PROGRAMMING THE GENOME:

Any Delivery Method. Any Nucleic Acid. Any Cell Type.

At Mirus Bio, our role in PROGRAMMING THE GENOME is to provide scientists turnkey tools to ADD, DELETE or MODIFY any gene at will. We accomplish this through support of ANY DELIVERY METHOD, capable of delivery of ANY NUCLEIC ACID into ANY CELL TYPE. Whether there is a need for high functional virus titers, or efficient knockdown of target genes, or effective, low toxicity solutions, our delivery systems for molecular and cell biology applications give researchers unprecedented genome control at their fingertips.

The products included in this brochure are our most robust transfection reagents that provide solutions for hard-to-transfect and common cell types, promoting effective workflow in a number of applications. Choosing a reagent that balances efficient nucleic acid delivery and low cellular toxicity is important to achieve consistent experimental outcomes.

Multifunctional Transfection Reagent

High Efficiency, Low Toxicity Transfection Reagents

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-
Call: +1-608-441-2852

*Trans*IT-X2® Dynamic Delivery System

Mirus Bio combines its chemistry and biology expertise to develop cutting edge transfection technologies, like the *Trans*IT-X2® Dynamic Delivery System; an advanced system for delivery of plasmid DNA, siRNA/miRNA, and CRISPR/Cas9 components.

- Broad range cell transfection performance
- Outperforms Lipofectamine® 2000 in 28 of 41 tested cell lines
- Cutting edge delivery of plasmid DNA and/or small RNAs (siRNA, miRNA, CRISPR guide RNA, and CRISPR ribonucleoprotein (RNP) complex)

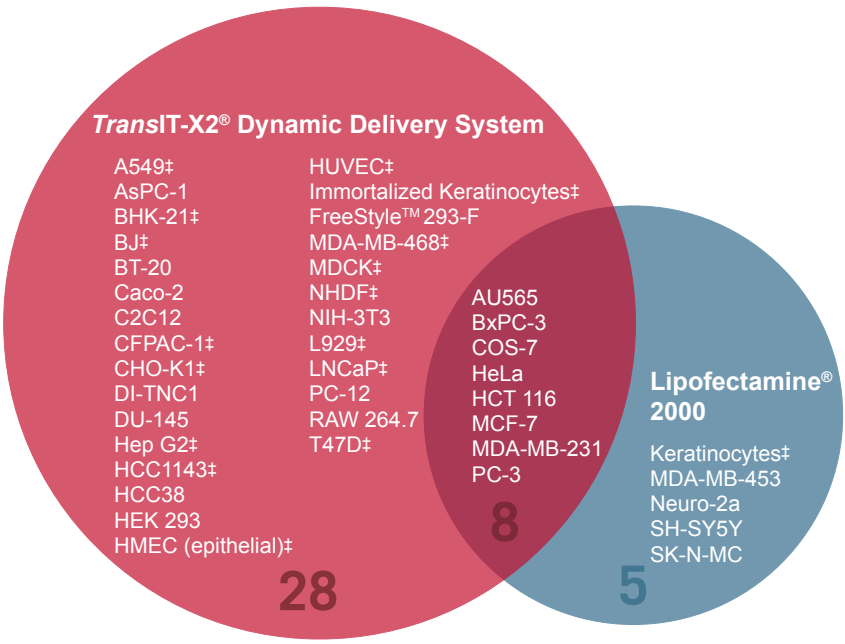
APPLICATION USES:

- CRISPR/Cas9 Genome Editing
- Stem Cell Transfection
- Gene Knockdown
- Co-transfection
- Stable Transfection
- Primary Cells
- Gene Expression

"I was recently tasked with developing a CRISPR protocol for primary and bone-derived cell lines. *Trans*IT-X2® Dynamic Delivery System 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."

Dr. Joshua Chou,
Harvard School of Dental Medicine

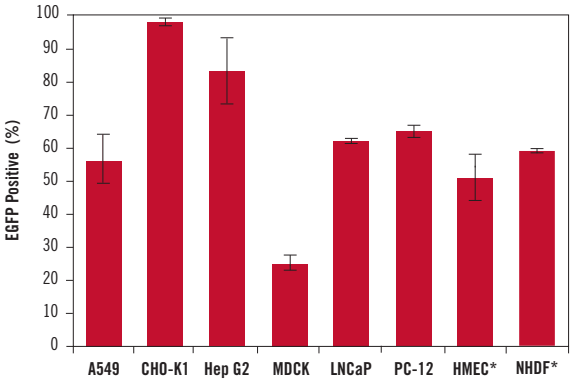
The *Trans*IT-X2® Dynamic Delivery System Enables Superior Gene Expression in a Variety of Cell Types



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

*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.

