THE TRANSFECTION EXPERTS

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THE TRANSFECTION EXPERTS

Our work at Mirus Bio began with a passion for science. That passion continues today with a brand that reflects our commitment to deliver the best results, support and technologies to scientists worldwide. It highlights innovative products that address the workflow and applications needed in today's research. Expanding upon our expertise in transfection, Mirus has crafted a more comprehensive offering for nucleic acid delivery. With chemical transfection reagents, electroporation products and virus production and transduction products, we can now provide the optimal delivery systems for molecular and cell biology applications.

Highlighted within this brochure is our portfolio of delivery methods that include chemical transfection, electroporation and viral transduction to support relevant cell culture workflows with the best possible experimental results.

MOST RECENT BREAKTHROUGHS

2021: *Trans*IT-VirusGEN® GMP Transfection Reagent and Kits– Large-scale virus production for late-phase clinical trials and commercial manufacturing

2019: *Trans*IT-VirusGEN® SELECT Transfection Reagent – Large-scale virus production for preclinical and early phase clinical trials

2019: CHOgro® High Yield Expression System – High titer transient transfection for suspension CHO cells

2018: Ingenio® EZporator® Electroporation System – Easy-to-use and cost-effective electroporation system for high efficiency transfection of mammalian cells

2017: *Trans*IT-VirusGEN® – Ideal for recombinant adeno-associated virus and lentivirus production

2016: *Trans*IT®-Lenti – Ideal for high titer recombinant lentivirus production

2014: *Trans*IT®-Insect – Optimal insect cell transfection for transgene & baculovirus expression

2013: *Trans*IT-X2® Dynamic Delivery System—Superior delivery of plasmid DNA, siRNA and/or Cas9 RNP

OUR PROMISE TO YOU: SERVICE THROUGH EXPERTISE

Order Online 24/7 (Credit Cards or Purchase Orders)
www.mirusbio.com

Customer Service
Hours of Operation: 8:00 AM–4:00 PM Central Time, Monday–Friday
Customer Service Email: sales@mirusbio.com
Customer Service Phone: 888.530.0801 (toll free in the U.S.) or +1.608.441.2852
Fax: +1.608.441.2849

Technical Support
Hours of Operation: 8:30 AM–4:00 PM Central Time, Monday–Friday
Technical Support Email: techsupport@mirusbio.com
Technical Support Hotline: 844.MIRUSBIO (toll free in the U.S.) or +1.608.441.2852

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Mail
Mirus Bio LLC
Attention: Customer Service
5602 Research Park Blvd, Ste 210
Madison, WI 53719 USA

Not sure where to start?

START WITH: Reagent Agent®
To determine the best reagent for your experiment, view citations, customer feedback, and in-house transfection data with the Reagent Agent® Transfection Database: www.mirusbio.com/RA

PROVE IT TO YOURSELF: Request a FREE Sample
Visit: www.mirusbio.com/sample -OR-
Email: techsupport@mirusbio.com

CONTENTS

CHEMICAL TRANSFECTION

ELECTROPORATION

VIRUS PRODUCTION

Mirus

Multiple Nucleic Acid Types Into a Broad Range of Cell Types

*Trans*IT-X2® DYNAMIC DELIVERY SYSTEM

- **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

To inquire about bulk pricing, please call +1.608.441.2852

> 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®. We are also pleased to hear that *Trans*IT-X2® will be offered in similar volume configurations to Lipofectamine® 2000.
>
> *Dr. Edwin Li,* Assistant Professor
> Saint Joseph's University

Description

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

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

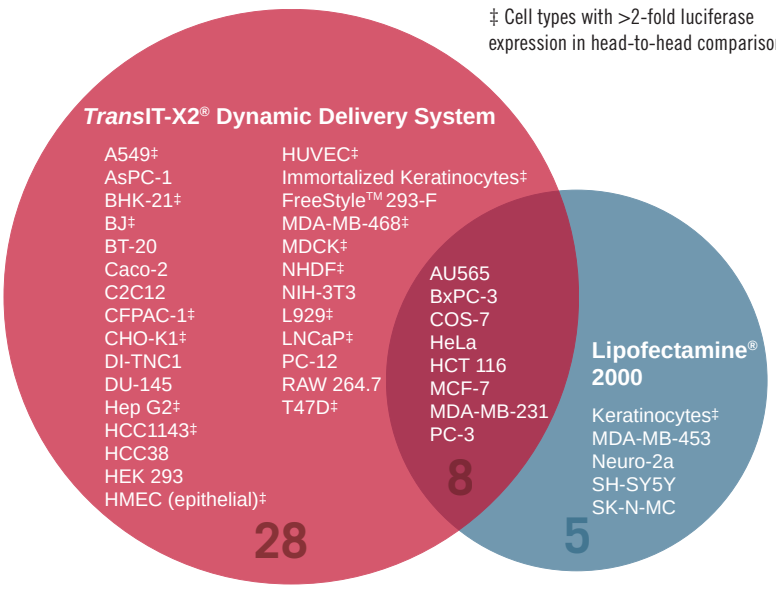


FIGURE 1. The *Trans*IT-X2® Dynamic Delivery System Enables Superior Gene Expression in a Variety of Cell Types. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect plasmid DNA encoding luciferase into 41 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 ‡.

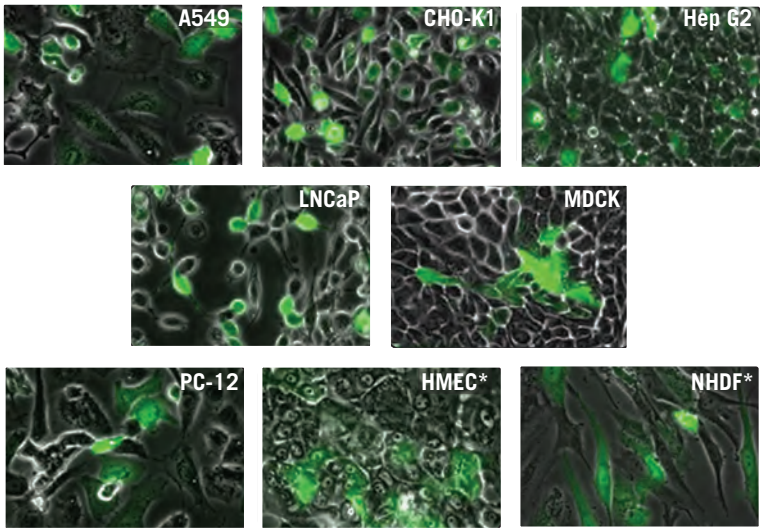


FIGURE 2. Visualization of High GFP Expression Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, LNCaP, MDCK, PC-12, primary human mammary epithelial cells (HMEC) and primary 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.

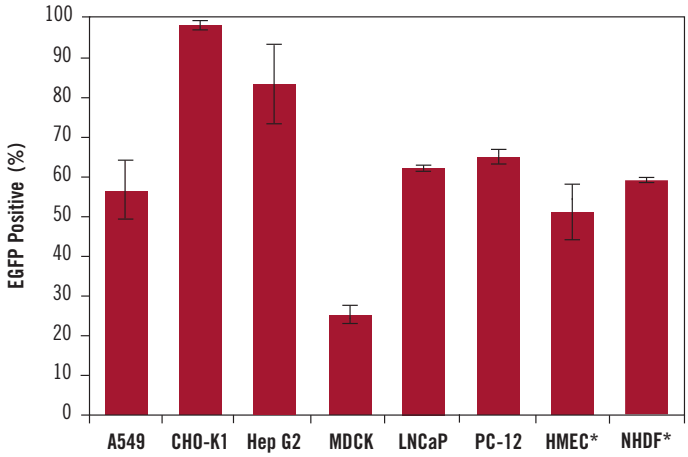


FIGURE 3. High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, MDCK, LNCaP, PC-12, primary human mammary 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.

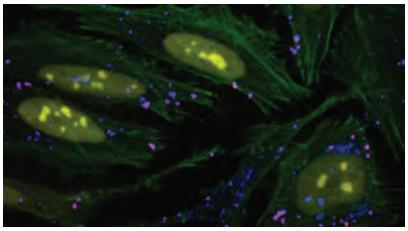


FIGURE 4. Functional Co-delivery of Plasmid DNA and siRNA Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid Cy®5 labeled DNA encoding nuclear YFP and Cy®3 labeled siRNA into HeLa cells. Transfection was performed in a 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,
University of Michigan
Comprehensive Cancer Center

"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."

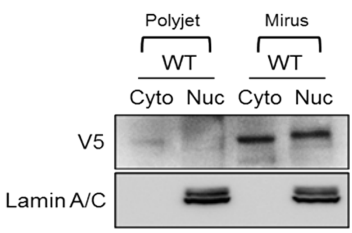
Dr. G. Du, Assistant Professor
Texas Medical Center

"We are pleased with the performance of the *Trans*IT-X2® Dynamic Delivery System when transfecting our renal carcinoma cell line 786-0."

Sathish Padi,
North Dakota State University

"The *Trans*IT-X2® Dynamic Delivery System performed better than our regular transfection reagent (Polyjet) for delivering DNA into the hard to transfect A549 cell line. *Trans*IT-X2® was able to show protein expression compared to Polyjet which failed to produce detectable levels of protein containing V5 tag."

Jason Liggett and Kyung-Won Min, Baek Lab
University of Tennessee



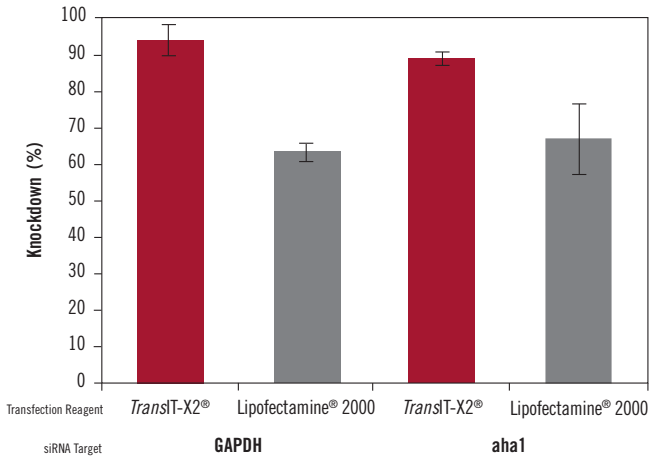


FIGURE 5. The *Trans*IT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect siRNA targeting endogenous proteins, GAPDH and aha1, 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.

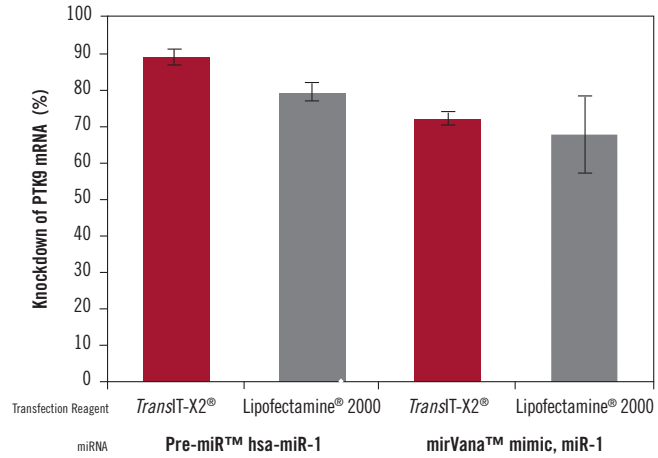
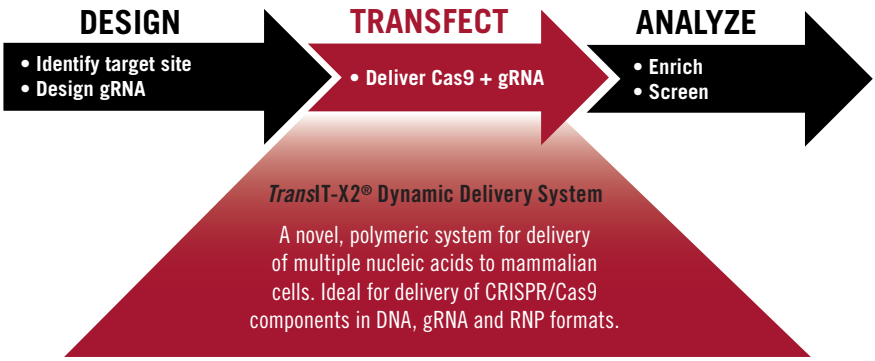


FIGURE 6. Effective miRNA Delivery Using the *Trans*IT-X2® Dynamic Delivery System Yields Decreased Levels of PTK9 mRNA. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect T47D cells with Pre-miR™ hsa-miR-1 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.

