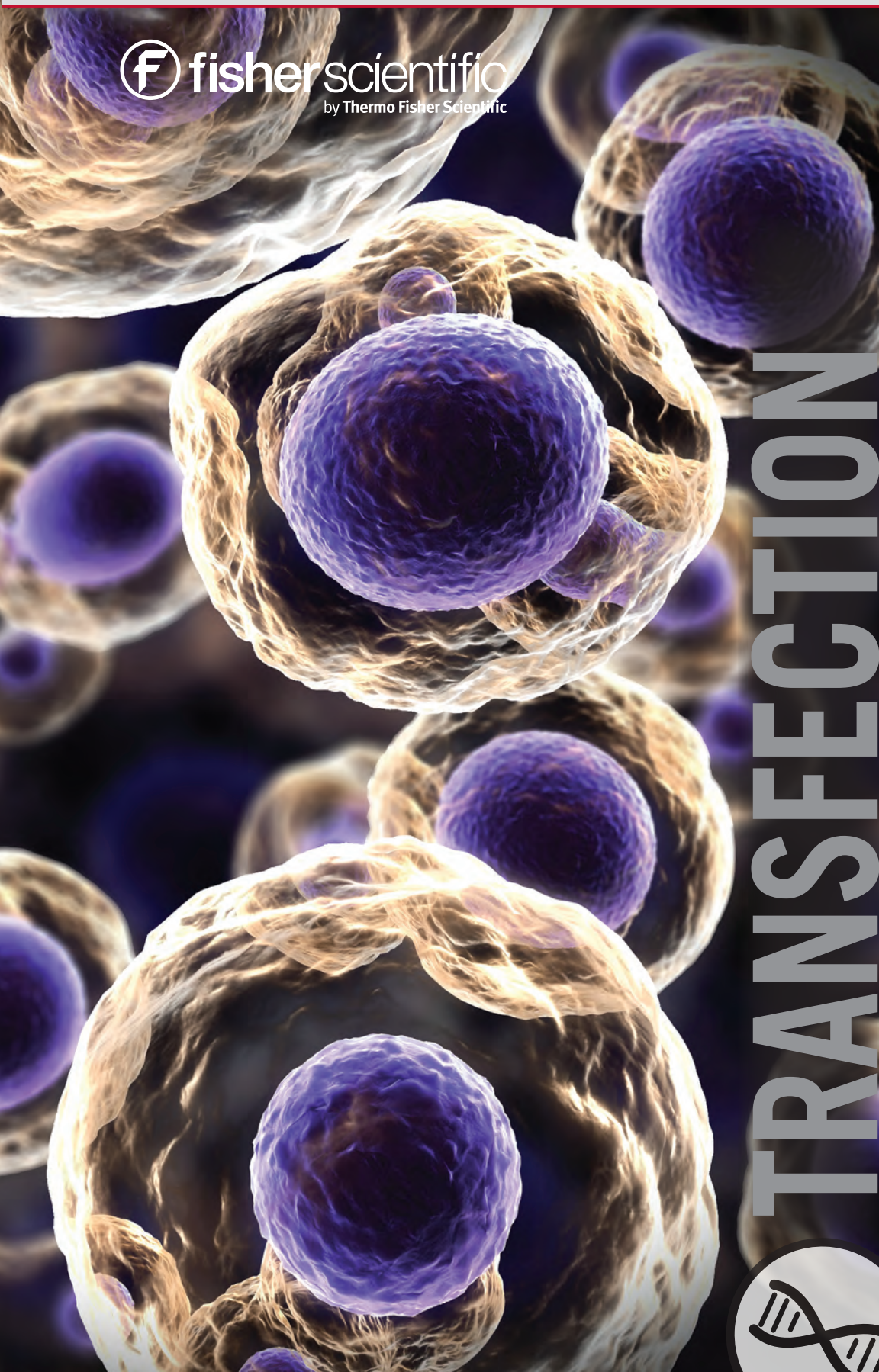
Delivery by Mirus

fisher scientific
by Thermo Fisher Scientific



# TRANSFECTION

# DELIVERY BY MIRUS

*Delivery by Mirus encompasses our mission to: deliver innovation, empower discovery and improve life. We established expertise by providing optimal delivery systems for the molecular and cellular biology applications used today. Mirus enables scientists to focus on their research by providing the support needed to better understand the world around us.*

*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

**2016:** *Trans*IT®-Lenti Transfection Reagent—Ideal for recombinant lentivirus production

**2015:** CHOgro® Expression System—High titer transient transfection for suspension CHO cells

**2014:** *Trans*IT®-Insect—Effective transient transfection for high yield baculovirus titers

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

*Trans*IT®-BrCa Transfection Reagent—The *first* breast cancer cell transfection reagent

**2010:** *Trans*IT-PRO® Transfection Kit—Large-scale, high yield antibody and protein production

**2008:** Ingenio® Electroporation Kits & Solution—Versatile, multi-platform electroporation solution

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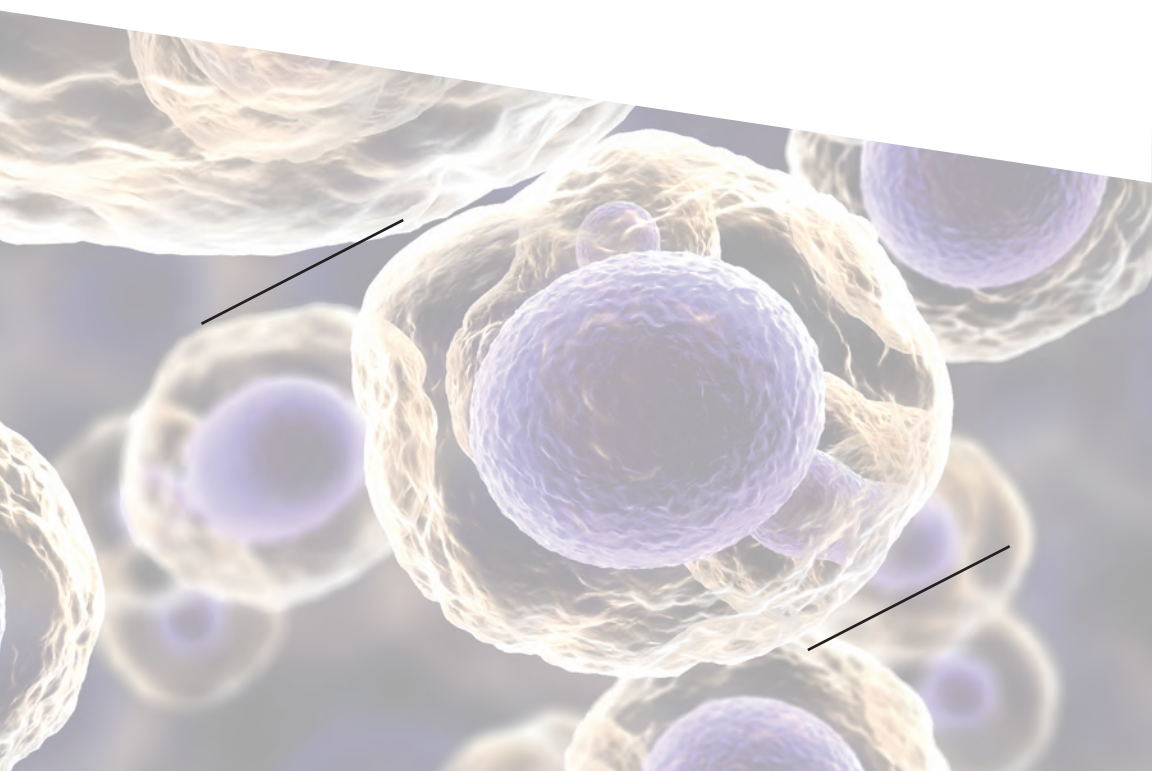

# CONTENTS

fisher scientific
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## CHEMICAL TRANSFECTION

### Ideal for: Broad Spectrum DNA, siRNA/miRNA & CRISPR/Cas9



### Ideal for: Broad Spectrum DNA



### Ideal for: Specific Cell Types



### Ideal for: siRNA/miRNA



### Ideal for: Large RNA (Viral RNA, mRNA & CRISPR/Cas9)



### Ideal for: Insect Cell Transfection & Baculovirus Production



### Ideal for: Protein & Antibody Production



## ELECTROPORATION

### Ideal for: PLASMID DNA, RNA, siRNA, miRNA and RNP Delivery



## VIRUS PRODUCTION

### Ideal for: LENTIVIRUS PRODUCTION

*Broad Spectrum DNA & siRNA/miRNA*



# *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

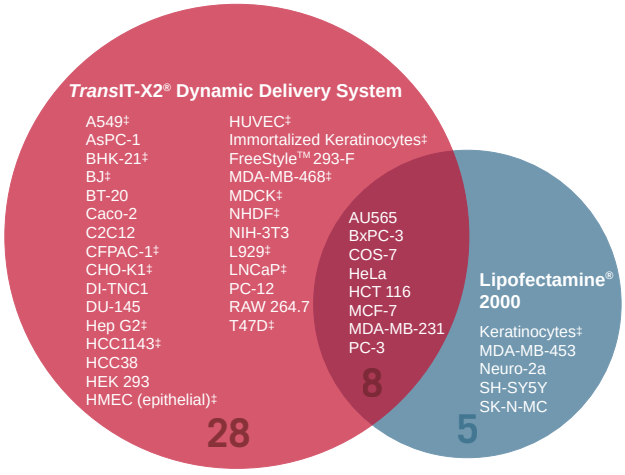
| PRODUCT NO. | QUANTITY |
|---|---|
| MIR6003 | 0.3 ml |
| MIR6004 | 0.75 ml |
| MIR6000 | 1.5 ml |
| MIR6005 | 5 x 1.5 ml |
| MIR6006 | 10 x 1.5 ml |

"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 ‡.