High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using *Trans*IT-X2® Dynamic Delivery System

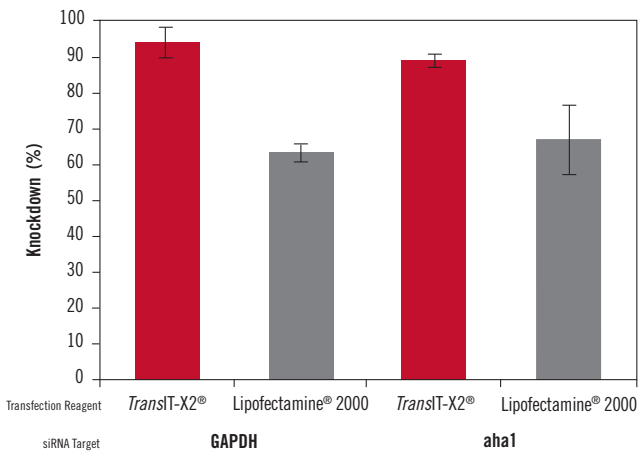


*Trans*IT-X2® (Mirus Bio) was used to transfect plasmid DNA encoding EGFP into the indicated cell lines. Transfections were performed in 96-well plates using 0.2-0.4 µl of TransIT-X2® (Mirus Bio) to deliver 0.1 µg of DNA. Triplicate wells were assayed 48 hours post-transfection.

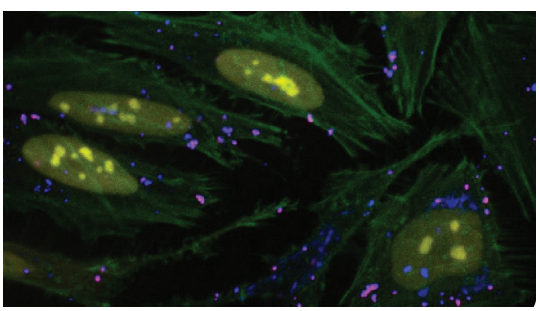
* Indicates primary cell type.

Achieve Higher siRNA Knockdown Than Lipofectamine® 2000

*Trans*IT-X2® (Mirus Bio) and Lipofectamine® 2000 (Thermo Fisher Scientific) were used to transfect siRNA targeting endogenous proteins – GAPDH and AHA1 in 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.