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

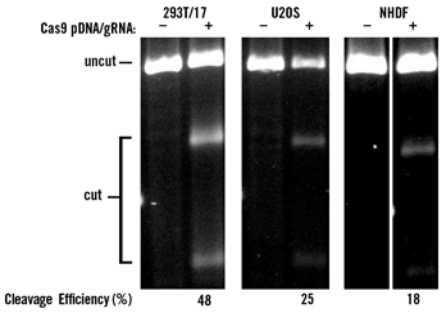


FIGURE 7. Efficient Genome Editing with Cas9 Plasmid DNA and Guide RNA Oligonucleotides. HEK 293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding pDNA (MilliporeSigma) and 50 nM PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using The *Trans*IT-X2® Dynamic Delivery System (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.

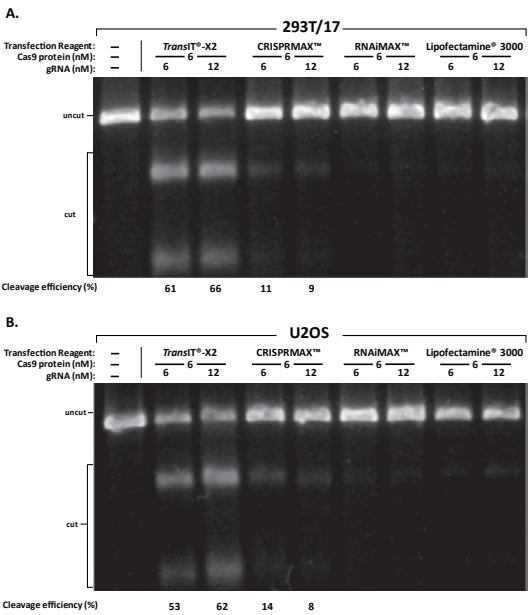
> "I was recently tasked with developing a CRISPR protocol for primary and bone-derived cell lines. *Trans*IT-X2® was simple to use, 2-3 times better for transfection and much gentler on my cells than other products! I feel I have hit the jackpot and have already passed this exciting information on to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

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** - No risk of insertional mutagenesis

FIGURE 8. *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.



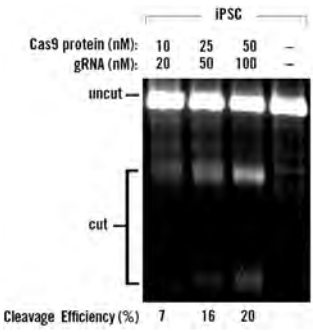


FIGURE 9. Genome Editing in IPS Cells with Cas9 and Guide RNA Ribonucleoprotein Complexes. The *Trans*IT-X2® Dynamic Delivery System was used to deliver Cas9 protein/guide RNA ribonucleoprotein (RNP) complexes in human induced pluripotent stem cells (iPSCs). A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection.

For more on CRISPR/Cas9 delivery, please see Page 17 for gRNA ribonucleoprotein delivery with *Trans*IT®-mRNA and Page 26 for RNP delivery with Ingenio® Electroporation Solution.

*Trans*IT®-LT1 TRANSFECTION REAGENT

- **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

To inquire about bulk pricing, please call +1.608.441.2852

> "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

Description

The *Trans*IT®-LT1 (**L**ow **T**oxicity) 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.

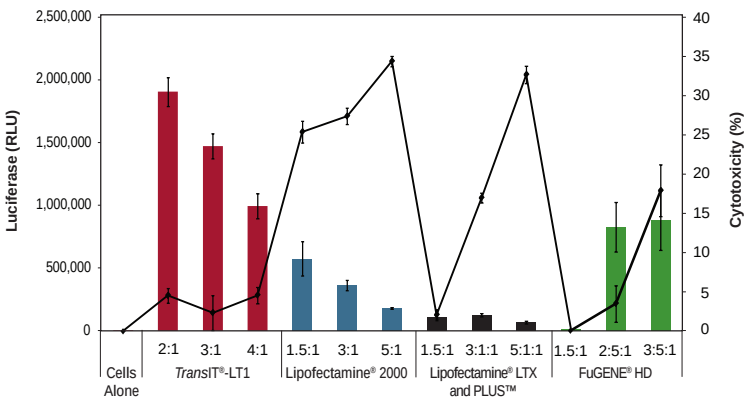


FIGURE 10. *Trans*IT®-LT1 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents. Hep G2 cells were transfected with a luciferase expression plasmid using the designated reagents at the manufacturers' recommended reagent-to-DNA ratio indicated beneath each 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.

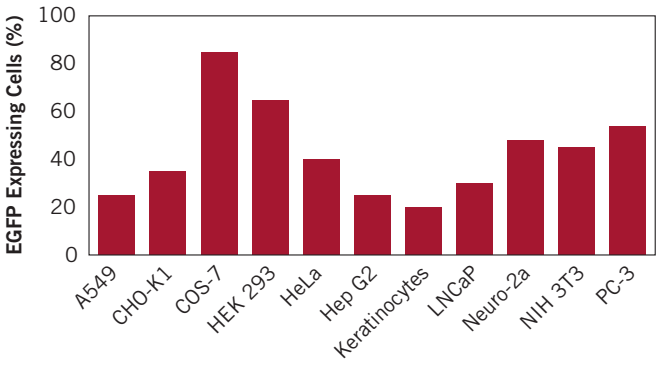


FIGURE 11. The *Trans*IT®-LT1 Reagent Efficiently Delivers DNA to a Wide Variety of Cell Lines. Using the *Trans*IT®-LT1 Transfection Reagent (Mirus Bio), cells were transfected with the pEGFP-C1 expression vector, and the percentage of EGFP expressing cells was determined 24-48 hours post-transfection by flow cytometry.

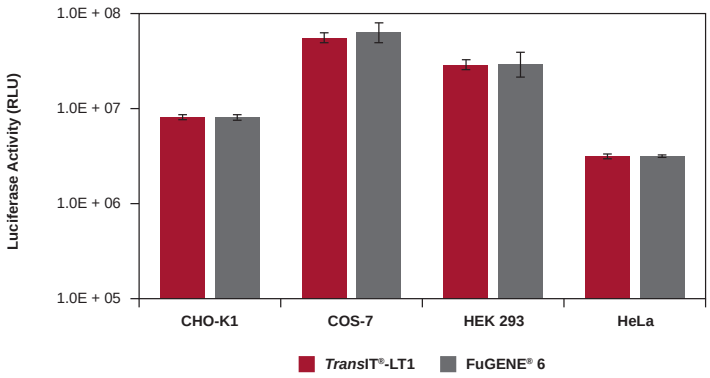


FIGURE 12. Comparable Luciferase Expression With *Trans*IT®-LT1 Reagent and FuGENE® 6 in Multiple Cell Types. The indicated cell lines were transfected in duplicate with 1 µg of a luciferase expression vector per well of a 12-well plate using either 3 µl of *Trans*IT®-LT1 (Mirus Bio) or FuGENE® 6 Reagents (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.

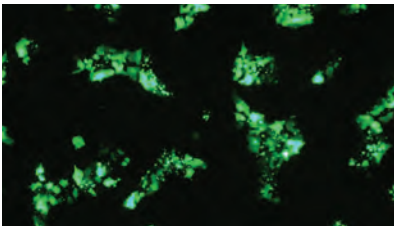


FIGURE 13. Exceptional Transfection Efficiency in Human Induced Pluripotent Stem Cells (iPSCs) via Reverse Transfection with the *Trans*IT®-LT1 Transfection Reagent. The *Trans*IT®-LT1 Transfection Reagent (Mirus Bio) was used to reverse transfect 1.3 x 10^6 iPSCs with a ZsGreen expressing plasmid (Clontech). Cells were visualized 48 hours post-transfection.

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

*Trans*IT®-2020 TRANSFECTION REAGENT

- **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 protein yield 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

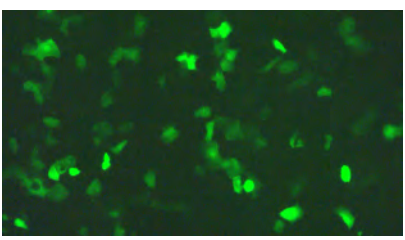
To inquire about bulk pricing, please call +1.608.441.2852

> 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 else would want to use anything else is just beyond me.
>
> *Aviva Joseph,*
> University of Massachusetts Medical School

Description

*Trans*IT-2020® Reagent is a versatile transfection solution for broad 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.

FIGURE 14. High Performance Plasmid Transfection. Primary Human Small 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).



> I recently tested *Trans*IT®-2020 and *Trans*IT®-LT1, and both reagents worked well in terms of their efficiency at transfecting human-derived iPS cells with CRISPR constructs and a fluorescent protein reporter. Through visual inspection, transfection efficiencies with *Trans*IT®-2020 and *Trans*IT®-LT1 were clearly higher than with Lipofectamine® 3000.
>
> *Fedir Kiskin,*
> University of Cambridge

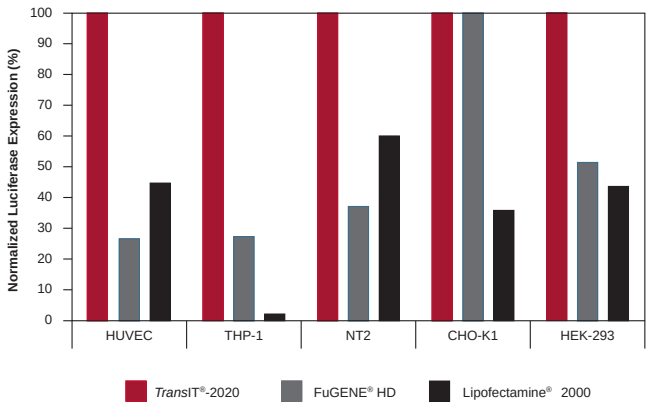


FIGURE 15. Superior Gene Expression in a Broad Spectrum of Cell Types. The indicated cell types were transfected in 96-well plates with a luciferase expression plasmid (0.1 µg/well) according to industry accepted testing protocols. Reagent-to-DNA ratios were optimized for each cell type: *Trans*IT®-2020 (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 *Trans*IT®-2020 and presented as a percentage of luciferase expression. FuGENE is a registered trademark of Fugent LLC.