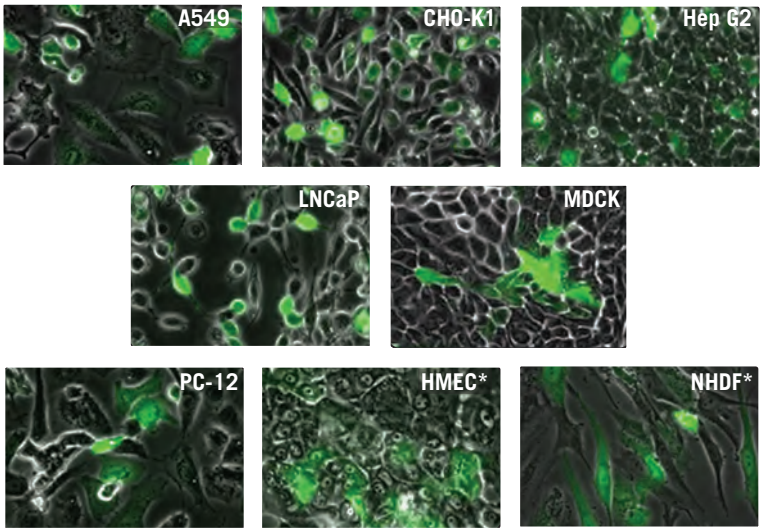



**FIGURE 2.** Visualization of High GFP Expression Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, LNCaP, MDCK, PC-12, primary human mammary epithelial cells (HMEC) and primary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

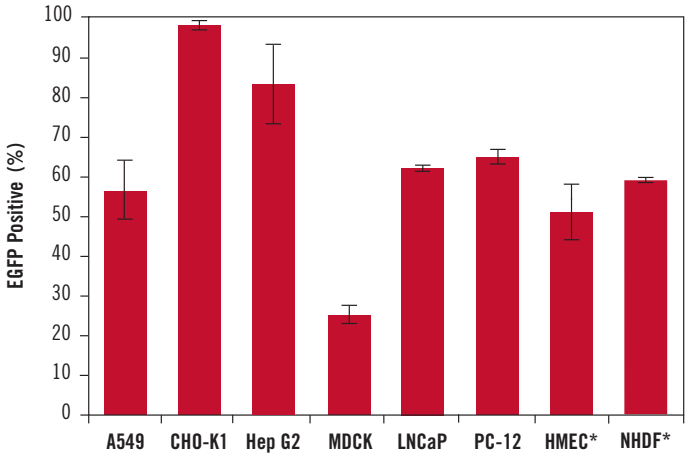



**FIGURE 3.** High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, MDCK, LNCaP, PC-12, primary human mammary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

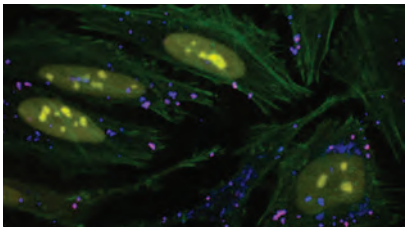


**FIGURE 4.** Functional Co-delivery of Plasmid DNA and siRNA Using the *Trans*IT-X2® Dynamic Delivery System. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid Cy®5 labeled DNA encoding nuclear YFP and Cy®3 labeled siRNA into HeLa cells. Transfection was performed in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

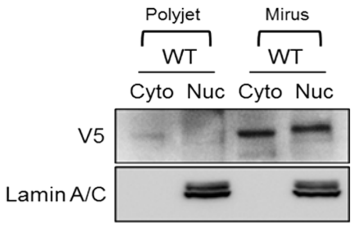
*Dr. G. Du,* Assistant Professor
Texas Medical Center

"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



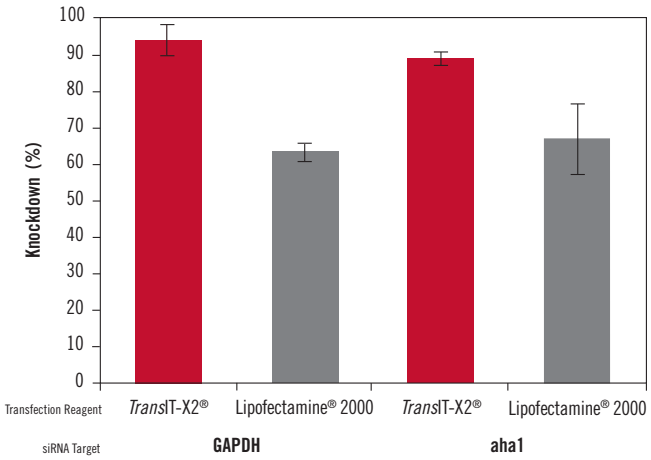



**FIGURE 5.** The *Trans*IT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect siRNA targeting endogenous proteins - GAPDH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

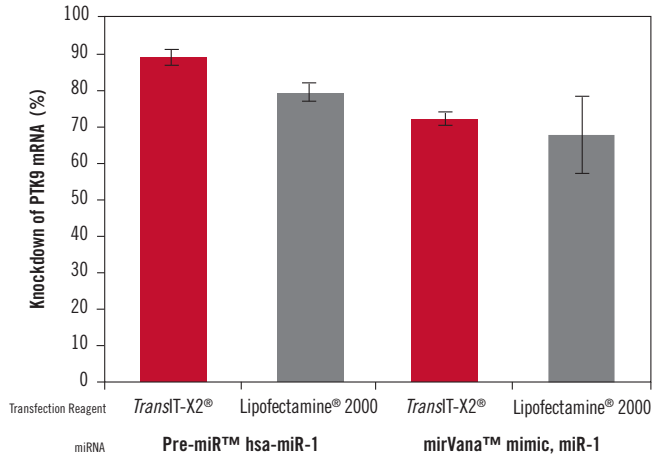
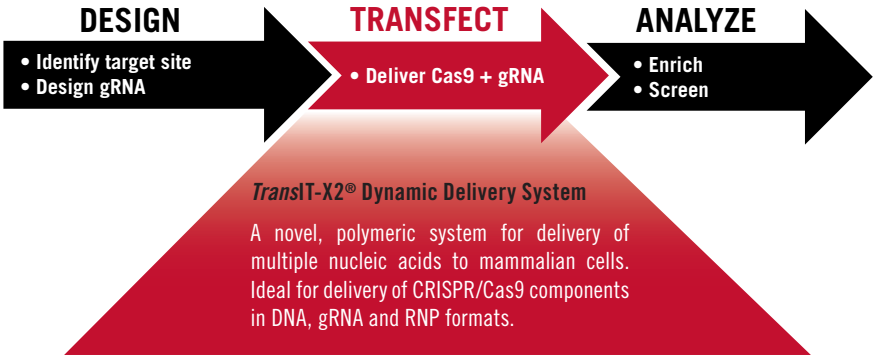



**FIGURE 6.** Effective miRNA Delivery Using The *Trans*IT-X2® Dynamic Delivery System Yields Decreased Levels of PTK9 mRNA. The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) and Lipofectamine® 2000 Transfection Reagent (Thermo Fisher Scientific) were used to transfect T47D cells with Pre-miR™ hsa-miR-1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

CHEMICAL TRANSFECTION

## 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

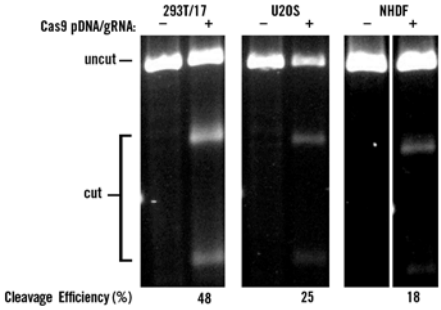



**FIGURE 7. Efficient Genome Editing with Cas9 Plasmid DNA and Guide RNA Oligonucleotides.** HEK293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding pDNA (MilliporeSigma) and 50nM PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using The *Trans*IT-X2® Dynamic Delivery 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

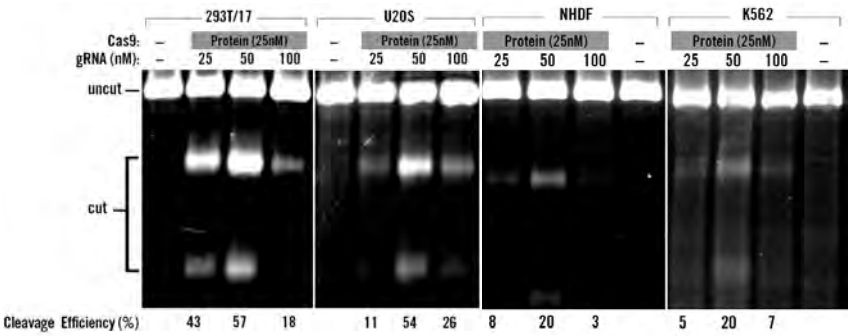



**FIGURE 8. Genome Editing with Cas9 + Guide RNA Ribonucleoprotein Complexes.** The RNP complex of PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) and Cas9 protein (PNA Bio) was delivered into HEK293T/17, U2OS, NHDF and K562 cells using the *Trans*IT-X2® Dynamic Delivery System (1 µl/well of a 24-well plate, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection. High levels of gene editing can be achieved in cells that were transfected with an RNP complex comprised of 50nM of gRNA and 25nM of Cas9 protein.

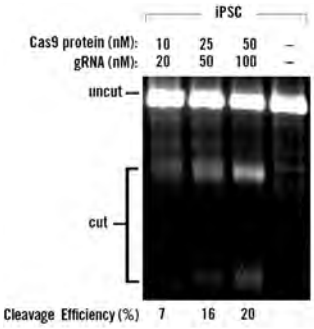



**FIGURE 9. Genome Editing in IPS Cells with Cas9 + Guide RNA Ribonucleoprotein Complexes.** The *Trans*IT-X2® Dynamic Delivery System was used to deliver Cas9 protein/guide RNA ribonucleoprotein (RNP) complexes in human induced pluripotent stem cells (iPSCs). A T7E1 mismatch assay was used to measure 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 |
|---|---|
| MIR2304 | 0.4 ml |
| MIR2300 | 1.0 ml |
| MIR2305 | 5 x 1.0 ml |
| MIR2306 | 10 x 1.0 ml |

IDEAL FOR USE IN VIRUS PRODUCTION

> "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.