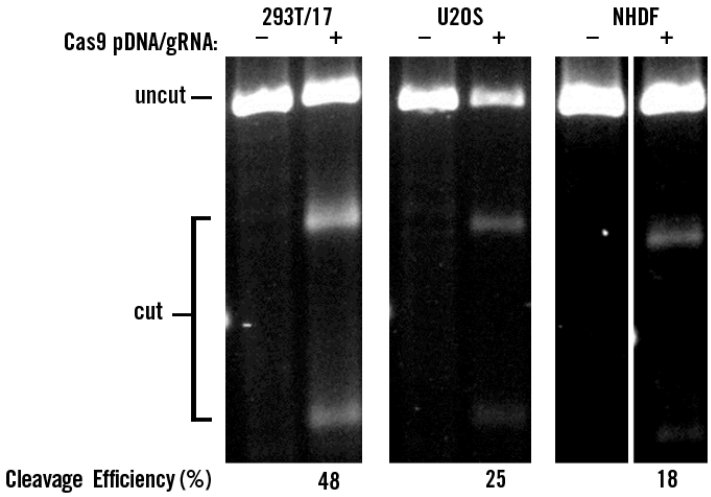


Functional Co-delivery of Plasmid DNA and siRNA



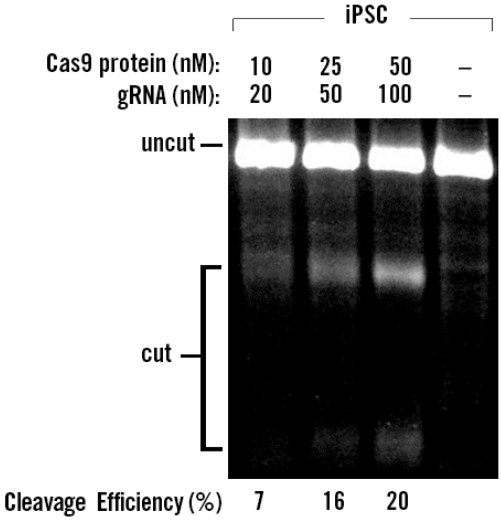
The *Trans*IT-X2® Dynamic Delivery System (Mirus Bio) was used to transfect plasmid Cy®5 labeled DNA encoding nuclear YFP and Cy®3 labeled siRNA into HeLa cells. Transfection was performed in a 6-well plate using 4 µl of *Trans*IT-X2® (Mirus Bio) to deliver 2 µg of DNA (2:1 reagent:DNA ratio) and 25 nM siRNA. Image key: yellow (nuclear YFP), blue (Cy®5 labeled DNA), red (Cy®3 labeled siRNA), green (actin cytoskeleton).

Efficient Genome Editing with Cas9 Plasmid DNA and Guide RNA Oligonucleotides



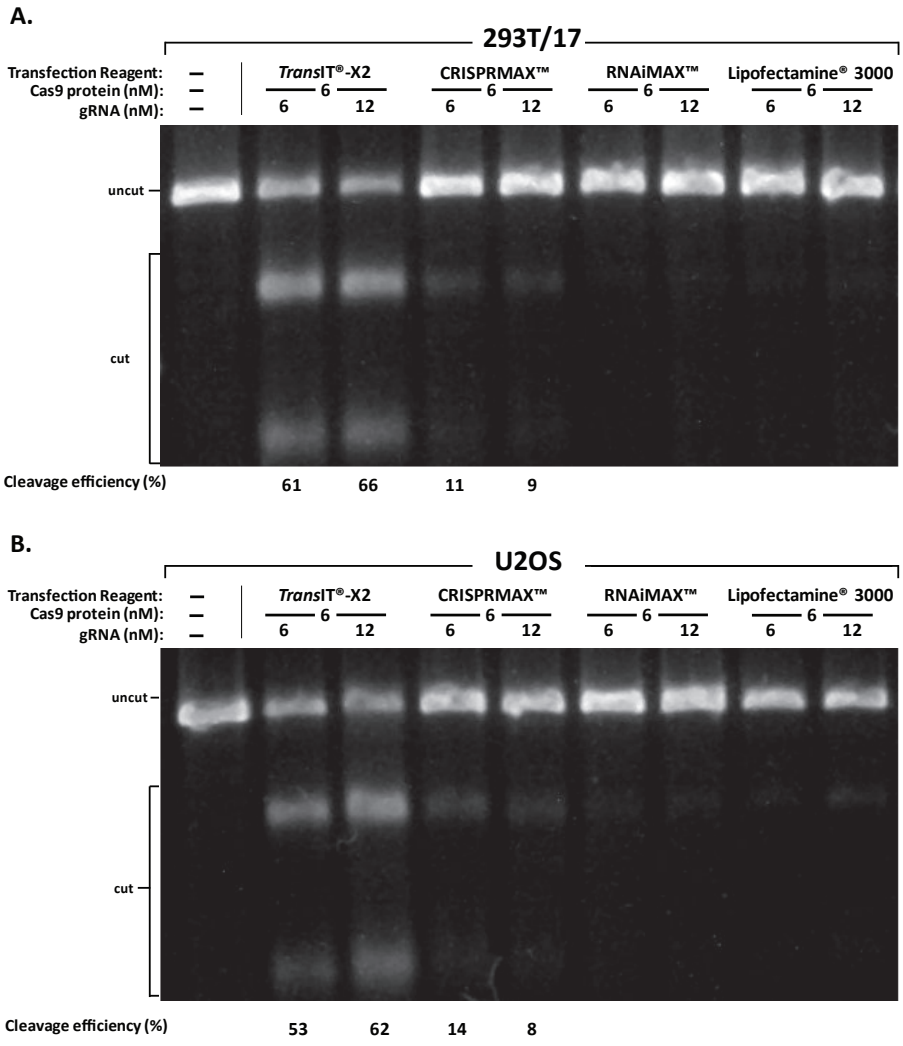
HEK293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding pDNA (MilliporeSigma) and 50nM PPIB targeting two-part gRNA (Dharmacon/GE Healthcare) using The *Trans*IT-X2® Dynamic Delivery System (2 µl/well of a 24-well plate, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.