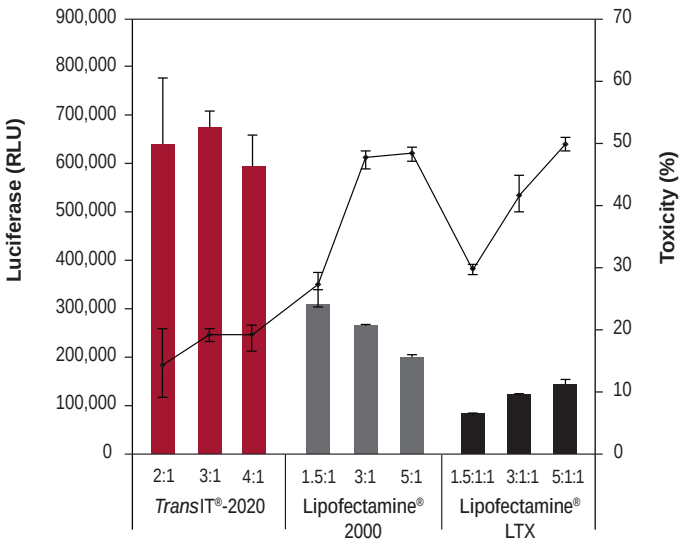


FIGURE 16. *Trans*IT®-2020 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents. Human Umbilical Vein Endothelial Cells (HUVEC) were transfected with a luciferase expression plasmid using the designated reagents at the indicated reagent-to-DNA ratios. 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.

Mirus

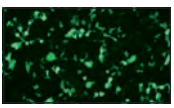
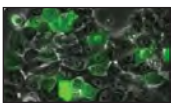
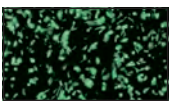
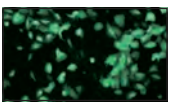
Cell Type Specific

*Trans*IT® CELL TYPE SPECIFIC TRANSFECTION REAGENTS

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

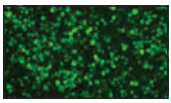
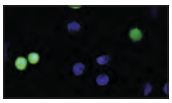
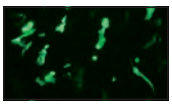
All of these reagents offer:

- **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 Line(s) or Cell Type(s)	Efficiency**	Product No.	Quantity
*Trans*IT®-293 Transfection Reagent				
	HEK 293, HEK 293T, 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-231, MDA-MB-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)				
	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

"Our lab has been satisfied with the routine use of the *Trans*IT-HelaMONSTER® Transfection Kit. 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,
The Ohio State University – Center for Retrovirus Research

Product*	Applicable Cell Line(s) or Cell Type(s)	Efficiency**	Product No.	Quantity
***Trans*IT®-Insect Transfection Reagent**				
	High Five™, S2, Sf9	–	MIR 6104	0.4 ml
			MIR 6100	1.0 ml
			MIR 6105	5 x 1.0 ml
			MIR 6106	10 x 1.0 ml
***Trans*IT®-Jurkat Transfection Reagent**				
	Jurkat, Jurkat-E6, RAW 264.7, THP-1, K562, and other lymphoid cell lines	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**				
	Immortalized 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

To inquire about bulk pricing, please call +1.608.441.2852

* 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

*Trans*IT® REAGENTS FOR OLIGONUCLEOTIDE DELIVERY

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

Oligonucleotides Tested

phosphodiester DNA, phosphothioate DNA (sDNA), phosphothioate RNA (sRNA), 2'OMe RNA, 2'OMe RNA/sDNA Chimerics, and Morpholino/DNA duplexes.

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

To inquire about bulk pricing, please call +1.608.441.2852

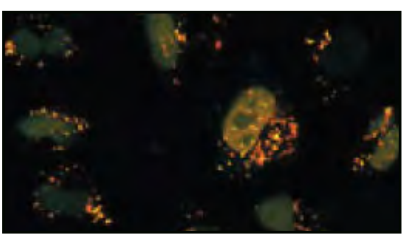


FIGURE 17. *Trans*IT®-Oligo Reagent Achieves High Transfection Efficiency. HeLa cells were transfected with Cy®3 and fluorescein labeled phosphothioate DNA oligos using *Trans*IT®-Oligo Reagent and observed 24 hours post-transfection. The yellow punctate fluorescence is the result of co-localization of the fluorescein and Cy®3 labeled oligonucleotides after transfection. The image was acquired using a confocal microscope.

Mirus

siRNA/miRNA

*Trans*IT-TKO® & *Trans*IT-siQUEST® TRANSFECTION REAGENTS

- **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

> "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-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

*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

To inquire about bulk pricing, please call +1.608.441.2852

Description

*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.

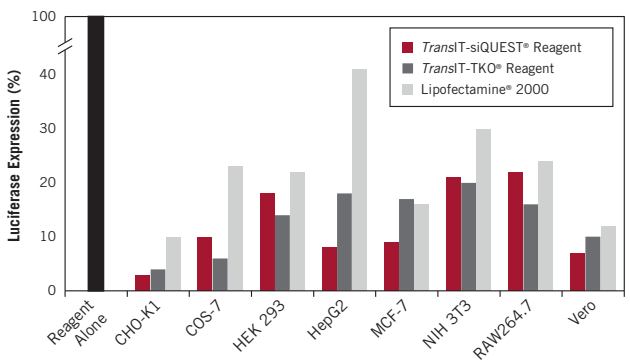


FIGURE 18. Knockdown Efficiencies Using *Trans*IT-siQUEST®, *Trans*IT-TKO® Reagents and Lipofectamine® 2000. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines using the *Trans*IT®-LT1 Reagent (Mirus Bio). Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either *Trans*IT-siQUEST® (red, (Mirus Bio)), *Trans*IT-TKO® (tan, (Mirus Bio)) or Lipofectamine® 2000 (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.

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%
HepG2 (human liver)	MAPK1	80%	--
NIH 3T3-luc (mouse fibroblast)	Luciferase*	85%	89%
NIH 3T3-L1	MAPK1	70%	--
	MAPK3	70%	--
Secondary Human Astrocytes	Lamin A/C	80%	--
Primary Mouse Hepatocytes	ABC A1	70%	--
	Lamin A/C	81%	--
	PPAR-alpha	--	82%

TABLE 1. 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.

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

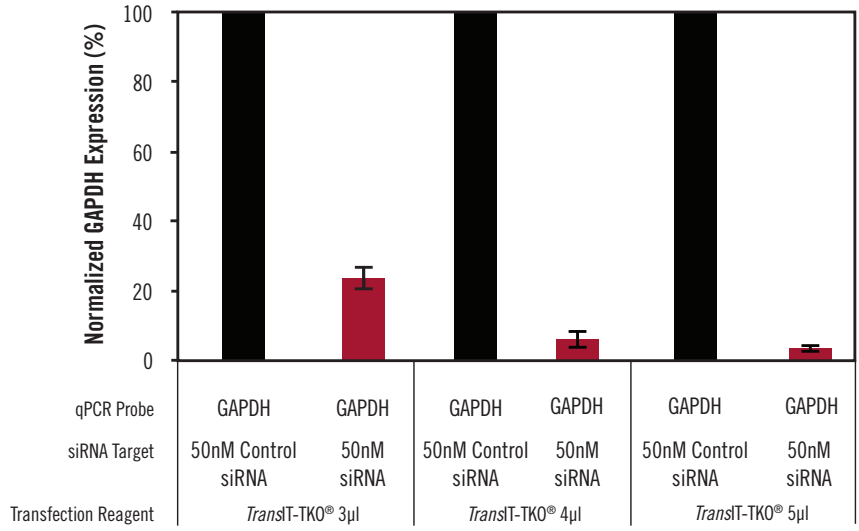


FIGURE 19. High Efficiency Endogenous Knockdown in iCell® Cardiomyocytes. The *Trans*IT-TKO® Transfection Reagent (Mirus Bio) was used to transfect iCell® Cardiomyocytes (Cellular Dynamics International (CDI), a FUJIFILM Company) plated at a density of 136,500 cells per well of a 12-well plate pre-coated 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.

Large RNA (Viral RNA and mRNA)

*Trans*IT®-mRNA TRANSFECTION KIT

- **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 ml
MIR 2256	10 x 1 ml

To inquire about bulk pricing, please call +1.608.441.2852

Description

The *Trans*IT®-mRNA Transfection Kit provides high efficiency transfection of large RNA molecules such as mRNA or viral RNA. The kit is easy to use and minimizes cellular toxicity due to its ability to transfect RNA in the presence of serum.

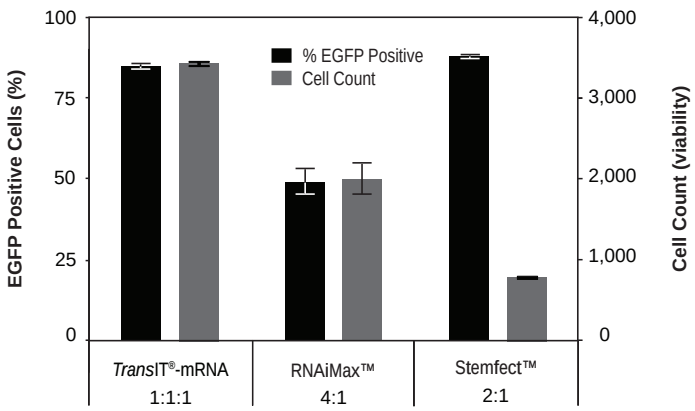


FIGURE 20. High Efficiency and Low Toxicity Transfection Following 14 Consecutive Transfections With the *Trans*IT®-mRNA Transfection Kit. Repeated daily transfections were performed in the same population of BJ fibroblasts using three commercially available transfection reagents – the *Trans*IT®-mRNA 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 (blue bars). Cell viability was determined using cell counts measured during flow cytometry (grey bars). Error bars represent the standard deviation of triplicate wells.

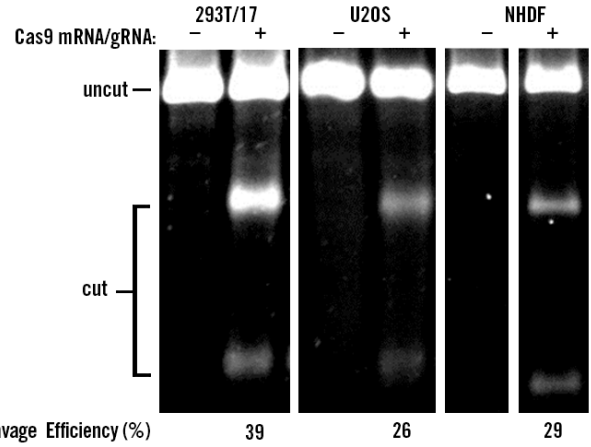


FIGURE 21. Efficient Genome Editing with Cas9 mRNA and Guide RNA Oligonucleotides. HEK 293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding mRNA, 5mC, (Trilink Biotechnologies) and 25 nM of PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using *Trans*IT®-mRNA Transfection Kit (0.5 µl/well of 24-well plate of both mRNA reagent and boost, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.

Please see pages 6-7 for CRISPR/Cas9 delivery with *Trans*IT-X2® and page 25 for Ingenio® Electroporation Solution.

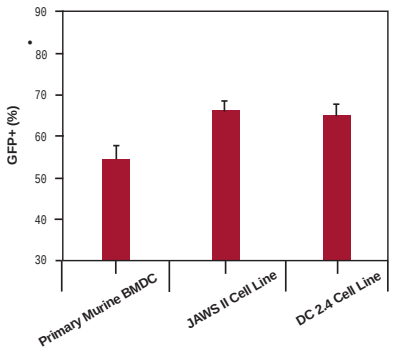
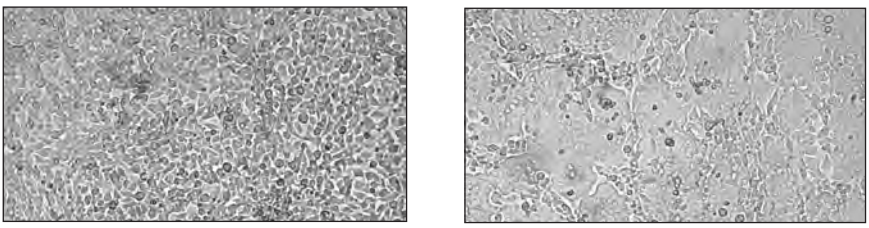


FIGURE 22. Multiple Dendritic Cell Types Express GFP from mRNA Transfected Using the *Trans*IT®-mRNA Transfection Kit. Murine primary bone marrow derived dendritic cells (BMDC) and murine dendritic cells (JAWS II and DC 2.4) were transfected with 1 µg of capped and polyadenlyated mRNA encoding GFP using 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.