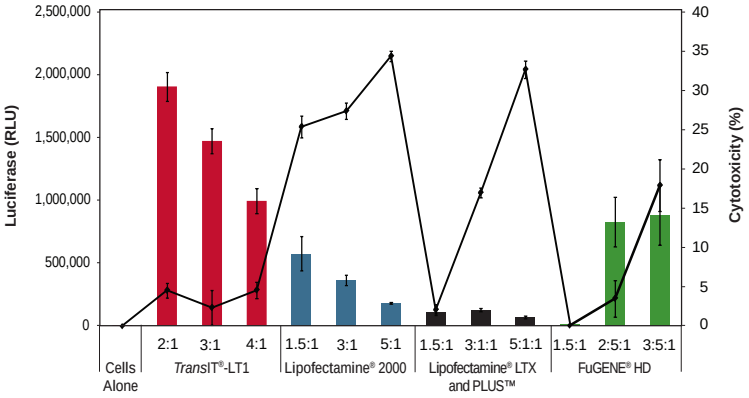



**FIGURE 10.** *Trans*IT®-LT1 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents. Hep G2 cells were transfected with a luciferase expression plasmid using the designated reagents at the manufacturers' recommended reagent-to-DNA ratio indicated beneath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

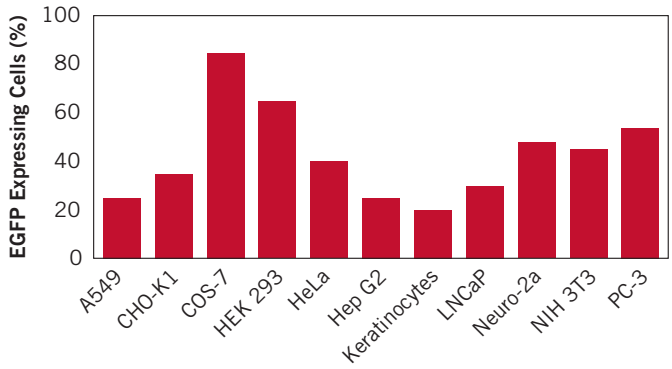



**FIGURE 11.** The *Trans*IT®-LT1 Reagent Efficiently Delivers DNA to a Wide Variety of Cell Lines. Using the *Trans*IT®-LT1 Transfection Reagent (Mirus Bio), cells were transfected with the pEGFP-C1 expression vector, and the percentage of EGFP expressing cells was determined 24-48 hours post-transfection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

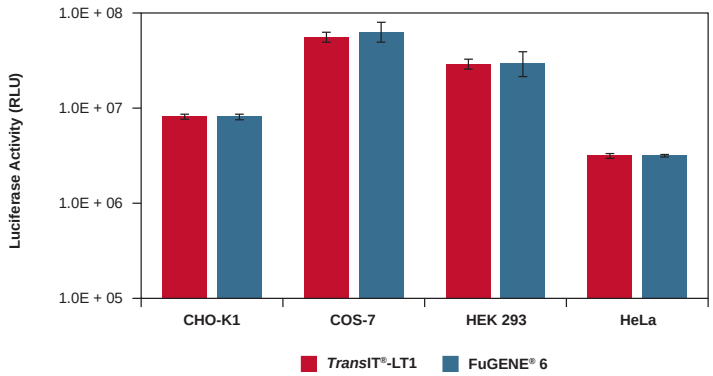



**FIGURE 12.** Comparable Luciferase Expression With *Trans*IT®-LT1 Reagent and FuGENE® 6 in Multiple Cell Types. The indicated cell lines were transfected in duplicate with 1 µg of a luciferase expression vector per well of a 12-well plate using either 3 µl of *Trans*IT®-LT1 (Mirus Bio) or FuGENE® 6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

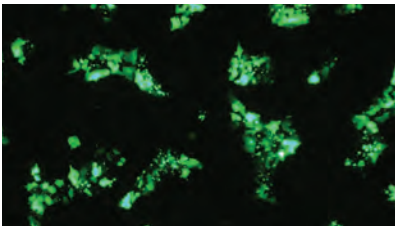


**FIGURE 13.** Exceptional Transfection Efficiency in Human Induced Pluripotent Stem Cells (iPSCs) via Reverse Transfection with the *Trans*IT®-LT1 Transfection Reagent. The *Trans*IT®-LT1 Transfection Reagent (Mirus Bio) was used to reverse transfect 1.3 x $10^6$ iPS cells with a ZsGreen expressing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

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

Delivery by Mirus

*Broad Spectrum DNA*

# *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 |
|---|---|
| MIR5404 | 0.4 ml |
| MIR5400 | 1.0 ml |
| MIR5405 | 5 x 1.0 ml |
| MIR5406 | 10 x 1.0 ml |

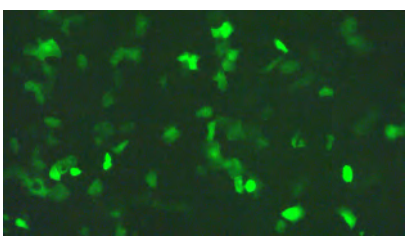
IDEAL FOR USE IN VIRUS PRODUCTION

> Using *Trans*IT®-2020, we transfected HeLa cells in 6-well plates with 1.25 µg of the Zheng 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 major competitors in most cell types.

**FIGURE 14.** High Performance Plasmid Transfection. Primary Human Small Epithelial cells (HSAEpic) 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

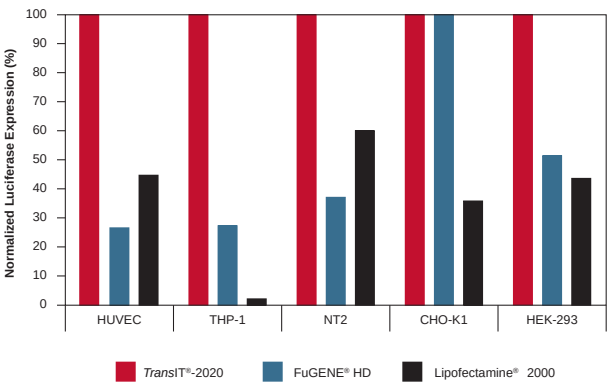



**FIGURE 15.** Superior Gene Expression in a Broad Spectrum of Cell Types. The indicated cell types were transfected in 96-well plates with a luciferase expression plasmid (0.1 µg/well) according to industry accepted testing protocols. Reagent-to-DNA ratios were optimized for each cell type: *Trans*IT®-2020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

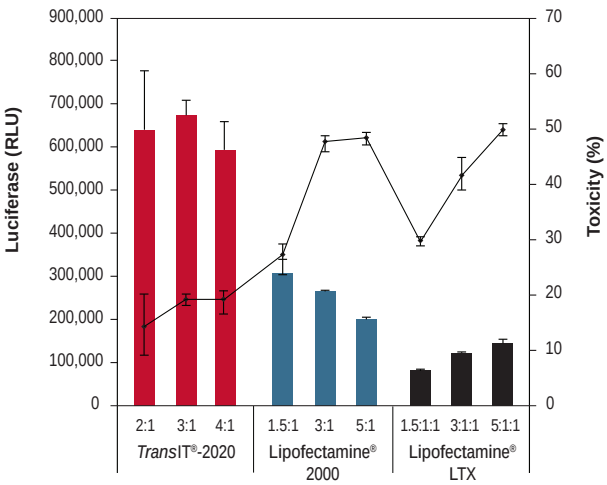


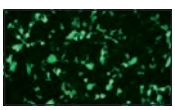
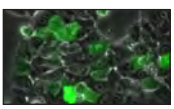
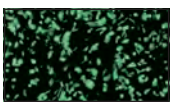
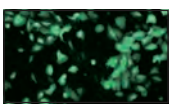

**FIGURE 16.** *Trans*IT®-2020 Reagent Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents. Human Umbilical Vein Endothelial Cells (HUVEC) were transfected with a luciferase expression plasmid using the designated reagents at the reagent-to-DNA ratios. Transfections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

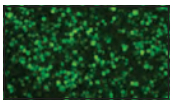
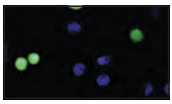
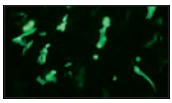
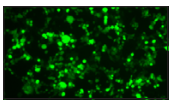
*Trans*IT® Cell Line Specific DNA Transfection Reagents are formulated to maximize transfection efficiency while maintaining cellular health in many popular or hard-to-transfect cell types.

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 | IDEAL FOR USE IN VIRUS PRODUCTION 75–85% | MIR2704 | 0.4 ml |
| | | | MIR2700 | 1.0 ml |
| | | | MIR2705 | 5 x 1.0 ml |
| | | | MIR2706 | 10 x 1.0 ml |
| *Trans*IT®-BrCa Transfection Reagent | | | | |
|  | MCF-7, MDA-MB-231, MDA-MB-453, MDA-MB-468, T47D | 40–80% | MIR5504 | 0.4 ml |
| | | | MIR5500 | 1.0 ml |
| | | | MIR5505 | 5 x 1.0 ml |
| | | | MIR5506 | 10 x 1.0 ml |
| *Trans*IT®-CHO Transfection Kit (*Trans*IT®-CHO Reagent & CHO Mojo Reagent) | | | | |
|  | CHO-K1 and related | 50–60% | MIR2174 | 0.4 ml |
| | | | MIR2170 | 1.0 ml |
| | | | MIR2175 | 5 x 1.0 ml |
| | | | MIR2176 | 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 | – | MIR6104 | 0.4 ml |
| | | | MIR6100 | 1.0 ml |
| | | | MIR6105 | 5 x 1.0 ml |
| | | | MIR6106 | 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% | MIR2124 | 0.4 ml |
| | | | MIR2120 | 1.0 ml |
| | | | MIR2125 | 5 x 1.0 ml |
| | | | MIR2126 | 10 x 1.0 ml |
| ***Trans*IT®-Keratinocyte Transfection Reagent** | | | | |
|  | Immortalized Keratinocyte | 20–30% | MIR2804 | 0.4 ml |
| | | | MIR2800 | 1.0 ml |
| | | | MIR2805 | 5 x 1.0 ml |
| | | | MIR2806 | 10 x 1.0 ml |
| ***Trans*IT®-Lenti Transfection Reagent** | | | | |
|  | Adherent HEK 293T | IDEAL FOR USE IN VIRUS PRODUCTION 80–90% | MIR6603 | 0.3 ml |
| | | | MIR6604 | 0.75 ml |
| | | | MIR6600 | 1.5 ml |
| | | | MIR6605 | 5 x 1.5 ml |
| | | | MIR6606 | 10 x 1.5 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.