Genome Editing in IPS Cells with Cas9 RNP Complexes



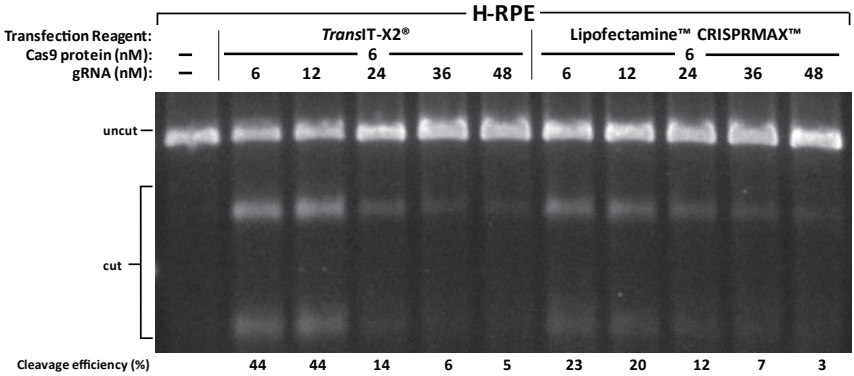
The *Trans*IT-X2® Dynamic Delivery System was used to deliver Cas9 protein/guide RNA ribonucleoprotein (RNP) complexes in human induced pluripotent stem cells (iPSCs). A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection.

*Trans*IT-X2® Outperforms Other Reagents for RNP Delivery

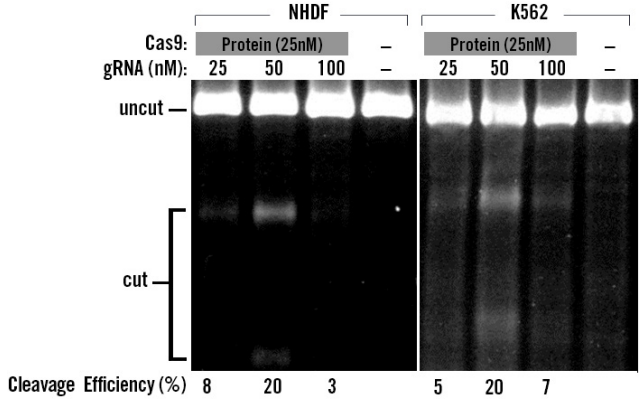


Ribonucleoprotein (RNP) complexes composed of PPIB (cyclophilin B) targeting 2-part gRNA (IDT) and Cas9 protein (PNA Bio) were delivered into (A) HEK293T/17 and (B) U2OS cells using *Trans*IT-X2® Dynamic Delivery System (1 µl/well, Mirus Bio) or Lipofectamine® CRISPRMAX™ (1.5 µl/well and 1 µl/well of Lipofectamine® Cas9 Plus™ Reagent, ThermoFisher) or Lipofectamine® RNAiMAX™ (1.5 µl/well, ThermoFisher) or Lipofectamine® 3000 (1.5 µl/well and 1 µl/well of P3000™ Reagent, ThermoFisher) 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.

*Trans*IT-X2® Provides Efficient Genome Editing in Multiple Cell Types



Ribonucleoprotein (RNP) complexes composed of PPIB targeting 2-part gRNA (IDT) and Cas9 protein (PNA Bio) were delivered into primary H-RPE 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, ThermoFisher) in a 24-well format according to the manufacturers' protocol. Varying levels of gRNA (6 nM – 60 nM) were tested with 6 nM Cas9 protein. A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.



The RNP complex of PPIB targeting 2-part gRNA (Dharmacon) and Cas9 protein (PNA Bio) was delivered into NHDF and K562 cells using *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.

*Trans*IT®-LT1 Transfection Reagent

Designated "LT" for its low toxicity, *Trans*IT®-LT1 has been the preferred reagent for researchers seeking a gentle and reliable solution for more than 20 years.