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



No RNA Control **MHV RNA Transfected**

FIGURE 23. 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.

Data courtesy of Mark Clemenz, Loyola University of Chicago.

*Trans*IT®-INSECT TRANSFECTION REAGENT

- **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

To inquire about bulk pricing, please call +1.608.441.2852

Description

Insect cell expression is a platform used to produce proteins with simple post-translational modifications. 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.

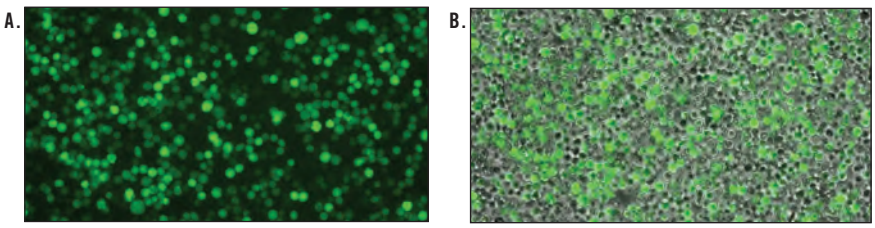


FIGURE 24. Efficient Transfection of Baculovirus Genomic DNA Using The *Trans*IT®-Insect Reagent. Transfections were performed in 6-well plates with 5 x 10^5 Sf9 cells per well using the *Trans*IT®-Insect Transfection Reagent (Mirus Bio) at the reagent-to-total DNA ratio of 3:1 (µl:µg). Cells were co-transfected 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).

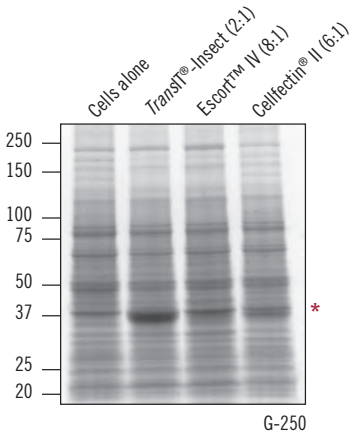


FIGURE 25. Superior Recombinant Protein Expression in High Five™ Cells Using *Trans*IT®-Insect. High Five™ cells (Thermo Fisher Scientific) were transfected in 6-well plates with 2.5 µg of a GFP expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated reagent at the indicated reagent-to-DNA ratios (µl:µg). Total soluble cell lysates were prepared from cells 72 hours post-transfection. Lysates from 100 µl culture 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).

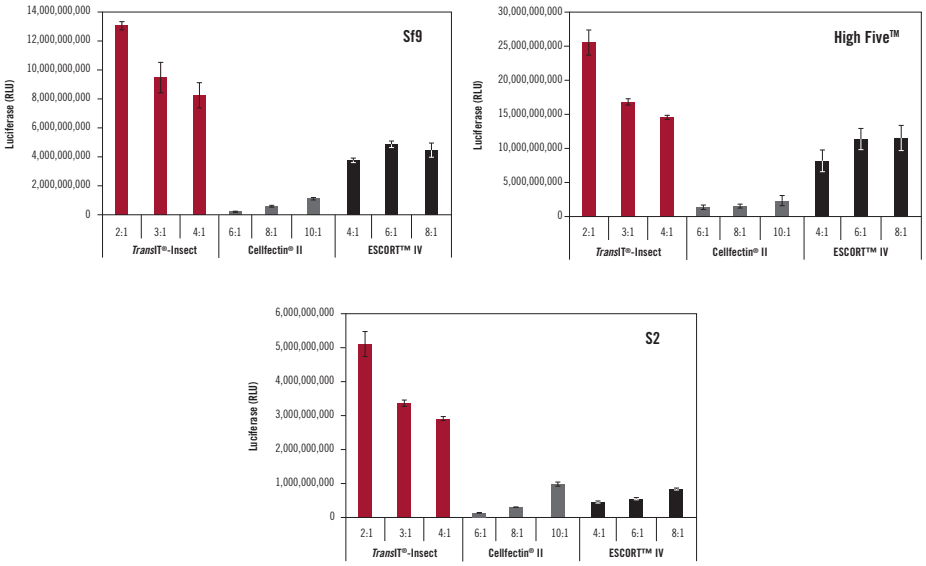


FIGURE 26. *Trans*IT®-Insect Outperforms Competitor Transfection Reagents. Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific) and Drosophila S2 cells were transfected in 96-well plates with 0.1 µg of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated 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.

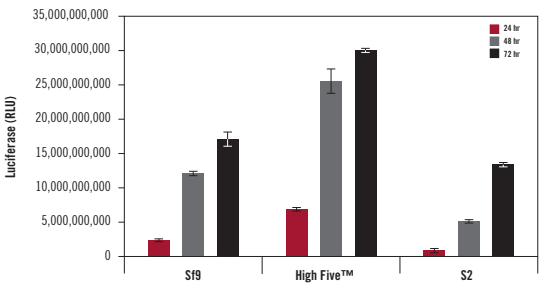


FIGURE 27. *Trans*IT®-Insect Yields Increased Protein Expression Over Time. Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific) and Drosophila S2 were transfected in a 96-well plate with 0.1 µg of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the *Trans*IT®-Insect 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

The Most Advanced System for Transient Transfection and Protein Production in Suspension CHO Cells

CHOgro® HIGH YIELD EXPRESSION SYSTEM

- **Efficient**—Enables high protein titers with simple workflow
- **Convenient**—Quick adaptation to CHO cell lineages
- **Optimized**—High density growth with minimal cell clumping post transfection
- **Worry-free**—No commercial license required; animal origin free

Description

The NEW CHOgro® High Yield Expression System is the most advanced and cost-effective transient transfection system for high-yield protein production in suspension CHO cells. Our 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

Complete CHOgro® High Yield Expression System

Complete CHOgro® Expression System
(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

PRODUCT	PRODUCT NO.	QUANTITY
CHOgro® Transfection and Titer Enhancer Kit *(Kit includes: Trans*IT-PRO® Transfection Reagent, *1 ml, CHOgro® Enhancer, 20 ml)*	MIR 6225	1 Kit
CHOgro® Expression Medium	MIR 6200	1 Liter
Liquid Polybag CHOgro® Expression Medium	MIR 6202	10 Liters
Dry Powder CHOgro® Expression Medium	MIR 6201	Prepares 10 Liters
*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

Accessory, Sold Separately
Not Included with Kit

PRODUCT	PRODUCT NO.	QUANTITY
Human IgG1 Expression Control	MIR 6250	100 µg

To inquire about bulk pricing, please call +1.608.441.2852

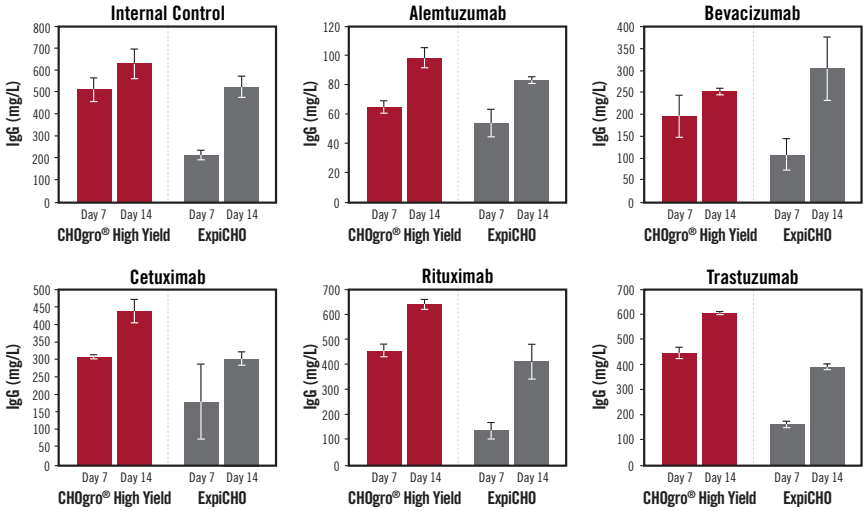


FIGURE 28. The CHOgro® High Yield Expression System Outperforms the ExpiCHO Expression System Using Multiple Antibody Constructs. Six different IgG1 antibody constructs, including five therapeutically relevant constructs synthesized in the same vector backbone were produced by transient transfection 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: www.mirusbio.com/products/transfection/chogro-high-yield-expression-system.

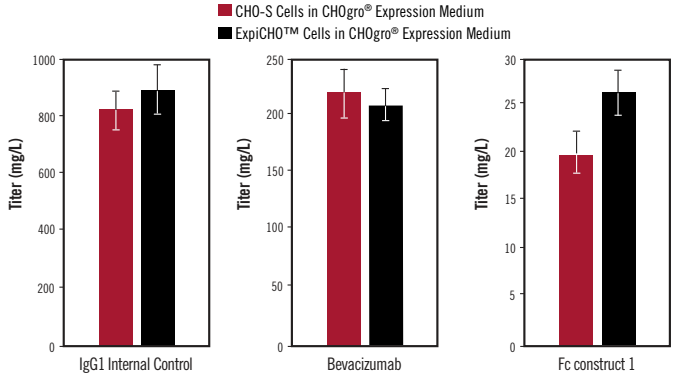


FIGURE 29. FreeStyle™ CHO-S and ExpiCHO Cell Grown in CHOgro® Expression Medium Yield Similar Titers. FreeStyle™ CHO-S cells (Thermo Fisher Scientific) or ExpiCHO-S cells (Thermo Fisher Scientific) were cultured in CHOgro® Expression Medium (Mirus Bio). Both cell lines were transiently transfected with 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.

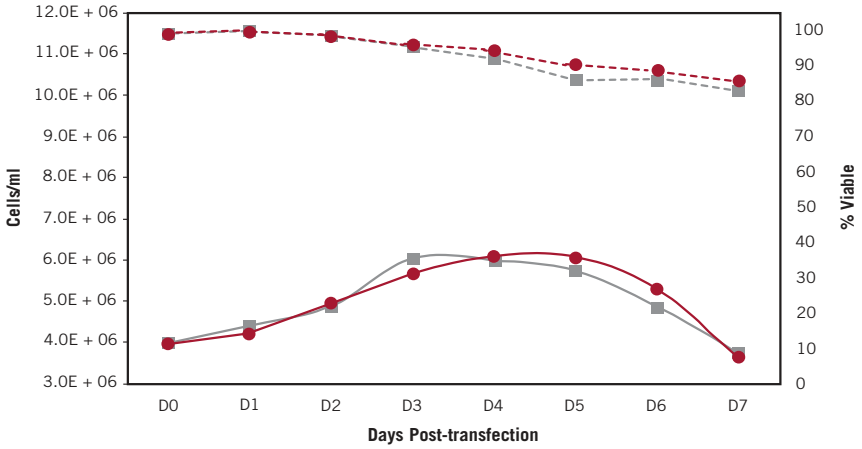


FIGURE 30. CHOgro® Titer Enhancer Does Not Adversely Affect Cell Growth and Viability Post-transfection. Triplicate flasks of FreeStyle™ CHO-S cells adapted to CHOgro® Expression Medium were transiently transfected with the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 reagent-to-DNA ratio (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).