> *Trans*IT®-CHO Transfection Kit is a great product. Easy to use, works well, and reasonably priced.
>
> *Matthew Nicotra,*
> University of Pittsburgh

# *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 |
|---|---|
| MIR2154 | 0.4 ml |
| MIR2150 | 1.5 ml |
| MIR2155 | 5 x 1.5 ml |
| MIR2156 | 10 x 1.5 ml |

*Trans*IT-siQUEST® Transfection Reagent

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR2114 | 0.4 ml |
| MIR2110 | 1.5 ml |
| MIR2115 | 5 x 1.5 ml |
| MIR2116 | 10 x 1.5 ml |

## 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. These two reagents allow the user to identify the best transfection reagent for their particular cell line.

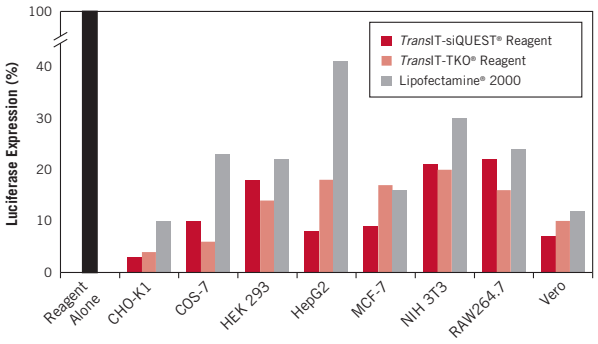



**FIGURE 17.** Knockdown Efficiencies Using *Trans*IT-siQUEST®, *Trans*IT-TKO® Reagents and Lipofectamine® 2000. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines using the *Trans*IT®-LT1 Reagent (Mirus Bio). Subsequently, firefly luciferase expression was 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-lux (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-lux (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.*

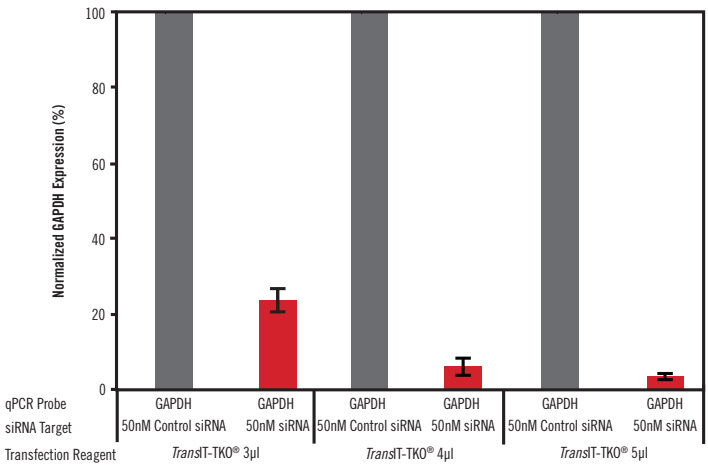



**FIGURE 18.** High Efficiency Endogenous Knockdown in iCell® Cardiomyocytes. The *Trans*IT-TKO® Transfection Reagent (Mirus Bio) was used to transfect iCell® Cardiomyocytes (Cellular Dynamics International (CDI), a FUJIFILM Company) plated at a density of 136,500 cells per well of a 12-well plate pre-coated 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 (50nM per well). Seventy-two hours post-transfection, the amount of GAPDH mRNA was measured relative to 18s rRNA mRNA 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 |
|---|---|
| MIR2225 | 0.4 ml |
| MIR2250 | 1.0 ml |
| MIR2255 | 5 x 1 ml |
| MIR2256 | 10 x 1 ml |

IDEAL FOR USE IN VIRUS PRODUCTION

## 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.

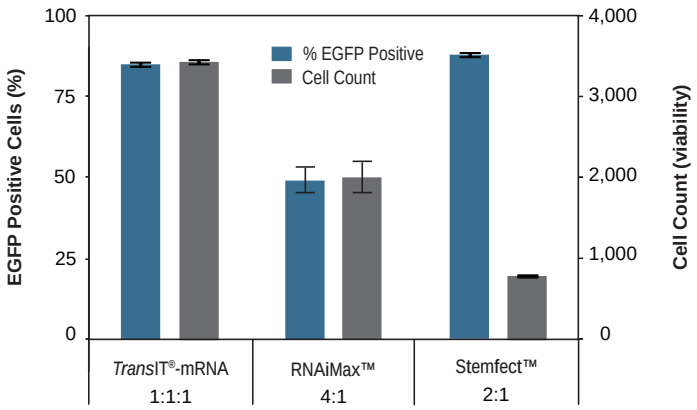



**FIGURE 19.** High Efficiency and Low Toxicity Transfection Following 14 Consecutive Transfections With The *Trans*IT®-mRNA Transfection Kit. Repeated daily transfections were performed in the same population of BJ fibroblasts using three commercially available transfection reagents – the *Trans*IT®-mRNA 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 (black line grey bars). Error bars represent the standard deviation of triplicate wells.

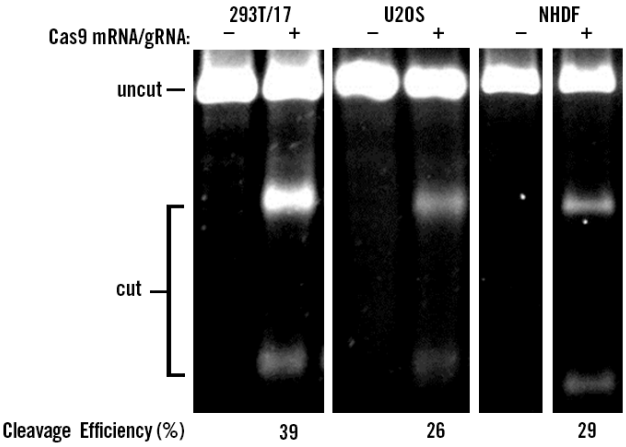



**FIGURE 20.** Efficient Genome Editing with Cas9 mRNA + Guide RNA Oligonucleotides. HEK293T/17, U2OS andNHDF cells were co-transfected with 0.5 µg of Cas9 encoding mRNA, 5meC, (Trilink Biotechnologies) and 25nM of PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using *Trans*IT®-mRNA Transfection 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 26 for RNP delivery with Ingenio® Electroporation Solution.

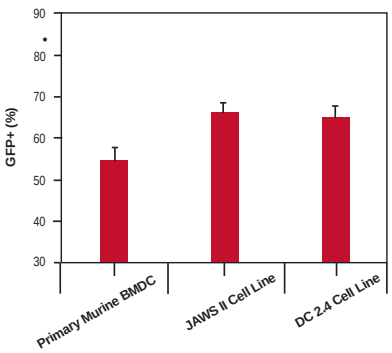
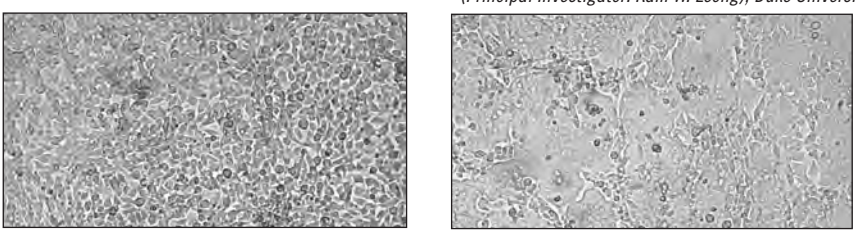



**FIGURE 21.** Multiple Dendritic Cell Types Express GFP From mRNA Transfected Using The *Trans*IT®-mRNA Transfection Kit. Murine primary bone marrow derived dendritic cells (BMDC) and murine dendritic cells types (JAWS II and DC 2.4) were transfected with 1 µg of capped and polyadenlyated mRNA encoding 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 cell/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 22.** The *Trans*IT®-mRNA Transfection Kit Successfully Delivers Viral RNAs 32 kb Long. A 32 kb *in vitro* transcript 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

- **Expectional 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 media change
- **Better Value**—Low reagent amounts required per transfection

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR6104 | 0.4 ml |
| MIR6100 | 1.0 ml |
| MIR6105 | 5 x 1.0 ml |
| MIR6106 | 10 x 1.0 ml |

IDEAL FOR USE IN BACULOVIRUS PRODUCTION

## 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.

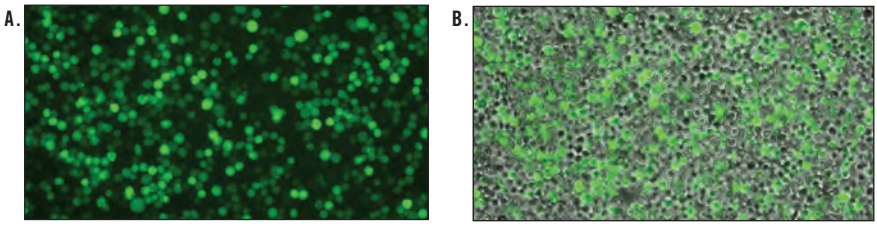



**FIGURE 23.** Efficienct Transfection of Baculovirus Genomic DNA Using The *Trans*IT®-Insect Reagent. Transfections were performed in 6-well plates with 5 x $10^5$ Sf9 cells per well using the *Trans*IT®-Insect Transfection Reagent (Mirus Bio) at the reagent-to-total DNA ratio of 3:1 (µl:µg). Cells 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).