- High efficiency, low toxicity transfections
- Utilize one reagent and protocol for a wide range of cell lines
- Deliver single or multiple plasmids

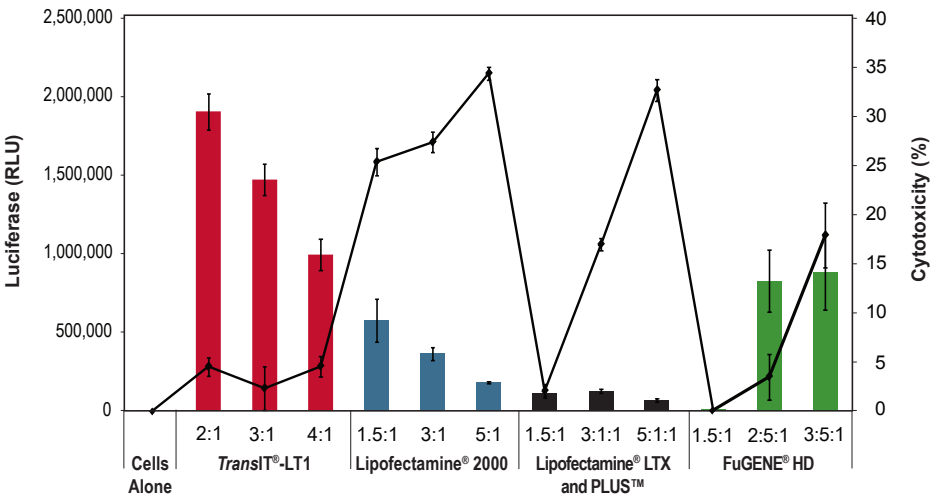
APPLICATION USES:

- Stem Cell Transfection
- Virus Production
- Gene Expression
- Low Toxicity
- Stable Transfection

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.

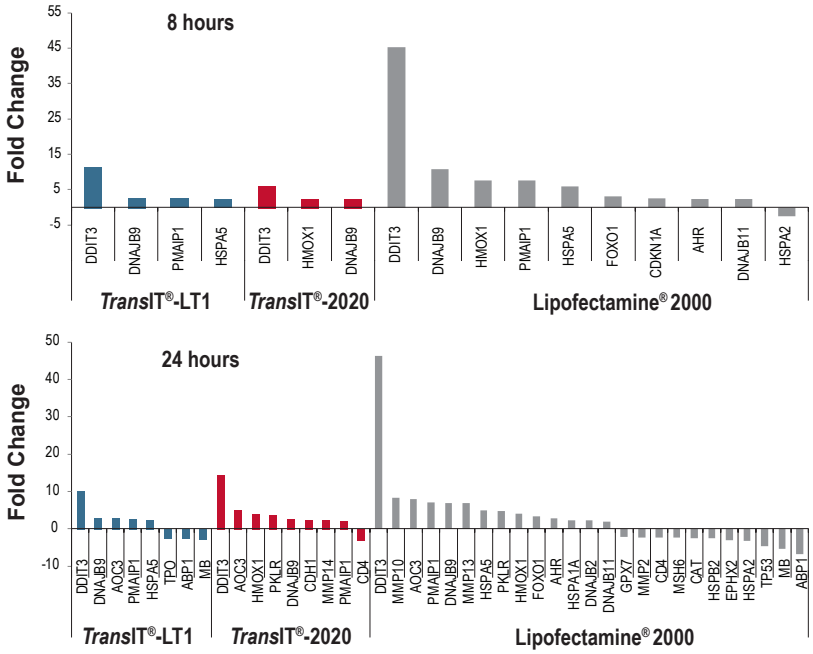
Fedir Kiskin,
University of Cambridge

*Trans*IT®-LT1 Exhibits Higher Expression and Lower Cellular Toxicity Compared to Other Transfection Reagents



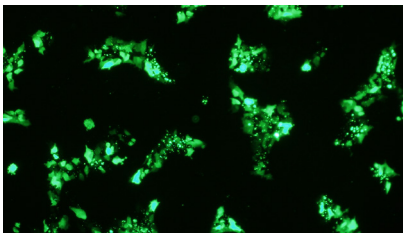
HepG2 cells were transfected with a luciferase expression plasmid using the designated reagents at the manufacturer's recommended reagent-to-DNA ratio indicated beneath each bar. Transfections were performed in 96-well plates using 0.1 µg of plasmid DNA per well. Luciferase expression (bar graph) and lactate dehydrogenase (LDH) levels (line graph) were measured at 24 hours post-transfection. FuGENE® is a registered trademark of Fugent LLC.