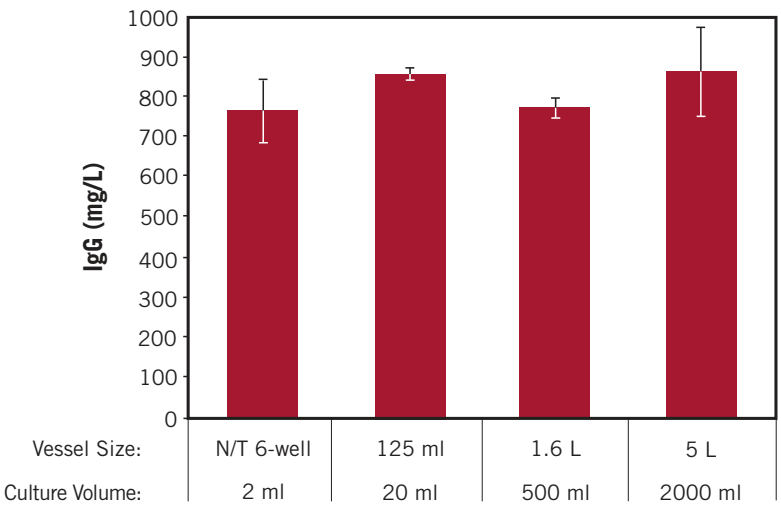


FIGURE 31. CHOgro® High Yield Expression System Enables 1,000-fold Scalability. Human IgG1 was produced by transient transfection with the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) and 1 µg plasmid DNA per milliliter of culture at a 1:1 reagent:DNA ratio. Freestyle CHO cells (Thermo Fisher Scientific) 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, 2000 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

- **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 ml
MIR 5760	10 ml

To inquire about bulk pricing, please call +1.608.441.2852

> "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.

Description

Decrease time to produce usable protein by maximizing target protein yields through 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. Since it is compatible with varied media formulations, the same media can be used for both transient and stable expression. *Trans*IT-PRO® outperforms linear PEI in protein yield, while providing a cost-effective alternative to FreeStyle™ MAX.

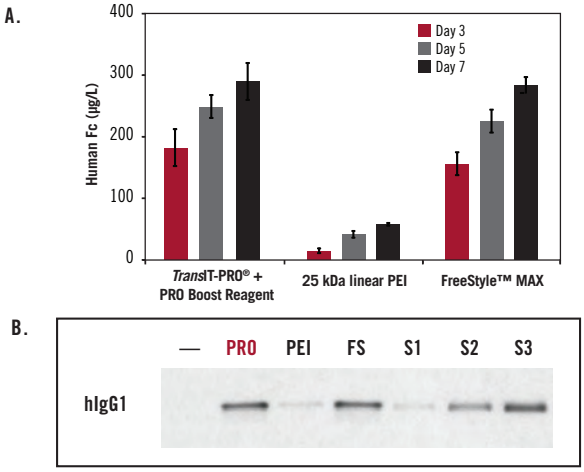


FIGURE 32. Achieve High Antibody Titers Using The *Trans*IT-PRO® Transfection Kit in Suspension CHO Cells. IgG1 was produced by transient transfection using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 25 kDa linear PEI (6:1) or FreeStyle™ MAX (1:1, Thermo Fisher Scientific) transfection 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 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).

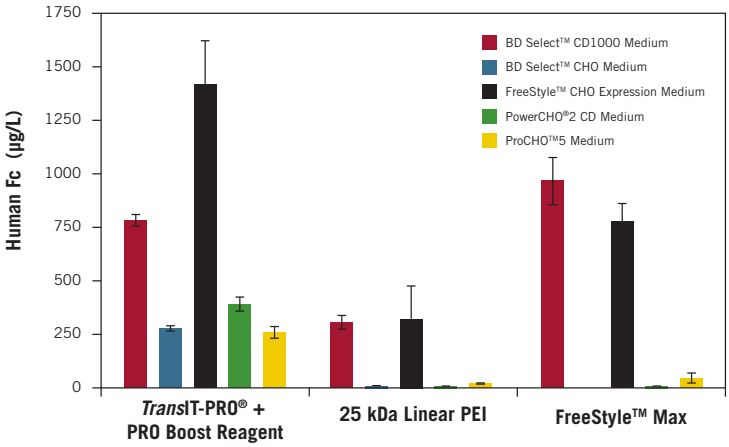


FIGURE 33. *Trans*IT-PRO® Provides High Performance Across Varied Media Formulations. FreeStyle™ CHO-S cells were adapted to five representative growth media as noted in the graph. Cells were transfected with an IgG encoding plasmid using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 25 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.

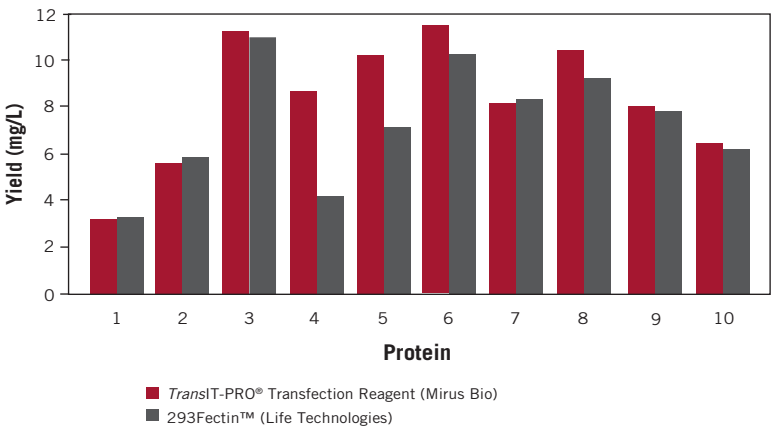


FIGURE 34. Achieve High Protein Yields Using the *Trans*IT-PRO® Transfection Kit in Suspension 293 Cells. Ten different secreted (non-antibody) proteins were transiently expressed in FreeStyle™ 293-F cells (Thermo Fisher Scientific) using the *Trans*IT-PRO® (1.5:1, Mirus Bio) or 293fectin™ (2:1, Thermo 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.

Data courtesy of a *Trans*IT-PRO® pharmaceutical customer.

Plasmid DNA, RNA, siRNA and miRNA

INGENIO® EZPORATOR® AND ELECTROPORATION KITS & SOLUTIONS

- **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

Description

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 and facilitate a wide range of applications requiring nucleic acid delivery to cells. All Ingenio® products can be purchased separately.

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)

PRODUCT NO.	QUANTITY
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)

PRODUCT NO.	QUANTITY
MIR 50113	25 RXN
MIR 50116	50 RXN
MIR 50119	100 RXN

Ingenio® Electroporation Solution

PRODUCT NO.	QUANTITY
MIR 50111	25 RXN
MIR 50114	50 RXN
MIR 50117	100 RXN

Ingenio® Electroporation Accessories

Cuvettes

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

Cell Droppers

PRODUCT NO.	QUANTITY
MIR 50125	50 PK

To inquire about bulk pricing, please call +1.608.441.2852

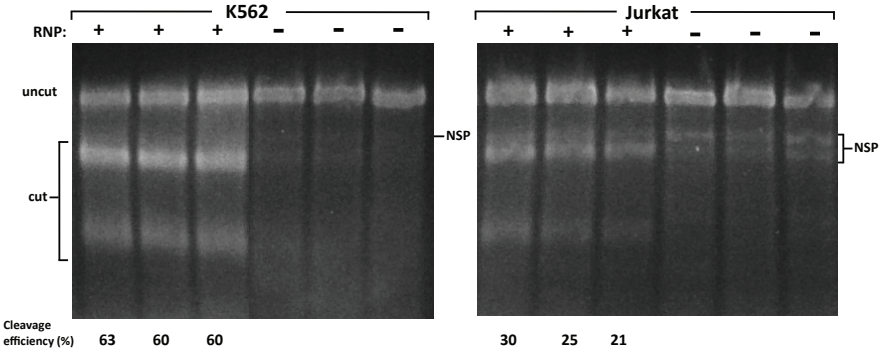


FIGURE 35. Efficient RNP Delivery with Electroporation Ingenio® Solution. K562 and Jurkat cells were electroporated with a Cas9 protein/gRNA, ribonucleoprotein (RNP) complex, comprised of 750 nM Cas9 protein (EnGen® Cas9 NLS, NEB) and 1500 nM pre-complexed two-part gRNA (IDT) targeting PPIB using the 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 150V, 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.

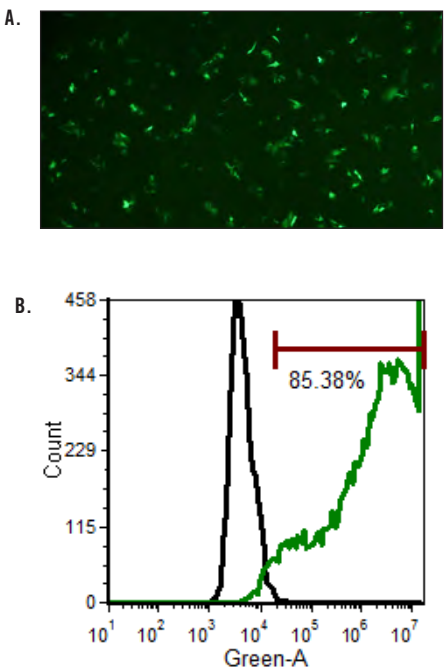


FIGURE 36. High Efficiency Plasmid DNA Electroporation of Human Induced Pluripotent Stem (iPS) Cells using Ingenio®. The Ingenio® Electroporation Kit (Mirus Bio) was used to transfect 2 x 10^6 iPS cells on the Amaxa® Nucleofector® II/2b Device (Lonza Group Ltd). Cells were electroporated with 8 µg ZsGreen 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.

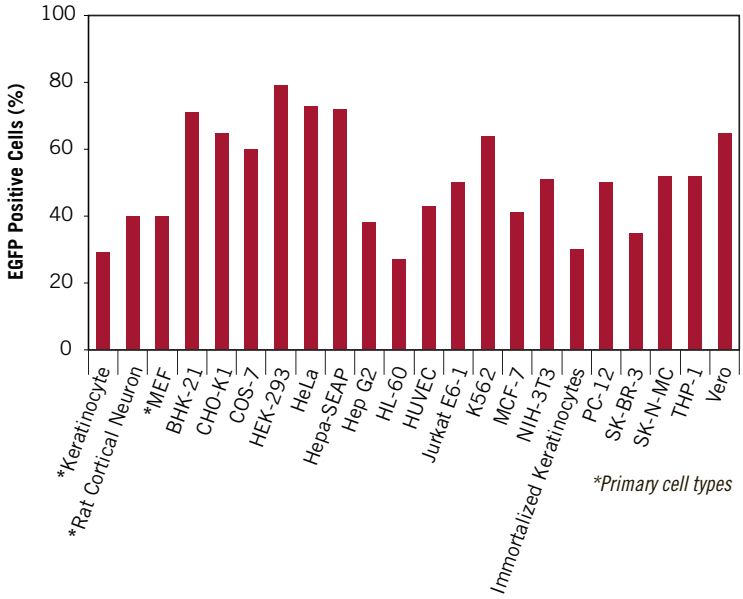


FIGURE 37. The Ingenio® Electroporation Kits are Ideal for Electroporation in Many Cell Types Using the Amaxa® Nucleofector® II/2b Device. Cells were assayed at 24 hours post-electroporation by flow cytometry and reported as percentage of live cell population. Visit www.mirusbio.com/applications/electroporation for ideal pulse conditions.