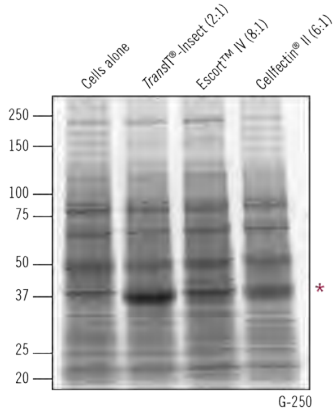



**FIGURE 24**. Superior Recombinant Protein Expression in High Five™ Cells Using *Trans*IT®-Insect. High Five™ cells (Thermo Fisher Scientific) were transfected in 6-well plates with 2.5 µg of a GFP expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated reagent at the indicated 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. Expressed GFP containing 6X His, S, and HSV tags (~38 kDa) was clearly detected in the lysate from the cells that were transfected (*) with the highest level of expression observed at *Trans*IT®-Insect (Mirus Bio):

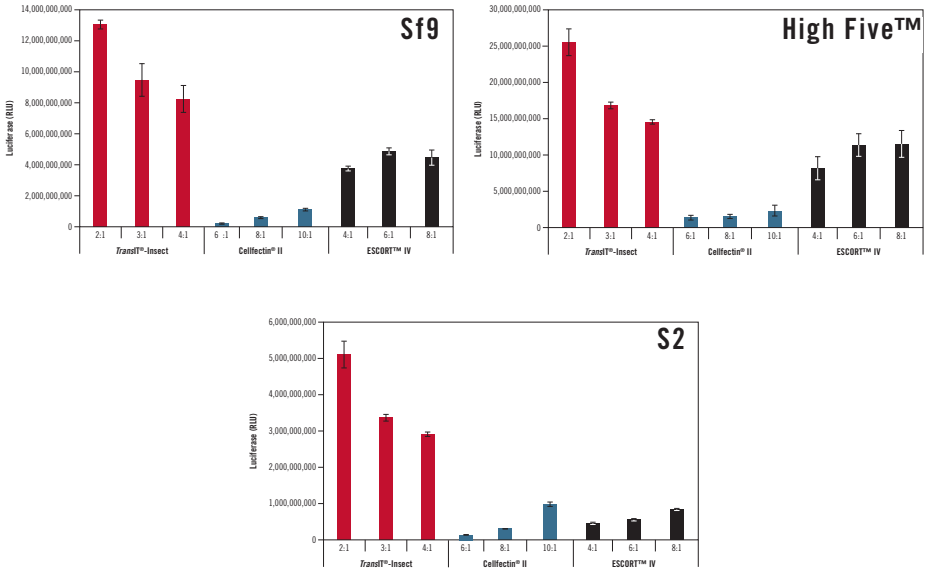



**FIGURE 25.** *Trans*IT®-Insect Outperforms Competitor Transfection Reagents. Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific), and Drosophila S2 cells were transfected in 96-well plates with 0.1 µg of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the designated 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.

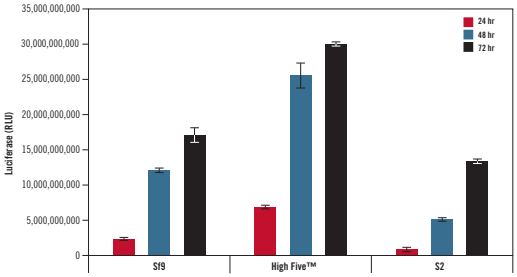



**FIGURE 26.** *Trans*IT®-Insect Yields Increased Protein Expression Over Time. Insect cell lines Sf9, High Five™ (Thermo Fisher Scientific), and Drosophila S2 were transfected in a 96-well plate with 0.1 ug of a luciferase expression plasmid driven by an hr5 enhancer/IE1 promoter using the *Trans*IT®-Insect 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

Delivery by Mirus

*Higher Titer Transient Transfection System for Suspension CHO Cells*

# CHOgro® 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 CHOgro® Expression System was developed through systematic optimization of transfection protocol parameters including: cell density, transfection reagent, media formulation and culture temperature. With the CHOgro® Expression System, high protein titers can now be achieved in suspension CHO cells through high density transient transfection.


Complete CHOgro® Expression System

Polybag and Dry Powder
Optional Media Formats

Complete CHOgro® Expression System
*(CHOgro® Expression Media, TransIT-PRO® Transfection Reagent, CHOgro® Complex Formation Solution, Poloxamer Solution and L-Glutamine Solution)*

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR6260 | 1 Kit |

Individual Components, Available Separately

| PRODUCT | PRODUCT NO. | QUANTITY |
|---|---|---|
| CHOgro® Expression Medium | MIR6200 | 1 Liter |
| Liquid Polybag CHOgro® Expression Medium | MIR6202 | 10 Liters |
| Dry Powder CHOgro® Expression Medium | MIR6201 | Prepares 10 Liters |
| *Trans*IT-PRO® Transfection Reagent *(Without boost; please see page 23 for kit with boost)* | MIR5740 | 1 ml |
| CHOgro® Complex Formation Solution | MIR6210 | 100 ml |
| Poloxamer 188 Solution | MIR6230 | 100 ml |
| L-Glutamine Solution | MIR6240 | 100 ml |

Accessory, Sold Separately
Not Included with Kit

| PRODUCT | PRODUCT NO. | QUANTITY |
|---|---|---|
| Human IgG1 Expression Control | MIR6250 | 1 µg |

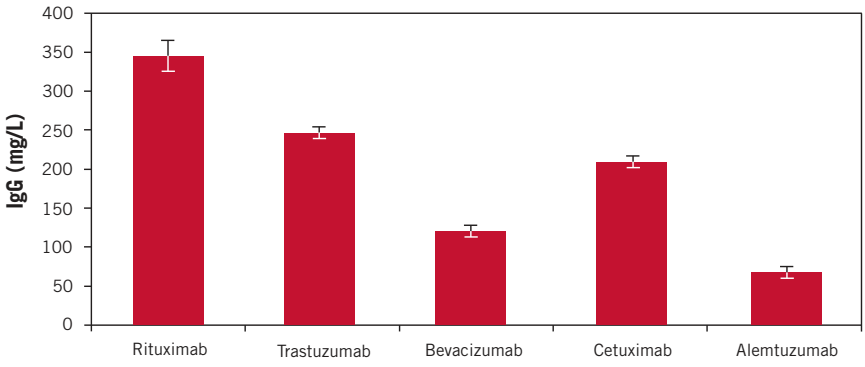



**FIGURE 27.** Titers of Different Antibody Vector Constructs. Five different antibody constructs were produced by transient transfection using a temperature shift to 32° C and *Trans*IT-PRO® (Mirus Bio). Day 11 supernatants were clarified and analyzed using a human IgG ELISA (ZeptoMetrix). Error bars represent the standard deviation of triplicate technical replicates.

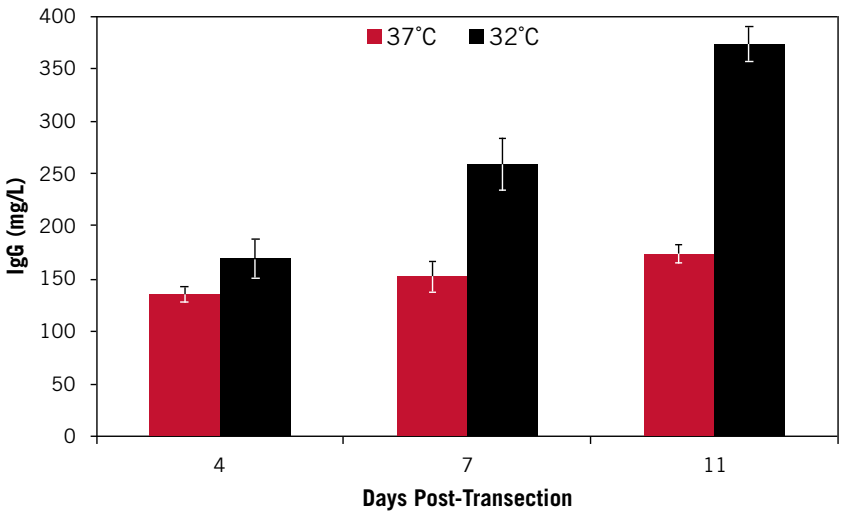



**FIGURE 28.** Increases in Product Titer are Observed at Longer Time Points with Mild Hypothermic Conditions. Cells were transfected at a density of 2 x $10^6$ cells/ml in 20 ml of CHOgro® Expression Medium (Mirus Bio) in 125 ml shake flasks (Thomson). Antibody levels were analyzed from day 4, 7 and 11 clarified supernatants using a human IgG ELISA (ZeptoMetrix). All flasks were incubated at 37°C for 24 hours; at the timepoint designated, parallel flasks were switched to 32°C for the remainder of the experiment. Error bars represent the standard deviation of triplicate technical replicates.

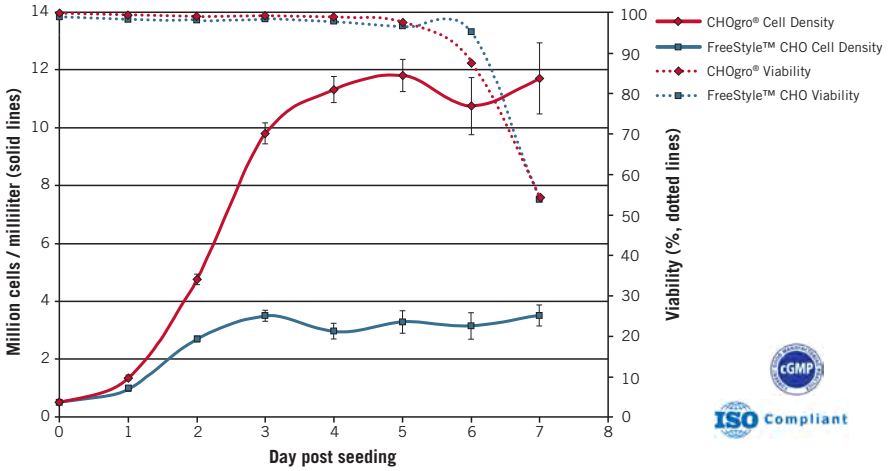



**FIGURE 29.** Suspension CHO Cells Grow to High Density in the CHOgro® Expression Medium. Triplicate flasks of FreeStyle™ CHO-S cells (Thermo Fisher Scientific) were seeded in CHOgro® Expression Medium (red line, Mirus Bio) or FreeStyle™ CHO Expression Medium (blue line, Thermo Fisher Scientific) at cell density of 0.5 x $10^6$ cells/ml, 40 ml per 125 ml shake flask (Thomson). Cell counts (solid line) and viability (propidium iodide staining, dotted line) were measured daily using a Guava easyCyte™ 5HT flow cytometer (MilliporeSigma). Error bars represent the standard deviation of three readings of biological triplicates.

