable protein by maximizing protein yields through efficient transient transfection. The *Trans*IT-PRO® Transfection Kit uses animal origin free components designed for high and reproducible protein yield in suspension CHO and 293 derived cells. It is compatible with different media formulations, so there is no need to switch media between transfection and stable expression. *Trans*IT-PRO® outperforms linear PEI in protein yield, while providing a cost-effective alternative to FreeStyle™ MAX.

High Performance—Achieve high protein yield in suspension CHO and 293 cell types

Easy to Use—Compatible with multiple media formulations

Total Cost Savings—Higher protein yield translates to lower material and labor costs

PRODUCT NO.	QUANTITY
MIR 5700	1.0 mL
MIR 5760	10 mL

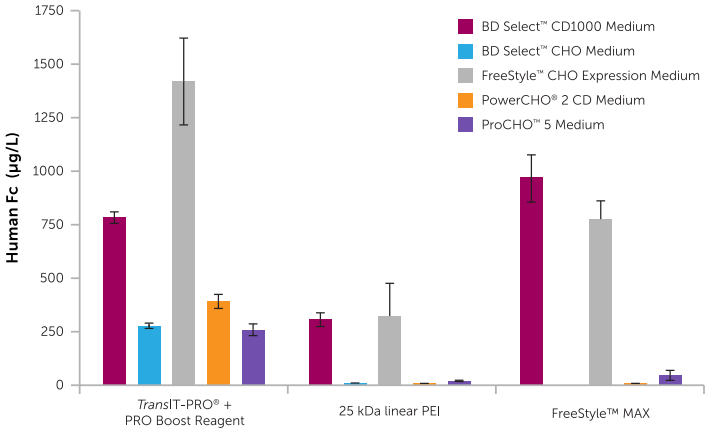


Achieve High Antibody Titers Using the *Trans*IT-PRO® Transfection Kit in Suspension CHO Cells. IgG1 was produced by transient transfection using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 25 kDa linear PEI (6:1) or FreeStyle™ MAX (1:1, Thermo Fisher Scientific) transfection reagents according to the manufacturers' or published protocol (reagent:DNA ratio). Transfections were performed using 1 µg plasmid DNA per milliliter of culture and 0.5 x 10^6 cells/ml at the time of transfection. FreeStyle™ CHO-S cells (Thermo Fisher Scientific) were cultured in 20 ml of FreeStyle™ CHO Expression medium (Thermo Fisher Scientific) in 125 ml shake flasks. (A) Day 3, 5 and 7 supernatants were clarified and analyzed using a human IgG-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate technical replicates for *Trans*IT-PRO® and FreeStyle™ MAX, and duplicate technical replicates for 25 kDa linear PEI. (B) Day 7 supernatants were clarified and analyzed by western blot. An IgG standard was included for quantification estimate (S1= 1.6 mg/L, S2= 3.2 mg/L, S3 = 6.3 mg/L).

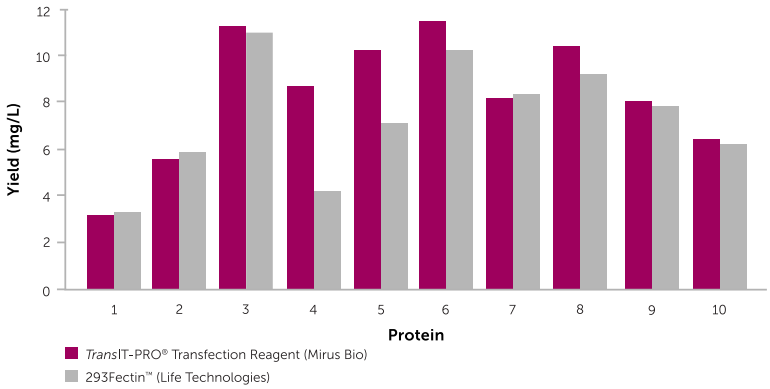
We recently engineered a bispecific immunofusion for the treatment and elimination of leukemia stem cells. For this work we chose *Trans*IT-PRO® for antibody production of CHO-S cells based on the high protein yield we obtained. (Kuo *et al*, *Protein Eng Des Sel.* Oct 2012)

Jen-Sing Liu, Ph.D.,
Molecular Templates Inc.

*Trans*IT-PRO® Transfection, *con't*



***Trans*IT-PRO® Provides High Performance Across Varied Media Formulations.** FreeStyle™ CHO-S cells were adapted to five representative growth media as noted in the graph. Cells were transfected with an IgG encoding plasmid using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 25 kDa linear PEI (6:1, Polysciences) or FreeStyle™ MAX (1:1, Thermo Fisher Scientific) transfection reagents according to published protocol (reagent:DNA ratio). Transfections were performed in 24-well deep well shaker blocks using 1 µg plasmid DNA per milliliter of culture and 0.5 x 10^6 cells/ml at the time of transfection. Human IgG1 was quantitated from day 5 clarified supernatants and analyzed by a human anti-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate wells.



Achieve High Protein Yields Using the *Trans*IT-PRO® Transfection Kit in Suspension 293 Cells. Ten different secreted (non-antibody) proteins were transiently expressed in FreeStyle™ 293-F cells (Thermo Fisher Scientific) using the *Trans*IT-PRO® (1.5:1, Mirus Bio) or 293fectin™ (2:1, Thermo Fisher Scientific) transfection reagents according to manufacturers' protocol. Cells were grown in FreeStyle™ 293 Expression Medium (Thermo Fisher Scientific) and transfected at a density of 4 x 10^6 cells/ml. The scale of the transfection for each protein varied between 1-6 L of culture.

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Madison, WI 53719 USA
Attention: Accounts Receivable

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