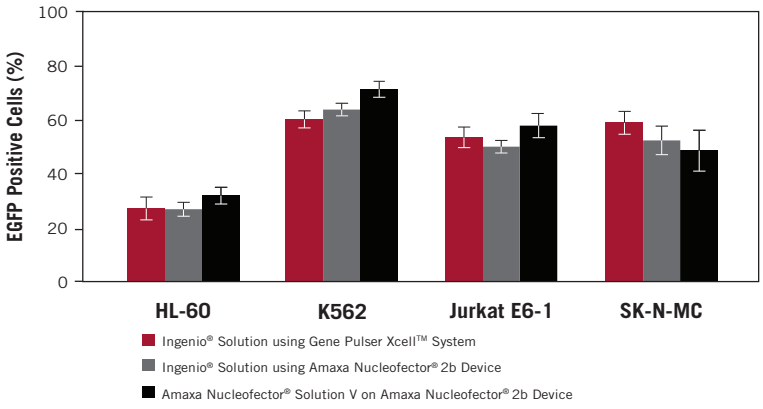


FIGURE 38. The Ingenio® Solution Provides Comparable Efficiency on the Amaxa® Nucleofector® II/2b Device. Cells were electroporated in parallel with an EGFP reporter vector. Two electroporators were used with different electroporation kits: the Ingenio® Electroporation Kit (Mirus Bio) was used in the Gene Pulser Xcell™ Eukaryotic System (Bio-Rad Laboratories, Inc,) and the Amaxa® Nucleofector® II/2b Device (Lonza Group Ltd); the Amaxa® Nucleofector® Kit V (Lonza Group Ltd) was used in the Amaxa® Nucleofector® II/2b Device (Lonza Group Ltd), all according to manufacturers' recommendations. EGFP expressing cells were identified 24 hours post-electroporation by flow cytometry and presented as a percentage of the live cell population. Experiments were performed in triplicate on three separate days and the data averaged.

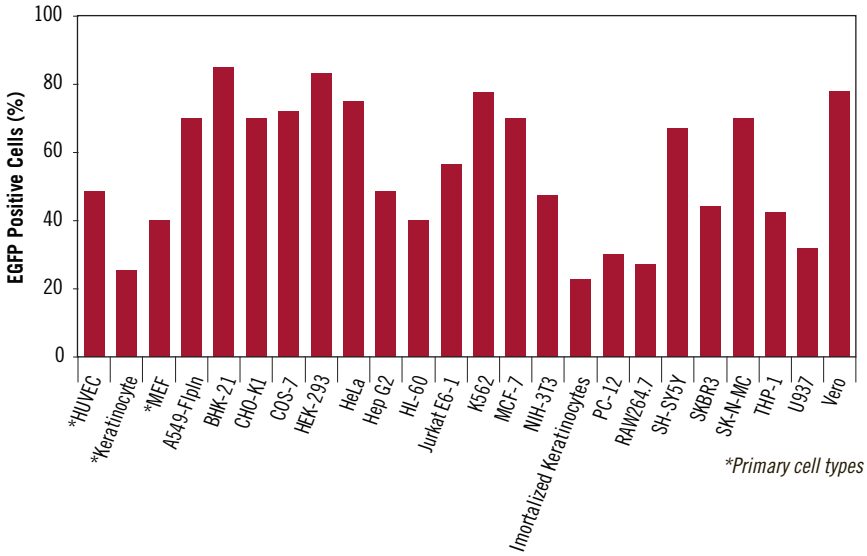


FIGURE 39. The Ingenio® Electroporation Kits are Ideal for Electroporation in Many Cell Types Using the Bio-Rad® GenePulser Xcell™ System. EGFP expressing cells were identified 24 hours post-electroporation by flow cytometry and presented as a percentage of the live cell population. Visit www.mirusbio.com/applications/electroporation for ideal pulse conditions.

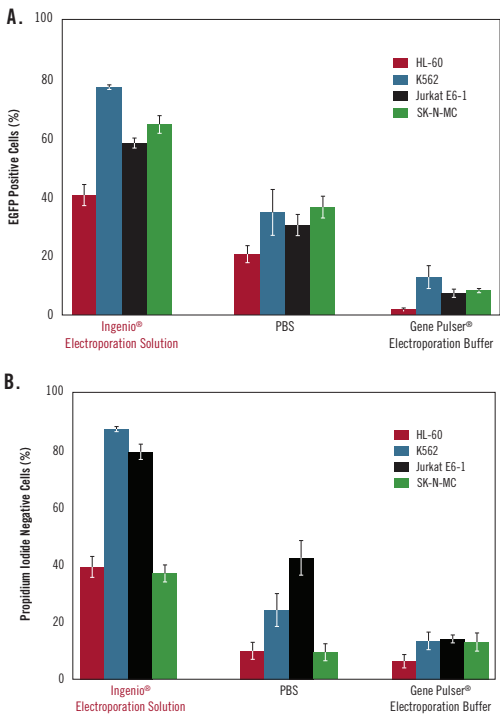


FIGURE 40. Ingenio® Kits Outperforms Other Electroporation Solutions in Efficiency and Viability. Cells were electroporated in parallel with an EGFP reporter vector using either Ingenio® Electroporation Solution (Mirus Bio), PBS or GenePulser® Electroporation Buffer (Bio-Rad Laboratories, Inc.) on the GenePulser Xcell™ Eukaryotic System (Bio-Rad Laboratories, Inc.). (A) EGFP expressing cells were identified 24 hours post-electroporation by flow cytometry and presented as a percentage of the live cell population. (B) Cells were assayed for viablility by propidium iodide staining and flow cytometry analysis. Error bars represent the standard deviation of triplicate wells.

Ideal for Large-scale Virus Production for Preclinical and Early Phase Clinical Trials

*Trans*IT-VirusGEN® SELECT TRANSFECTION REAGENT

- **Performance**—Efficient DNA delivery for large-scale production of high-titer viral vectors
- **Quality**—Tested for performance, identity, sterility, endotoxin and mycoplasma
- **Reliability**—Exceptional lot-to-lot consistency
- **Flexibility**—Compatible with different virus production platforms and repeat filtration

PRODUCT NO.	QUANTITY
MIR 6730	30 ml
MIR 6735	150 ml

To inquire about bulk pricing, please call +1.608.441.2852

IDEAL FOR USE IN BACULOVIRUS PRODUCTION

Description

The *Trans*IT-VirusGEN® SELECT Transfection Reagent is designed for large-scale virus production in preclinical and early-phase clinical trials and is identical in formulation to the fully synthetic and animal origin free, research-grade *Trans*IT-VirusGEN® Transfection Reagent. *Trans*IT-VirusGEN® SELECT is tested for performance, identity, sterility, endotoxin and mycoplasma to streamline ancillary material qualification in viral vector manufacturing.

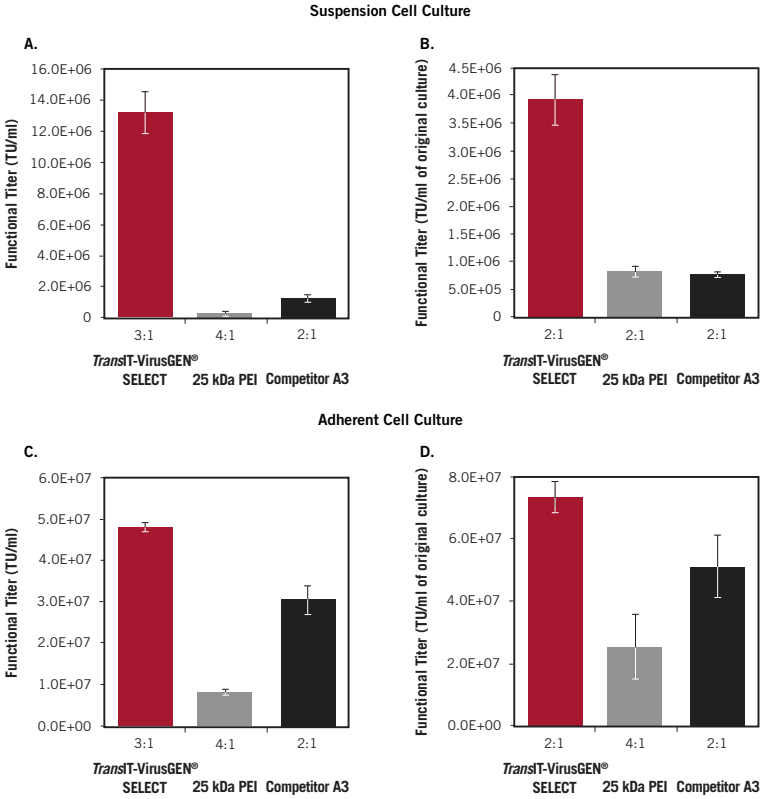


FIGURE 41. *Trans*IT-VirusGEN® SELECT Outperforms Competitor Reagents. For complete experimental details, please visit: www.mirusbio.com/products/transfection/virus-production-transit-virusgen-select

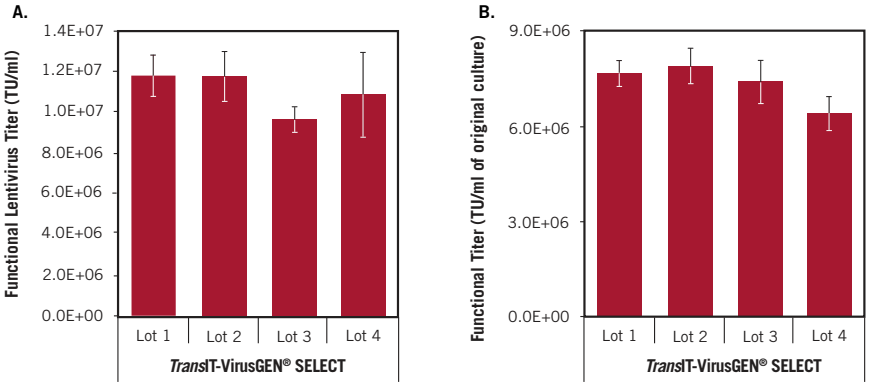


FIGURE 42. Reliable Lot-to-Lot Consistency. **(A) Lentivirus** was produced using suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium and transfected with 3rd generation vectors pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma) and ViraSafe™ Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 ug/ml total plasmid, using the *Trans*IT-VirusGEN® SELECT Transfection Reagent (3:1, vol:wt). Virus containing supernatant was used to transduce 293T/17 cells and GFP expression was measured at 72 hours post-transduction using a Guava® easyCyte™ 5HT Flow Cytometer.

(B) AAV2 was produced using suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium and transfected using pAAV-hrGFP, pAAV-RC and pAAV-Helper plasmids (1:1:1 DNA ratio, 1.5 µg/ml, Agilent Technologies) using *Trans*IT-VirusGEN® SELECT Transfection Reagent (2:1, vol:wt). Harvested virus was used to transduce HT1080 cells and GFP expression was measured 48 hours post-transduction using a Guava® easyCyte™ 5HT Flow Cytometer. For both lentivirus and AAV, functional titers were measured from virus dilutions with less than 20% GFP positive cells.*

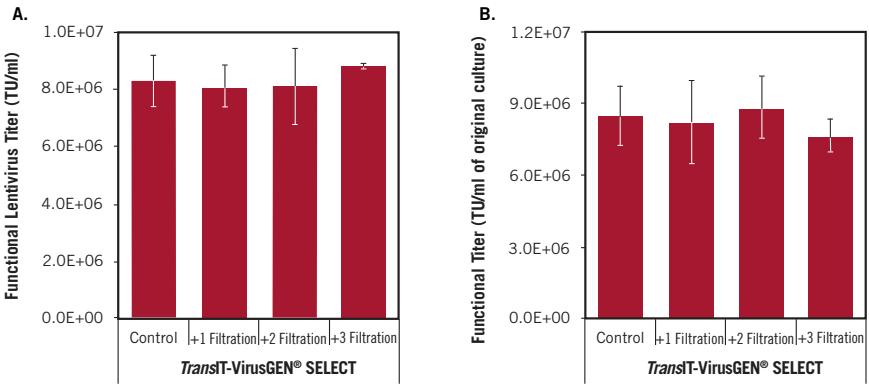


FIGURE 43. Compatible with Multiple Filtrations. **(A) Lentivirus** was produced using suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium and transfected with 3rd generation vectors pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma) and ViraSafe™ Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 ug/ml total plasmid, using the *Trans*IT-VirusGEN® SELECT Transfection Reagent (3:1, vol:wt) that was filtered through a 0.22 µm polyethersulfone (PES) filter unit (Millipore Sigma) for the indicated number of times. Virus-containing supernatant was used to transduce 293T/17 cells and GFP expression was measured at 72 hours post-transduction using a Guava® easyCyte™ 5HT Flow Cytometer.