*Trans*IT® Transfection Reagents Minimize the Stress Response in Transfected HeLa Cells



Stress-related gene expression changes were determined by RT-qPCR from total RNA samples harvested from HeLa cells that were transfected with *Trans*IT®-LT1 (Mirus Bio), *Trans*IT®-2020 (Mirus Bio) or Lipofectamine® 2000 (Thermo Fisher Scientific) at 8 and 24 hours. Eighty-four genes were analyzed using the Human Stress Response 96 StellARray™ (Lonza Group, Ltd.).

Exceptional Transfection Efficiency in Human Induced Pluripotent Stem Cells (iPSCs) via Reverse Transfection With *Trans*IT®-LT1



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). Reverse transfections were performed in 6-well plates using 12 µl of *Trans*IT®-LT1 (Mirus Bio) to deliver 4 µg of DNA (3:1, reagent: DNA). Cells were visualized 48 hours post-transfection.

Data courtesy of: Cellular Dynamics international, a FUJIFILM company

*Trans*IT®-2020 Transfection Reagent

A unique DNA transfection reagent that enables high expression in many cell types, including a subset that are typically resistant to chemical transfection.

- Achieve high expression in difficult to transfect cells, including HUVEC, THP-1, and MEF
- Balance high efficiency nucleic acid delivery and low cellular toxicity
- Animal origin free formulation

APPLICATION USES:

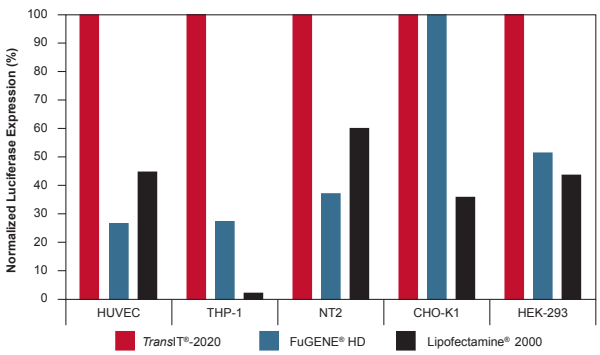
- Stem Cell Transfection
- Stable Transfection
- Low Toxicity
- Gene Expression
- Primary Cells

...We have been able to transfect BMDM's with the *Trans*IT®-2020 Transfection Reagent. Not only are my cells healthier, but I get good expression, and it is more cost effective to the Neon® Transfection System that my lab traditionally uses. I have also been able to share these results with others that are now trying it with great success. It makes me look like a hero!

Jeffrey Fay,
Hoffman Lab, University of Hawaii at Manoa

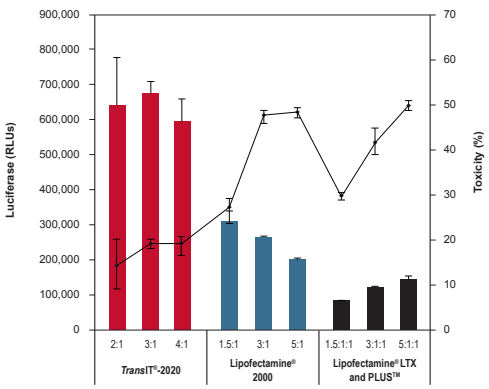
Superior Gene Expression

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 (Fugent LLC, 3.5:1), Lipofectamine® 2000 (Thermo Fisher Scientific, 1.5:1, 3:1 or 5:1). Values were normalized to *Trans*IT®-2020 (Mirus Bio) and presented as a percentage of luciferase expression. FuGENE® is a registered trademark of Fugent LLC.



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 indicated beneath each bar. Transfections were performed in 96-well plates using 0.1 µg of plasmid DNA per well. Luciferase expression (bar graph) and lactate dehydrogenase (LDH) levels (line graph) were measured at 24 hours post-transfection.



*Trans*IT®-mRNA Transfection Reagent

A high efficiency, low toxicity transfection reagent for large RNA and CRISPR guide RNA delivery.