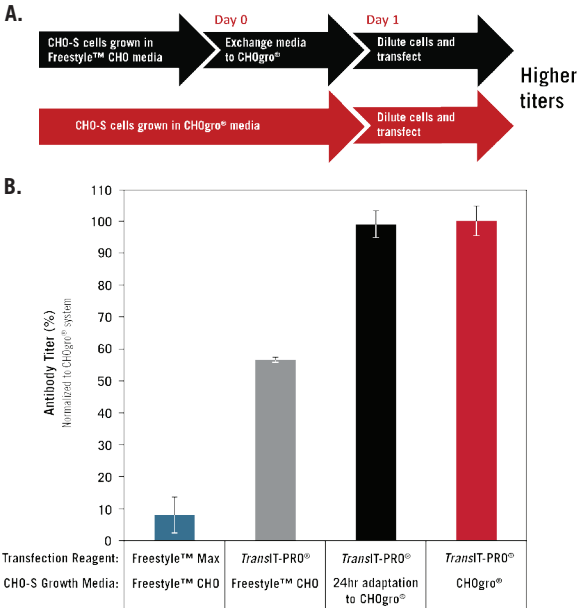



**FIGURE 30.** CHOgro® Media Exchange Leads to Higher Protein Production. FreeStyle™ CHO-S cells (Thermo Fisher Scientific) were cultured in FreeStyle™ CHO Expression Medium (Thermo Fisher Scientific) or CHOgro® Expression Medium (Mirus Bio). (A) Workflow schematic of media exchange of CHO-S cells from FreeStyle™ CHO Expression Medium (Thermo Fisher Scientific) to CHOgro® Expression Medium (black arrow, Mirus Bio) or the normal CHOgro® Expression System (red arrow, Mirus Bio) (B) Day 6 supernatants were clarified and analyzed using a human IgG ELISA (ZeptoMetrix). Data is normalized to the complete CHOgro® Expression System (red bar, Mirus Bio). 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 |
|---|---|
| MIR5700 | 1 ml |
| MIR5760 | 10 ml |

> 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.

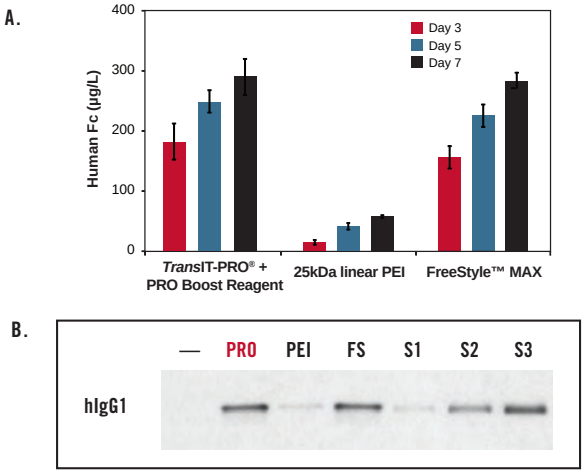



**FIGURE 31.** Achieve High Antibody Titers Using The *Trans*IT-PRO® Transfection Kit in Suspension CHO Cells. IgG1 was produced by transient transfection using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 25 kDa linear PEI (6:1) or FreeStyle™ MAX (1:1, Thermo Fisher Scientific) transfection 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, 25kDa linear PEI is duplicate technical replicates. (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).

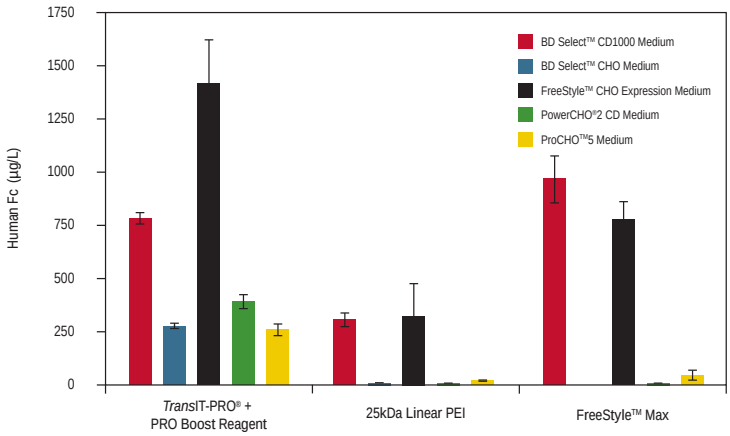



**FIGURE 32.** *Trans*IT-PRO® Provides High Performance Across Varied Media Formulations. FreeStyle™ CHO-S cells were adapted to five representative growth media as noted in the graph. Cells were transfected with an IgG encoding plasmid using the *Trans*IT-PRO® and PRO Boost Reagent (1:1:1, Mirus Bio), 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.

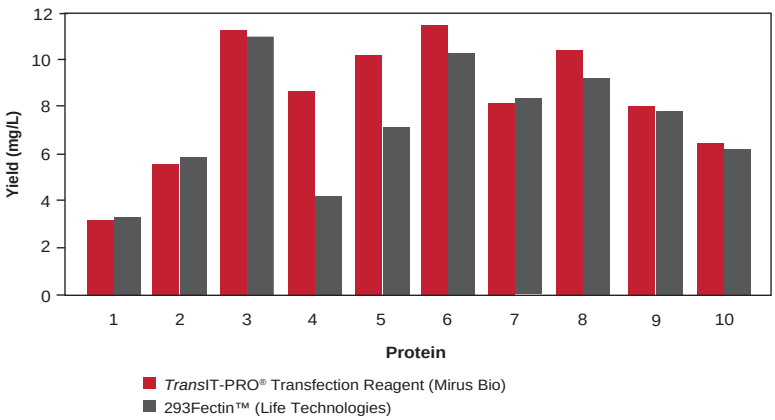



**FIGURE 33.** Achieve High Protein Yields Using The *Trans*IT-PRO® Transfection Kit in Suspension 293 Cells. Ten different secreted (non-antibody) proteins were transiently expressed in FreeStyle™ 293-F cells (Thermo Fisher Scientific) using the *Trans*IT-PRO® (1.5:1, Mirus Bio) or 293fectin™ (2:1, 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 TransIT-PRO® pharmaceutical customer.*

*Plasmid DNA, RNA, siRNA and miRNA*

# INGENIO® 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. It is also available as a stand alone solution.

> "I was very depressed for the last 6 months because I was unable to transfect my rat cell line with various transfection reagents. I tried 5 Nucleofection® programs, 2 buffers and several different cell densities. But nothing worked. I am very happy to inform you, Ingenio® is a life saver!"
>
> *Sanal Madhusudana Girija,*
> Albert Einstein College of Medicine

Ingenio® Electroporation Kits for Amaxa® Nucleofector® II/2b Nucleofector Devices
*(solution, 0.2 cm cuvettes, cell droppers)*

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR50112 | 25 RXN |
| MIR50115 | 50 RXN |
| MIR50118 | 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 |
|---|---|
| MIR50113 | 25 RXN |
| MIR50116 | 50 RXN |
| MIR50119 | 100 RXN |

Ingenio® Electroporation Solution

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR50111 | 25 RXN |
| MIR50114 | 50 RXN |
| MIR50117 | 100 RXN |

Ingenio® Electroporation Accessories

*Cuvettes*

| PRODUCT NO. | SIZE | QUANTITY |
|---|---|---|
| MIR50120 | 0.2 cm | 25 PK |
| MIR50121 | 0.2 cm | 50 PK |
| MIR50122 | 0.4 cm | 25 PK |
| MIR50123 | 0.4 cm | 50 PK |

*Cell Droppers*

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR50124 | 25 PK |
| MIR50125 | 50 PK |

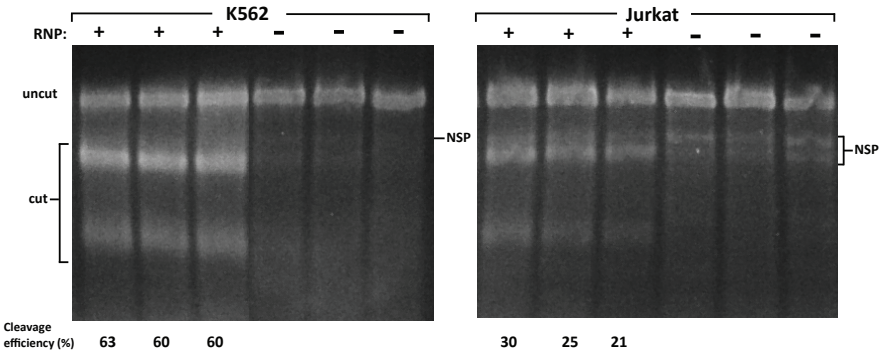



**FIGURE 34.** Efficient RNP Delivery with Electroporation Ingenio® Solution. K562 and Jurkat cells were electroporated with a Cas9 protein/gRNA, ribonucleoprotein (RNP) complex, comprised of 750 nM Cas9 protein (EnGen® Cas9 NLS, NEB) and 1500 nM pre-complexed two-part gRNA (IDT) targeting PPIB using 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.

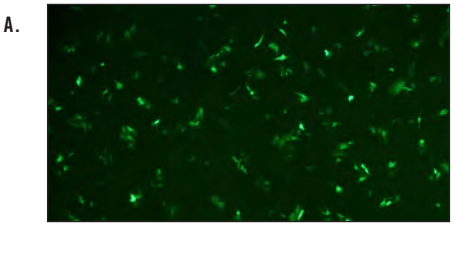



**FIGURE 35.** High Efficiency Plasmid DNA Electroporation of Human Induced Pluripotent Stem (iPS) Cells using Ingenio®. The Ingenio® Electroporation Kit (Mirus Bio) was used to transfect 2 x $10^6$ iPS cells on the Amaxa® Nucleofector® II/2b Device (Lonza Group Ltd). Cells were electroporated with 8 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.*

Ingenio® Electroporation Kits and Solutions *continued*

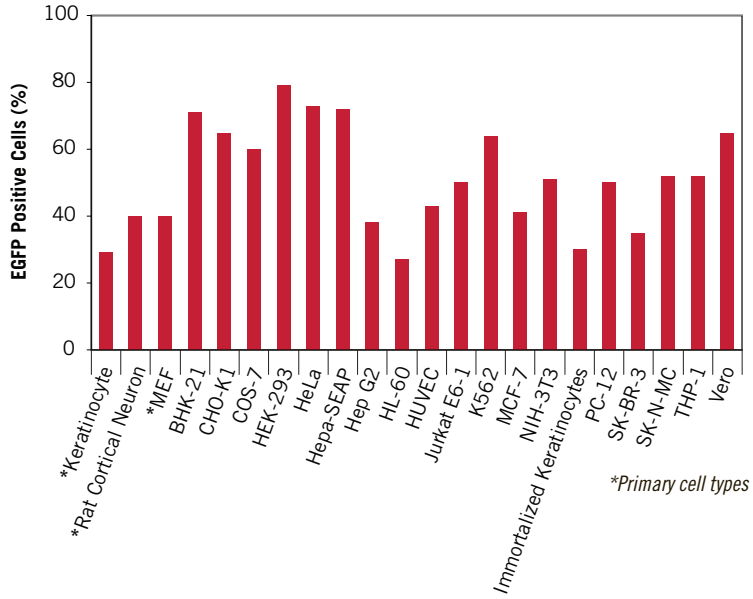



**FIGURE 36.** The Ingenio® Electroporation Kits are Ideal for Electroporation in Many Cell Types Using the Amaxa® Nucleofector® II/2b Device. Cells were assayed at 24 hours post-electroporation by flow cytometry and reported as percentage of live cell population. Visit www.mirusbio.com/applications/electroporation for ideal pulse conditions.