(B) AAV2 was produced using suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium and transfected using pAAV-hrGFP, pAAV-RC and pAAV-Helper plasmids (1:1:1 DNA ratio, 1.5 µg/ml, Agilent Technologies) using *Trans*IT-VirusGEN® SELECT Transfection Reagent (2:1, vol:wt). Harvested virus was used to transduce HT1080 cells and GFP expression was measured 48 hours post-transduction using a Guava® easyCyte™ 5HT Flow Cytometer. For both lentivirus and AAV, functional titers were measured from virus dilutions with less than 20% GFP positive cells.*

*The error bars represent the range of triplicate wells. Suspension cells were at a density of 2 x10^6 cells/ml and adherent cells were approximately 85% confluent at the time of transfection.

Compare *Trans*IT-VirusGEN® Reagents

Therapeutic Development Pipeline	Research & Development	Preclinical & Early Phase Clinical Trial	Late Phase Clinical Trial & Commercial Manufacturing
***Trans*IT-VirusGEN® Reagents**	 *Trans*IT-VirusGEN®	 *Trans*IT-VirusGEN® SELECT	 *Trans*IT-VirusGEN® GMP
Composition	**Identical *Trans*IT-VirusGEN® Reagent Formulation** (ready to use, chemically defined, animal origin free in 77.5% ethanol)		
AAV and LV Enhancer Kits Available	Available	Coming Soon	Available
Quality	R&D	Preclinical	GMP
Quality Control	Functional AAV titer assay	• Functional AAV titer assay • Formulation identity • Appearance • Sterility: per USP <71> • Bacterial endotoxin: per USP <85> • Mycoplasma: per USP <63>	• Validated formulation identity assay • Appearance • Sterility: per USP <71> • Bacterial endotoxin: per USP <85> • Mycoplasma: per USP <63>
Configuration	0.3 ml; 0.75 ml; 1.5 ml; 5 and 10 pack, 1.5 ml vials	• 30 ml bottle • 150 ml bottle	150 ml bottle
Manufacturing	Research grade manufacturing with aseptic filtration	Research grade manufacturing with aseptic filtration	GMP validated manufacturing process with sterile filtration
Raw Materials	Research grade		GMP grade critical raw materials
Available Documentation	• Certificate of Analysis • Certificate of Origin	• Certificate of Analysis • Certificate of Origin Includes: TSE/BSE Statement	• Certificate of Analysis • Certificate of Origin Includes: TSE/BSE Statement • DMF Available in 2022 with Quality Agreement
Available Analytical Assays	—		• Validated identity assay • Residual reagent assay

All *Trans*IT-VirusGEN® reagents are 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 and adeno-associated virus AAV.

*Trans*IT-VirusGEN® SELECT Transfection Reagent is identical in formulation to our research-grade *Trans*IT-VirusGEN® and includes release testing and quality documentation to streamline the process of ancillary material qualification, ensuring seamless transitions from discovery through large-scale manufacturing, making it the superior choice for large-scale virus production.

Animal Origin Free and Quality Control Documentation

Below is an example of the Certificate of Origin and BSE/TSE Statement for *Trans*IT-VirusGEN® SELECT Transfection Reagent documenting that it is chemically synthesized and is not manufactured with any animal-derived components.

Also pictured is an example of the Certificate of Analysis for *Trans*IT-VirusGEN® SELECT Transfection Reagent that includes a functional assay and testing for: sterility, endotoxin and mycoplasma.

CERTIFICATE OF ORIGIN

Product Name: *Trans*IT-VirusGEN® SELECT Transfection Reagent
Product Number: MIR 6730
Lot Number:
Animal Derived Components: None
Type of Manufacture: Chemical Synthesis
Country of Manufacture: USA

This information is to be used for the purpose of determining animal origin only and not to be confused with "country of origin" for import/export purposes.

Certified By:
Susan Elder, Quality Control Supe

This product is sold to the Buyer with a limited l
product, may not be re-packaged or re-sold with

Rev. Example

Mirus Bio LLC | 5602 Research Park

BSE TSE Statement

Product Name: *Trans*IT-VirusGEN® SELECT Transfection Reagent
Product Number: MIR 6730
Lot Number:
Issue Date:

This product is manufactured in Madison, WI (USA) entirely from material of non-animal origin. The manufacture, packaging, storage and transportation of these products have minimal risk of contamination w Encephalopathy (TSE).

Mirus Bio LLC does not have plans to change or TSE contamination.

Signed on behalf of Mirus Bio LLC By:

Susan Elder, Quality Supervisor

Mirus Bio LLC | 5602 Research Park Blvd, Ste 21

CERTIFICATE OF ANALYSIS

Product Name: *Trans*IT-VirusGEN® SELECT Transfection Reagent
Product Number: MIR 6730
Lot Number:
Retest Date:
Storage Condition: -10 to -30°C
Issue Date:

Quality Control Testing and Results

Test Description	Specification	Result
Functional Assay[1]	> 500,000 TU/mL	Pass
Sterility Testing[2]	No growth observed	Pass
Endotoxin Testing[3]	≤ 1 EU/mL	Pass
Mycoplasma Testing[4]	None detected	Pass

References

[1] *Trans*IT-VirusGEN® Transfection Reagent is tested for adeno-associated virus (AAV) production in suspension 293 cells using a GFP encoding transfer vector. Functional virus titer is determined by transducing HT1080 cells and measuring GFP expression by flow cytometry.

[2] Performed per USP <71> guidelines.

[3] Performed per USP <85> guidelines.

[4] Performed per USP <63> guidelines.

Certified By:
Susan Elder, Quality Control Supervisor

This product is sold to the Buyer with a limited license for research use and further manufacturing; not for administration into humans. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio LLC. For bulk inquiries, contact Mirus Bio LLC, 545 Science Drive, Madison, WI 53711; Email: sales@mirusbio.com

*Trans*IT-VirusGEN® is a registered trademark of Mirus Bio LLC.

Mirus Bio LLC | 5602 Research Park Blvd, Ste 210 | Madison, Wisconsin | 53719 | 608.441.2852 | FAX 608.441.2849 | www.mirusbio.com

Ideal for High Titer Virus Production

IDEAL FOR USE IN BACULOVIRUS PRODUCTION

*Trans*IT-VirusGEN® TRANSFECTION REAGENT

- **Reliable**—Consistent high titer virus production
- **Scalable**—Efficient across different formats
- **Flexible**—Address different virus and cell culture systems

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

To inquire about bulk pricing, please call +1.608.441.2852

Description

*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 production.

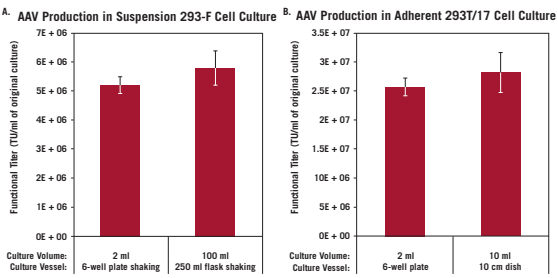


FIGURE 44. *Trans*IT-VirusGEN® Enables Broad Scalability (AAV). Suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium (A) or adherent 293T/17 cells (B) were transfected with pAAV-hrGFP, pAAV-RC and pAAV-Helper (1:1:1 ratio, 1.5 total µg/ml, Agilent Technologies) using the *Trans*IT-VirusGEN® Transfection Reagent (2:1 reagent-to-DNA ratio (vol:wt), Mirus) at the indicated volumes per culture vessel. For experimental details, please visit www.mirusbio.com/virusgen

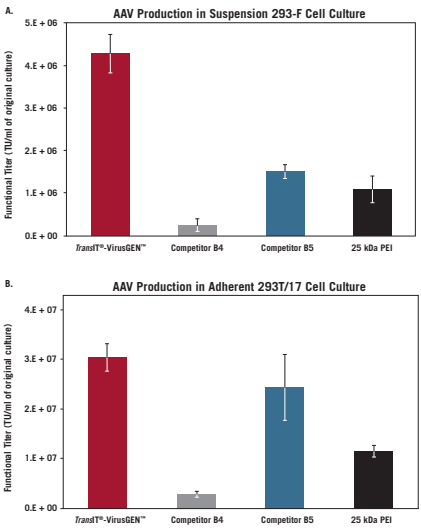


FIGURE 45. *Trans*IT-VirusGEN® Outperforms Competitor Reagents in Suspension and Adherent AAV Cell Cultures. For experimental details, please visit www.mirusbio.com/virusgen

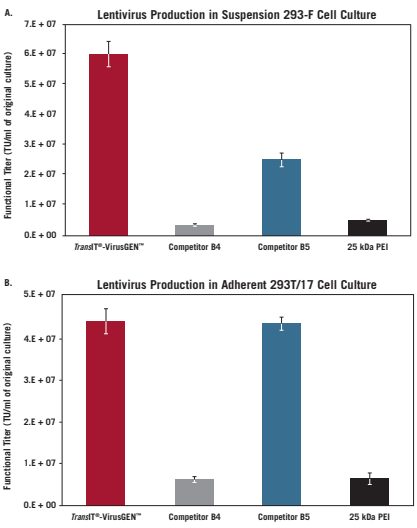


FIGURE 46. *Trans*IT-VirusGEN® Outperforms Competitor Reagents in Suspension and Adherent Lentivirus Cell Cultures. For experimental details, please visit www.mirusbio.com/virusgen

VIRUS PRODUCTION

Ideal for Recombinant Lentivirus Production

*Trans*IT®-LENTI TRANSFECTION REAGENT

- **High Performance**—Provide up to eight-fold higher functional titers
- **Simple Protocol**—No media change required, single harvest
- **Animal Origin Free**—Regulatory friendly

Description

The *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.

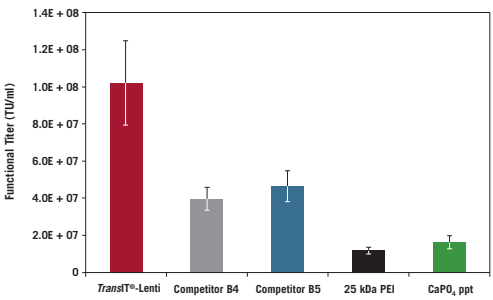


FIGURE 47. High Functional Titers with the *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate with pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma-Aldrich, Inc. LLC) and the Lentivirus Packaging Mix Powered by MISSION® (1:1 ratio, 2 µg/well) with the following 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.