- Ideal for specialized applications, such as virus production, protein expression and CRISPR/Cas9 genome editing
- Achieve RNA delivery in a large population of cells to ensure experimental success
- Perform transfections in the presence of serum, which eliminates the need for a media change

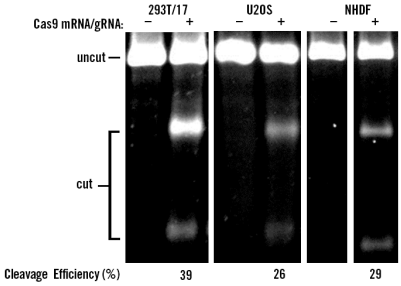
APPLICATION USES:

- CRISPR/Cas9 Genome Editing
- Stem Cell Transfection
- Hard-to-transfect
- Low Toxicity
- Large RNA Delivery

Our lab recently used the *Trans*IT®-mRNA Transfection Kit to show that intracellular delivery of HPLC-purified and pseudouridine-containing mRNA can translate very efficiently without immune activation which is ideal for mRNA-based gene therapy applications. *Trans*IT®-mRNA further facilitated this work through low toxicity transfections of HEK 293T, human dendritic cells (DCs) and primary keratinocytes.

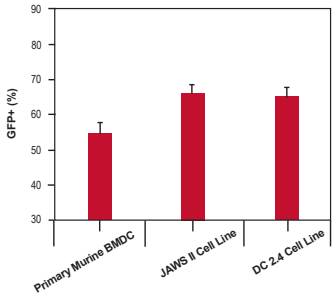
Dr. Katalin Karikó, Department of Neurology, University of Pennsylvania

Efficient Genome Editing With Cas9 mRNA + gRNA Oligonucleotides



Indicated cell types were co-transfected with 0.5 µg of Cas9 encoding mRNA, 5meC, ψ (Trilink Biotechnologies) and 25nM of PPIB targeting 2-part gRNA (Dharmacon) 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.

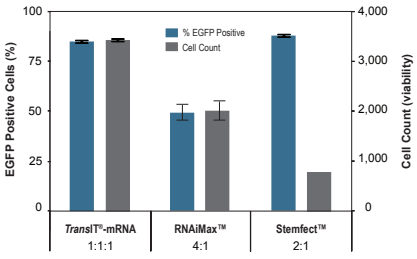
Multiple Dentritic Cell Types Express GFP From mRNA Transfection



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: Boost: mRNA ratio of 1:1:1 (µl:µl:µg) (Mirus Bio).

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

High Efficiency, Low Toxicity After 14 Consecutive Transfections



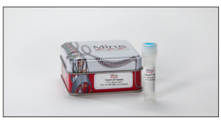
Repeated daily transfections were performed in the same population of BJ fibroblasts using *Trans*IT®-mRNA Transfection Kit (Mirus Bio), Lipofectamine® RNAiMAX (Thermo Fisher Scientific) and Stemfect™ RNA Transfection Kit (Stemgent).

Mirus

	*Trans*IT-X2®*	*Trans*IT®-LT1	*Trans*IT®-2020*	*Trans*IT®-mRNA*
Plasmid DNA	●●●	●●○	●●○	○○○
siRNA	●●●	○○○	○○○	○○○
Cas9 RNP	●●●	○○○	○○○	○○○
CRISPR gRNA	●●●	○○○	○○○	●●○
Low Cellular Toxicity	●●○	●●●	●●○	●●●
DNA Virus Production	●○○	●●●	●●○	○○○
mRNA, Viral RNA	○○○	○○○	○○○	●●●

*Animal origin free formulation.

*Trans*IT® Transfection Reagents

PRODUCT	DESCRIPTION	PRODUCT NO.	QUANTITY
*Trans*IT-X2® Dynamic Delivery System 	The premier reagent from Mirus Bio: *Trans*IT-X2® is an advanced system for delivery of plasmid DNA, siRNA/miRNA and CRISPR/Cas9 components to mammalian cells.	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
*Trans*IT®-LT1 Transfection Reagent 	A very low toxicity DNA transfection reagent for a broad range of mammalian cells.	MIR 2304	0.4 ml
		MIR 2300	1 ml
		MIR 2305	5 x 1 ml
		MIR 2306	10 x 1 ml
*Trans*IT®-2020 Transfection Reagent 	A high performance, animal-origin-free, transfection reagent developed expecially for hard-to-transfect cells.	MIR 5404	0.4 ml
		MIR 5400	1 ml
		MIR 5405	5 x 1 ml
		MIR 5406	10 x 1 ml
*Trans*IT®-mRNA Transfection Kit 	A high efficiency, low toxicity, transfection reagent for large RNA and CRISPR guide RNA	MIR 2225	0.4 ml
		MIR 2250	1 ml
		MIR 2255	5 x 1 ml
		MIR 2256	10 x 1 ml

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

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