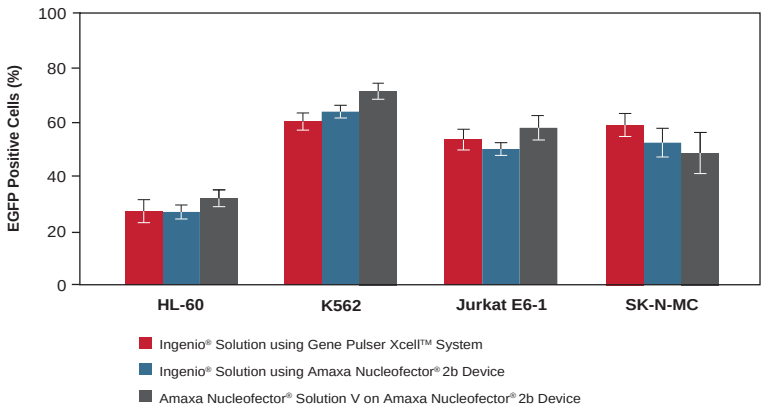



**FIGURE 37.** The Ingenio® Solution Provides Comparable Efficiency on the Amaxa® Nucleofector® II/2b Device. Cells were electroporated in parallel with an EGFP reporter vector. Two electroporators were used with different electroporation kits: the Ingenio® Electroporation Kit (Mirus Bio) was used in 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.

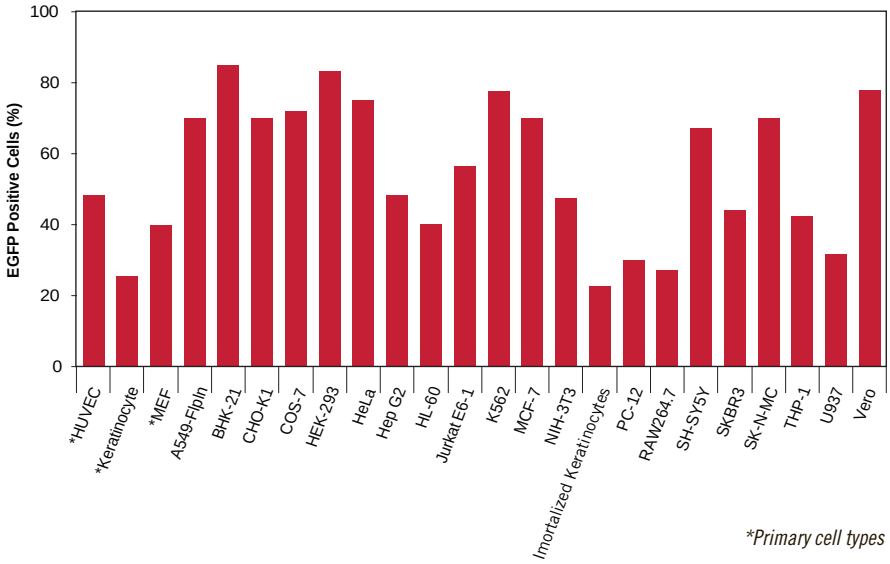



**FIGURE 38.** The Ingenio® Electroporation Kits are Ideal for Electroporation in Many Cell Types Using the Bio-Rad® GenePulser Xcell™ System. EGFP expressing cells were identified 24 hours post-electroporation by flow cytometry and presented as a percentage of the live cell population. Visit www.mirusbio.com/applications/electroporation for ideal pulse conditions.

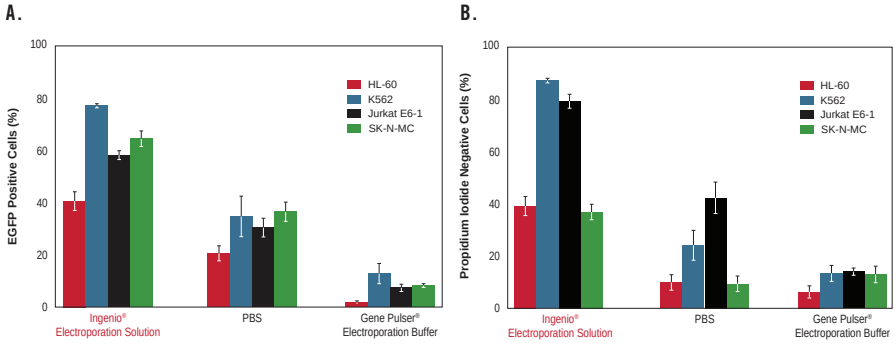



**FIGURE 39.** Ingenio® Kits Outperforms Other Electroporation Solutions in Efficiency and Viability. Cells were electroporated in parallel with an EGFP reporter vector using either Ingenio® Electroporation Solution (Mirus Bio), PBS or GenePulser® Electroporation Buffer (Bio-Rad Laboratories, Inc.) on 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 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

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR6603 | 0.3 ml |
| MIR6604 | 0.75 ml |
| MIR6600 | 1.5 ml |
| MIR6605 | 5 x 1.5 ml |
| MIR6606 | 10 x 1.5 ml |

*Transduce*IT™ Transduction Reagent

| PRODUCT NO. | QUANTITY |
|---|---|
| MIR6620 | 1 ml |

## 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.

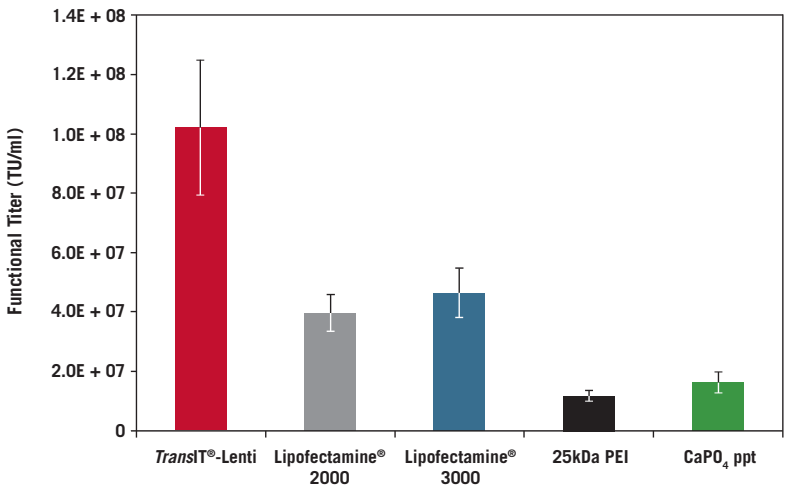



**FIGURE 40.** High Functional Titers With The *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate with pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma-Aldrich, Inc. LLC) and the Lentivirus Packaging Mix powered by MISSION® (1:1 ratio, 2 µg/well) with the following reagents: *Trans*IT®-Lenti (3:1, vol:wt; Mirus Bio), Lipofectamine® 2000 (3:1; Thermo Fisher Scientific), Lipofectamine® 3000 (3:1:1; Thermo Fisher Scientific), 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.

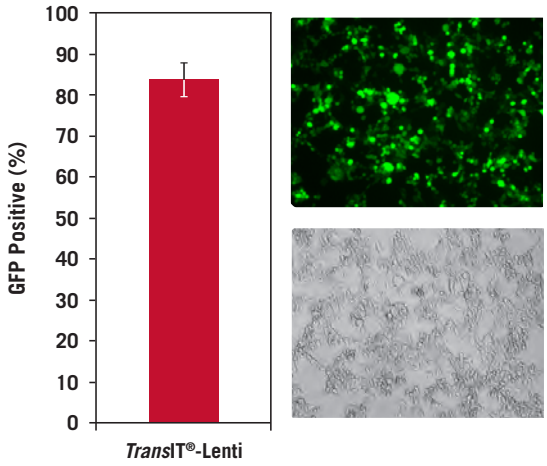



**FIGURE 41.** High Efficiency Transfection With the *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate format using MISSION® pLKO.1-puro-CMV-TurboGFP™ transfer vector and Lentivirus Packaging Mix (Sigma-Aldrich, Inc. LLC) using the *Trans*IT®-Lenti Transfection 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.

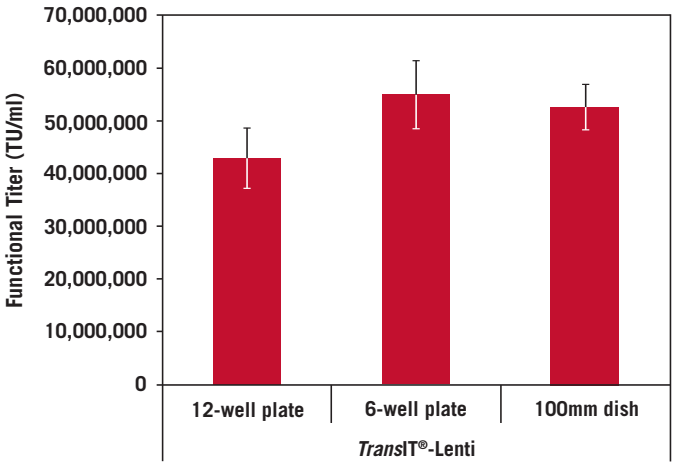



**FIGURE 42.** Lentivirus Production is Scalable. Adherent 293T/17 cells were transfected in a 12-well, 6-well or 100 mm plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix at a 1:1 ratio; Sigma-Aldrich, Inc. LLC) and the *Trans*IT®-Lenti 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 *continued*

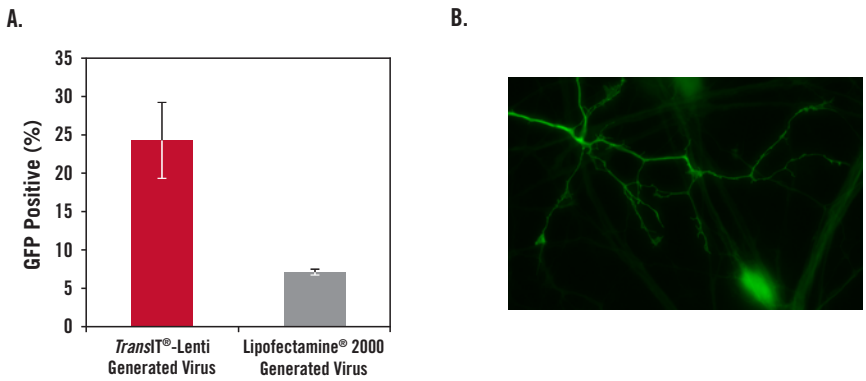



**FIGURE 43.** High Transduction Efficiency with Unconcentrated Lentivirus Using *Trans*IT®-Lenti. (A) Lentivirus was produced with the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt; Mirus Bio) or Lipofectamine® 2000 (Thermo Fisher Scientific) using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ 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), one microliter 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 35mm dishes (Ibidy) 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.