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, TransduceIT™ Transduction Reagent, Lentivirus Packaging Mix Powered by MISSION©*)*

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

Lentivirus Packaging Mix Powered by MISSION®

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

To inquire about bulk pricing, please call +1.608.441.2852

IDEAL FOR USE IN BACULOVIRUS PRODUCTION

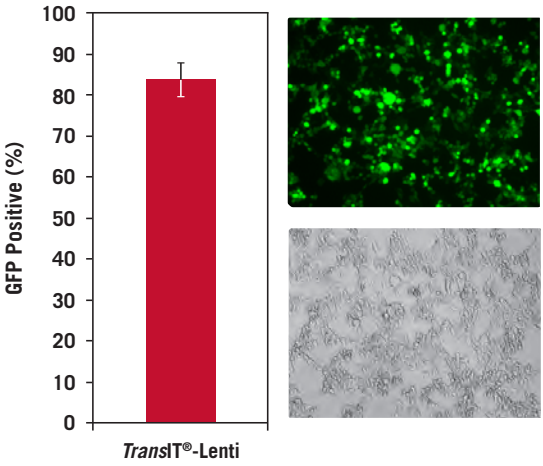


FIGURE 48. High Efficiency Transfection with the *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate format using MISSION® pLKO.1-puro-CMV-TurboGFP™ transfer vector and Lentivirus Packaging Mix Powered by MISSION® (Sigma-Aldrich, Inc. LLC) using the *Trans*IT®-Lenti 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.

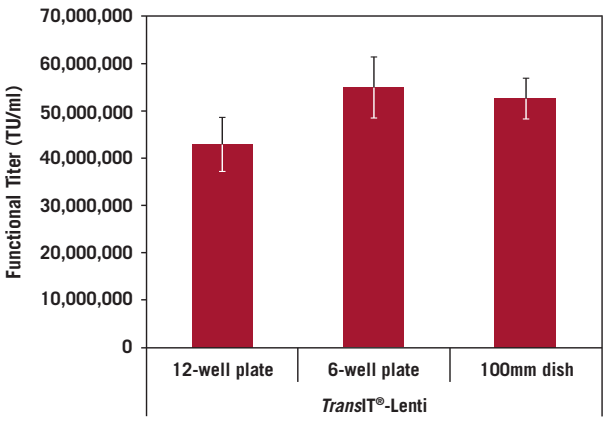


FIGURE 49. Lentivirus Production is Scalable. Adherent 293T/17 cells were transfected in a 12-well, 6-well or 100 mm plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix Powered by MISSION® at a 1:1 ratio; Sigma-Aldrich, Inc. LLC) and the *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

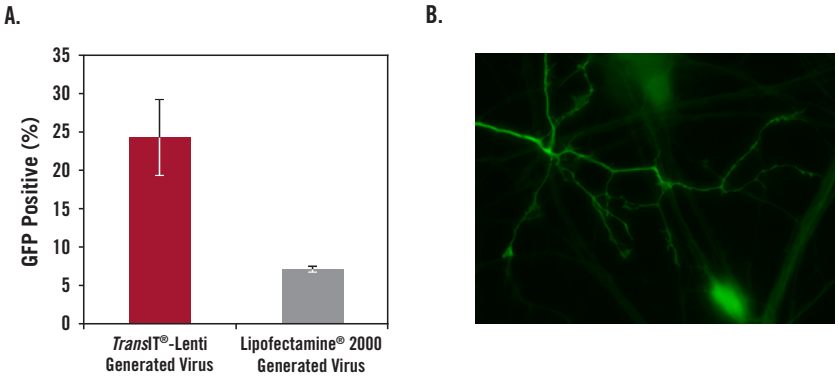


FIGURE 50. High Transduction Efficiency with Unconcentrated Lentivirus Using *Trans*IT®-Lenti. (A) Lentivirus was produced with the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt; Mirus Bio) or Lipofectamine® 2000 (Thermo Fisher Scientific) using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer 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.

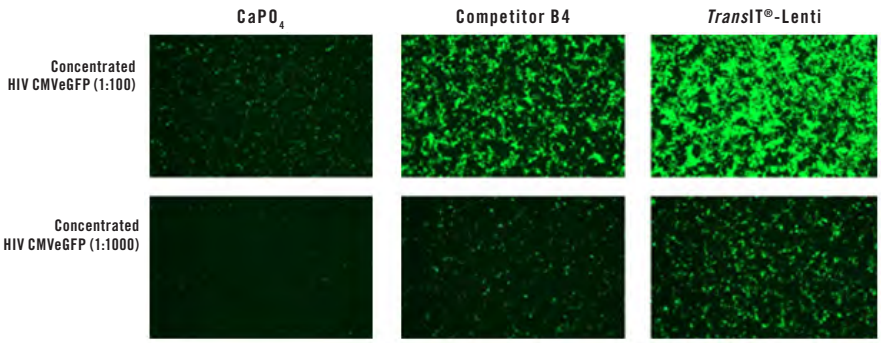


FIGURE 51. Comparing Functionality of $CaPO_4$, Competitor B4 or *Trans*IT®-Lenti Generated Lentivirus. HIV CMVeGFP virus was produced in HEK 293FT cells using either $CaPO_4$, Competitor B4 or *Trans*IT®-Lenti Transfection Reagent (Mirus Bio) per manufacturers' protocol. Lentivirus was collected 48 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 (above) were captured 48 hours post-transduction.

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

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PRODUCT LIST

CHEMICAL TRANSFECTION

Transfection Into Any Cell Type With Any Nucleic Acid

Product	Product No.	Quantity
*Trans*IT-X2® Dynamic Delivery System	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

Plasmid DNA Transfection

Product	Product No.	Quantity
*Trans*IT®-2020 Transfection Reagent	MIR 5404	0.4 ml
	MIR 5400	1 ml
	MIR 5405	5 x 1 ml
	MIR 5406	10 x 1 ml
*Trans*IT®-LT1 Transfection Reagent	MIR 2304	0.4 ml
	MIR 2300	1 ml
	MIR 2305	5 x 1 ml
	MIR 2306	10 x 1 ml

Cell Line Specific

Product	Product No.	Quantity
*Trans*IT®-293 Transfection Reagent	MIR 2704	0.4 ml
	MIR 2700	1 ml
	MIR 2705	5 x 1 ml
	MIR 2706	10 x 1 ml
*Trans*IT®-BrCa Transfection Reagent	MIR 5504	0.4 ml
	MIR 5500	1 ml
	MIR 5505	5 x 1 ml
	MIR 5506	10 x 1 ml
*Trans*IT®-CHO Transfection Kit*	MIR 2174	0.4 ml
	MIR 2170	1 ml
	MIR 2175	5 x 1 ml
	MIR 2176	10 x 1 ml
*Trans*IT-HeLaMONSTER® Transfection Kit*	MIR 2904	0.4 ml
	MIR 2900	1 ml
	MIR 2905	5 x 1 ml
	MIR 2906	10 x 1 ml

Product	Product No.	Quantity
*Trans*IT®-Jurkat Transfection Reagent	MIR 2124	0.4 ml
	MIR 2120	1 ml
	MIR 2125	5 x 1 ml
	MIR 2126	10 x 1 ml
*Trans*IT®-Keratinocyte Transfection Reagent	MIR 2804	0.4 ml
	MIR 2800	1 ml
	MIR 2805	5 x 1 ml
	MIR 2806	10 x 1 ml
*Trans*IT®-Oligo Transfection Reagent	MIR 2164	0.4 ml
	MIR 2160	1 ml
	MIR 2165	5 x 1 ml
	MIR 2166	10 x 1 ml

Insect Cell Transfection & Baculovirus Production

Product	Product No.	Quantity
*Trans*IT®-Insect Transfection Reagent	MIR 6104	0.4 ml
	MIR 6100	1 ml
	MIR 6105	5 x 1 ml
	MIR 6106	10 x 1 ml

siRNA/miRNA

Product	Product No.	Quantity
*Trans*IT-TKO® Transfection Reagent	MIR 2154	0.4 ml
	MIR 2150	1.5 ml
	MIR 2155	5 x 1.5 ml
	MIR 2156	10 x 1.5 ml
*Trans*IT-siQUEST® Transfection Reagent	MIR 2114	0.4 ml
	MIR 2110	1.5 ml
	MIR 2115	5 x 1.5 ml
	MIR 2116	10 x 1.5 ml

Large RNA (Viral and mRNA)

Product	Product No.	Quantity
*Trans*IT®-mRNA Transfection Kit*	MIR 2225	0.4 ml
	MIR 2250	1 ml
	MIR 2255	5 x 1 ml
	MIR 2256	10 x 1 ml

Large-Scale Protein Production in Suspension CHO Cells

Complete System	Product No.	Quantity
CHOgro® High Yield Expression System	MIR 6270	1 System
CHOgro® Expression System	MIR 6260	1 System

CHOgro® Components	Product No.	Quantity
CHOgro® Expression Medium	MIR 6200	1 Liter
CHOgro® Liquid Polybag Format	MIR 6202	10 Liters
CHOgro® Dry Powder Format	MIR 6201	Prepares 10L
CHOgro® Transfection & Titer Enhancer Kit	MIR 6225	1 Kit

CHOgro® Components	Product No.	Quantity
*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

Large-Scale Protein Production in Suspension CHO & HEK293 Cells

Product	Product No.	Quantity
*Trans*IT-PRO® Transfection Kit*	MIR 5700	1 ml
	MIR 5760	10 ml

ELECTROPORATION

Product	Product No.	Size
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 including EZporator®, Bio-Rad® and Harvard BTX® (solution, 0.4 cm cuvettes, cell droppers)	MIR 50113	25 RXN
	MIR 50116	50 RXN
	MIR 50119	100 RXN

Product	Product No.	Size
Ingenio® Electroporation Solution	MIR 50111	25 RXN (6.25 ml)
	MIR 50114	50 RXN (12.5 ml)
	MIR 50117	100 RXN (25 ml)
Ingenio® Electroporation Accessories	MIR 50121	0.2 cm cuvettes (50 PK)
	MIR 50123	0.4 cm cuvettes (50 PK)
	MIR 50125	Cell Droppers (50 PK)
Ingenio® EZporator® Electroporation System	MIR 51000	Complete System

VIRUS PRODUCTION

Research-Grade Reagents and Kits	Product No.	Quantity
*Trans*IT-VirusGEN® Transfection Reagent	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
*Trans*IT-VirusGEN® AAV Transfection Kit	MIR 6750	1 Kit
*Trans*IT-VirusGEN® LV Transfection Kit	MIR 6760	1 Kit

SELECT-Grade Reagents and Kits	Product No.	Quantity
*Trans*IT-VirusGEN® SELECT Transfection Reagent	MIR 6730	30 ml
	MIR 6735	150 ml
*Trans*IT-VirusGEN® SELECT AAV Transfection Kit	MIR 6770	1 Kit
	MIR 6730	30 ml
	MIR 6771	1 L
*Trans*IT-VirusGEN® SELECT AAV Transfection Kit	MIR 6775	1 Kit
	MIR 6735	150 ml
	MIR 6771	1 L
*Trans*IT-VirusGEN® SELECT LV Transfection Kit	MIR 6780	1 Kit
	MIR 6730	30 ml
	MIR 6781	1 L
	MIR 6782	1 L
*Trans*IT-VirusGEN® SELECT LV Transfection Kit	MIR 6785	1 Kit
	MIR 6735	150 ml
	MIR 6781	1 L
	MIR 6782	1 L

GMP-Grade Reagents and Kits	Product No.	Quantity
*Trans*IT-VirusGEN® GMP Transfection Reagent	MIR 6845-GMP	150 ml
*Trans*IT-VirusGEN® GMP AAV Transfection Kit	MIR 6815-GMP	1 Kit
	MIR 6845-GMP	150 ml
	MIR 6816-GMP	1 L
*Trans*IT-VirusGEN® GMP LV Transfection Kit	MIR 6825-GMP	1 Kit
	MIR 6845-GMP	150 ml
	MIR 6826-GMP	1 L
	MIR 6827-GMP	1 L

Accessory Products	Product No.	Quantity
*Trans*IT®-Lenti Transfection Reagent	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	MIR 6620	1 ml
*Trans*IT® Lentivirus System	MIR 6650	5 RXN
	MIR 6655	34 RXN
Lentivirus Packaging Mix Powered by MISSION® Genomics	MIR 6630	5 RXN
	MIR 6640	34 RXN