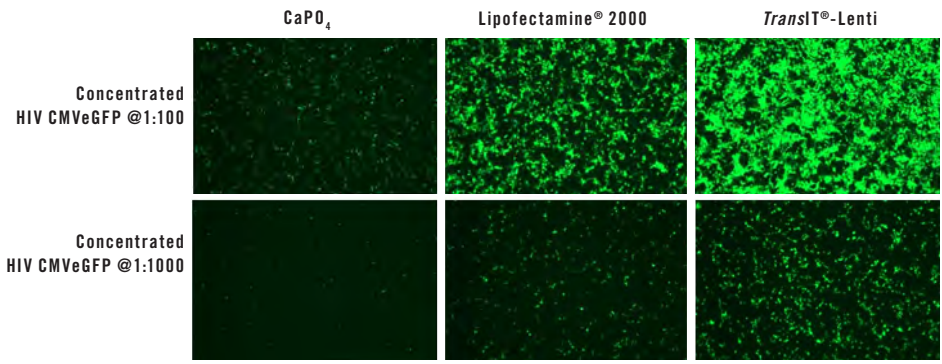



**FIGURE 44.** Comparing Functionality of $CaPO_4$, Lipofectamine® 2000 or *Trans*IT®-Lenti Generated Lentivirus. HIV CMVeGFP virus was produced in HEK 293FT cells using either $CaPO_4$, Lipofectamine® 2000 (Thermo Fisher Scientific)or *Trans*IT®-Lenti Transfection Reagent (Mirus Bio) per manufacturers' 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.*

fisher scientific
by Thermo Fisher Scientific

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545 Science Drive
Madison, WI 53711 USA
Attention: Accounts Payable
Fax: 608.441.2849

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Use of Mirus Bio *Trans*IT® polyamine transfection reagents are covered by U.S. Patent No. 7,101,995, No. 7,601,367, No. 8,921,448, No. 9,290,779 and patents pending. Mirus Bio *Label*IT® nucleic acid labeling and modifying reagents are covered by U.S. Patent No. 6,262,252, No. 6,593,465, No. 7,049,142, No. 7,326,780 and No. 7,491,538. This product is sold to the Buyer with a limited license for Research Use Only and is not for clinical, therapeutic or diagnostic use in humans or animals. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio LLC. The buyer agrees not to infringe upon Mirus patents or to attempt to reverse engineer, reconstruct, synthesize or otherwise modify Mirus Bio products.

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For commercial use of flashBAC™ or pOET products, please contact Oxford Expression Technologies, Ltd.

For further information, contact Mirus Bio LLC, 545 Science Drive, Madison WI 53711. Email license@mirusbio.com.

Delivery by Mirus

# PRODUCT LIST

## CHEMICAL TRANSFECTION

### Broad Spectrum DNA & siRNA/miRNA

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT-X2® Dynamic Delivery System | MIR6003 | 0.3 ml |
| | MIR6004 | 0.75 ml |
| | MIR6000 | 1.5 ml |
| | MIR6005 | 5 x 1.5 ml |
| | MIR6006 | 10 x 1.5 ml |

### Broad Spectrum DNA

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-2020 Transfection Reagent | MIR5404 | 0.4 ml |
| | MIR5400 | 1 ml |
| | MIR5405 | 5 x 1 ml |
| | MIR5406 | 10 x 1 ml |
| *Trans*IT®-LT1 Transfection Reagent | MIR2304 | 0.4 ml |
| | MIR2300 | 1 ml |
| | MIR2305 | 5 x 1 ml |
| | MIR2306 | 10 x 1 ml |

### Cell Line Specific

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-293 Transfection Reagent | MIR2704 | 0.4 ml |
| | MIR2700 | 1 ml |
| | MIR2705 | 5 x 1 ml |
| | MIR2706 | 10 x 1 ml |
| *Trans*IT®-BrCa Transfection Reagent | MIR5504 | 0.4 ml |
| | MIR5500 | 1 ml |
| | MIR5505 | 5 x 1 ml |
| | MIR5506 | 10 x 1 ml |
| *Trans*IT®-CHO Transfection Kit* | MIR2174 | 0.4 ml |
| | MIR2170 | 1 ml |
| | MIR2175 | 5 x 1 ml |
| | MIR2176 | 10 x 1 ml |
| *Trans*IT-HeLaMONSTER® Transfection Kit* | MIR2904 | 0.4 ml |
| | MIR2900 | 1 ml |
| | MIR2905 | 5 x 1 ml |
| | MIR2906 | 10 x 1 ml |

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-Jurkat Transfection Reagent | MIR2124 | 0.4 ml |
| | MIR2120 | 1 ml |
| | MIR2125 | 5 x 1 ml |
| | MIR2126 | 10 x 1 ml |
| *Trans*IT®-Keratinocyte Transfection Reagent | MIR2804 | 0.4 ml |
| | MIR2800 | 1 ml |
| | MIR2805 | 5 x 1 ml |
| | MIR2806 | 10 x 1 ml |

### Insect Cell Transfection & Baculovirus Production

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-Insect Transfection Reagent | MIR6104 | 0.4 ml |
| | MIR6100 | 1 ml |
| | MIR6105 | 5 x 1 ml |
| | MIR6106 | 10 x 1 ml |

### siRNA/miRNA

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT-TKO® Transfection Reagent | MIR2154 | 0.4 ml |
| | MIR2150 | 1.5 ml |
| | MIR2155 | 5 x 1.5 ml |
| | MIR2156 | 10 x 1.5 ml |
| *Trans*IT-siQUEST® Transfection Reagent | MIR2114 | 0.4 ml |
| | MIR2110 | 1.5 ml |
| | MIR2115 | 5 x 1.5 ml |
| | MIR2116 | 10 x 1.5 ml |

### Large RNA (Viral and mRNA)

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-mRNA Transfection Kit* | MIR2225 | 0.4 ml |
| | MIR2250 | 1 ml |
| | MIR2255 | 5 x 1 ml |
| | MIR2256 | 10 x 1 ml |

### Large Scale Protein Production in Suspension CHO Cells

| Complete System | Product No. | Quantity |
|---|---|---|
| CHOgro® Expression System | MIR6260 | 10 ml |

| CHOgro® Components | Product No. | Quantity |
|---|---|---|
| CHOgro® Expression Medium | MIR6200 | 1 Liter |
| CHOgro® Liquid Polybag Format | MIR6202 | 10 Liters |
| CHOgro® Dry Powder Format | MIR6201 | Prepares 10L |
| *Trans*IT-PRO® Transfection Reagent | MIR5740 | 1 ml |
| | MIR5750 | 10 ml |
| CHOgro® Complex Formation Solution | MIR6210 | 100 ml |

| CHOgro® Components | Product No. | Quantity |
|---|---|---|
| Poloxamer 188 Solution | MIR6230 | 100 ml |
| L-Glutamine Solution | MIR6240 | 100 ml |

| Accessory, Sold Separately | Product No. | Quantity |
|---|---|---|
| Human IgG1 Expression Control | MIR6250 | 1 µg |

### Large Scale Protein Production in Suspension CHO & HEK293 Cells

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT-PRO® Transfection Kit* | MIR5700 | 1 ml |
| | MIR5760 | 10 ml |

## ELECTROPORATION

| Product | Product No. | Size |
|---|---|---|
| Ingenio® Electroporation Kits (solution, 0.4 cm cuvettes, cell droppers) | | |
| | MIR50113 | 25 RXN |
| | MIR50116 | 50 RXN |
| | MIR50119 | 100 RXN |
| Ingenio® Electroporation Kits (solution, 0.2 cm cuvettes, cell droppers) | | |
| | MIR50112 | 25 RXN |
| | MIR50115 | 50 RXN |
| | MIR50118 | 100 RXN |

| Product | Product No. | Size |
|---|---|---|
| Ingenio® Electroporation Solution | MIR50111 | 25 RXN (6.25 ml) |
| | MIR50114 | 50 RXN (12.5 ml) |
| | MIR50117 | 100 RXN (25 ml) |
| Ingenio® Electroporation Accessories | MIR50120 | 0.2 cm cuvettes (25PK) |
| | MIR50121 | 0.2 cm cuvettes (50PK) |
| | MIR50122 | 0.4 cm cuvettes (25PK) |
| | MIR50123 | 0.4 cm cuvettes (50PK) |
| | MIR50124 | Cell Droppers (25 PK) |
| | MIR50125 | Cell Droppers (50 PK) |

## VIRUS PRODUCTION

| Product | Product No. | Quantity |
|---|---|---|
| *Trans*IT®-Lenti Transfection Reagent | MIR6603 | 0.3 ml |
| | MIR6604 | 0.75 ml |
| | MIR6600 | 1.5 ml |
| | MIR6605 | 5 x 1.5 ml |
| | MIR6606 | 10 x 1.5 ml |

| Product | Product No. | Quantity |
|---|---|---|
| *Transduce*IT™ Transduction Reagent | MIR6620 | 1 ml |

**Trans*IT Transfection Kits supplied with a transfection and booster reagent.



**In the United States:**
For customer service, call 1-800-766-7000
To fax an order, use 1-800-926-1166
To order online: fishersci.com

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