

The Transfection Experts



# THE TRANSFECTION EXPERTS

From basic research to product development, Mirus Bio scientists utilize their expertise in nucleic acid chemistry, cellular and molecular biology to establish innovative technologies and products for use in life science research. Over a decade ago, our first products reflected advancements achieved in our non-viral gene therapy research. Since then, our scientists have made significant contributions providing new and better tools for a variety of research applications in the life sciences.

Our technologies and expertise precipitated the acquisition of Mirus Bio Corporation's therapeutic division by Hoffman-La Roche in 2008. However, Mirus Bio's biological research products division remains as a stand-alone entity, called Mirus Bio LLC.

### At Mirus Bio LLC, we will continue to grow our expertise in nucleic acid delivery research and products to better serve you.



# WHAT IS TRANSFECTION?

Transfection is the introduction of any nucleic acid molecule by non-viral means into cultured mammalian cells. It is a common and convenient technology that aids in the study of gene function. Transfection methods can be divided into physical or direct transfer methods such as electroporation or chemical mediated transfection.

### Optimize Transfection Performance For All Reagents, Cells and Nucleic Acids

### 1. Ratio of transfection reagent to nucleic acid

In parallel for each cell type, transfect different amounts of nucleic acid with various amounts of transfection reagent. Titrate the amount of reagent while maintaining constant DNA concentration. Additionally, DNA concentration can be scaled.

# Transfection complex formation time Test different transfection complex formation times (reagent + nucleic acid incubation time) for each reagent and cell type being transfected.

- Cell density (confluency) at time of transfection Transfect each cell type at various densities ranging from 50% to 90% and monitor transfection performance.
- 4. Post-transfection incubation time (time after addition of transfection complexes to cells)

Harvest cells at different times post-transfection to determine the optimal assay time that meets your experimental goals.

For specific recommendations, please visit www.mirusbio.com or contact our transfection experts at techsupport@mirusbio.com.



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### $\triangleright$ LARGE RNA (VIRAL RNA and mRNA)

# **OUR PROMISE TO YOU: SERVICE THROUGH EXPERTISE**

### Order Online 24 Hours a Day (Credit Cards or Purchase Orders) www.mirusbio.com

### **Customer Service**

Hours of Operation: 8:00 AM-4:00 PM Central Time, Monday-Friday email: sales@mirusbio.com phone: 888.530.0801 (toll free within the U.S.) or +1.608.441.2852 fax: +1.608.441.2849

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Mirus Bio LLC Attention: Customer Service 545 Science Drive Madison, WI 53711 USA

**U.S. Distribution** Fisher Scientific



### International Distribution Mirus Bio products are available worldwide through our international distributors. For a complete list, please visit:

www.mirusbio.com/contact/distributors.aspx and contact the distributor in your region.

### **Broad Spectrum**

# **TransIT®-2020 TRANSFECTION REAGENT**

- Superior Performance—TransIT-2020 outperforms FuGENE<sup>®</sup> HD, Lipofectamine<sup>™</sup> 2000, and Lipofectamine<sup>™</sup> 2000 CD
- Broad Spectrum DNA Delivery—Achieve high expression in many cell types, including hard-to-transfect cells and insect cells
- Animal Origin Free—TransIT-2020 provides high performance with maximum compatibility

### Description

*Trans*IT-2020 Reagent is a new and versatile transfection solution for broad spectrum DNA delivery into mammalian cells. This premium, broad spectrum reagent is animal component free allowing maximum compatibility for all downstream applications while outperforming major competitors in many cell types.

### **PRODUCT SPECIFICATIONS**

PRODUCT NO.	QUANTITY
MIR 5400	1.0 ml
MIR 5404	0.4 ml
MIR 5405	5 × 1.0 ml
MIR 5406	$10 \times 1.0$ ml

To inquire about bulk pricing, please call 888.530.0801.



**Transfection Condition** 

**FIGURE 1.** The *Trans*IT<sup>®</sup>-2020 Reagent Effectively Transfects *Drosophila* S2 Cells. Cells were transfected with a plasmid construct expressing a secreted form of the Dscam extracellular domain fused to alkaline phosphatase (AP) in a 24-well plate. The ratio of transfection reagent to DNA and micrograms of DNA per well is noted beneath each bar. All products were used according to manufacturers' protocol. All transfections were performed in serum-free media for four hours followed by complete media supplementation. An AP enzymatic assay was used to measure the AP levels 24 hours post-transfection.

Data courtesy of Woj Wojtowicz, University of California, Berkeley



# **DNA Transfection**





FIGURE 2. Superior Gene Expression in a Broad Spectrum of Cell Types Using TransIT-2020®

Transfection Reagent. Luciferase gene expression was compared in HEK 293, K562, CHO-K1, and primary MEF cells transfected with a luciferase expression plasmid using *Trans*IT®-2020 (Mirus Bio, 3:1 reagent to DNA ratio), FuGENE HD (Roche, 7:2 reagent to DNA ratio), Lipofectamine<sup>™</sup> 2000 (Life Technologies Corporation, 5:2 reagent to DNA ratio), and Lipofectamine<sup>™</sup> 2000 CD (Invitrogen, 5:2 reagent to DNA ratio). Transfections were performed in 24-well plates using 0.5 µg of plasmid DNA per well. The optimal level of each transfection reagent was determined empirically and all reagents were used according to manufacturer's protocol. Cells were harvested at 24 hours post-transfection and assayed for luciferase activity. Error bars represent the standard deviation of triplicate wells.

### **Broad Spectrum**

# TransIT®-LT1 TRANSFECTION REAGENT

- Deliver Single or Multiple Plasmids—Suitable for many applications such as siRNA/shRNA expression, gene expression, viral production, and promoter analysis
- High Efficiency Delivery—Achieve expression in a large population of cells to ensure experimental success
- Low Cellular Toxicity—Maintain cell density and reduce experimental biases due to toxicity induced cellular changes
- ▷ Save Time—No media changes or extensive optimization required
- Save Money—Utilize one transfection reagent and protocol for a variety of cells

### Description

*Trans*IT<sup>®</sup>-LT1 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 histone and cationic lipids that efficiently transfects cells in the presence or absence of serum.

**PRODUCT SPECIFICATIONS** 

PRODUCT NO.	QUANTITY
MIR 2304	0.4 ml
MIR 2300	1.0 ml
MIR 2305	$5 \times 1.0$ ml
MIR 2306	$10 \times 1.0$ ml

To inquire about bulk pricing, please call 888.530.0801.

FIGURE 3. High Efficiency Transfection on a Broad Range of Cell Lines Using *Trans*IT<sup>®</sup>-LT1 Reagent. Various cell lines were transfected with an EGFP expression vector using *Trans*IT<sup>®</sup>-LT1 Transfection Reagent in complete growth media with no media changes and analyzed by flow cytometry.



# TransIT®-LT1: High Efficiency, Low Toxicity Broad Spectrum Reagent

- Physiologically relevant data you can trust—Due to the exceptionally low cellular toxicity of *Trans*IT-LT1 Reagent
- ▷ Higher transfection efficiency than Lipofectamine<sup>™</sup> 2000—At least twice the expression level regardless of cell density



**FIGURE 4.** High Cell Viability with *TransIT®*-LT1 Reagent, as Close to Nontransfected as Possible. The impact of the *TransIT®*-LT1 and Lipofectamine<sup>TM</sup> 2000 (LF2K) transfection reagents on cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). This assay measures the levels of ATP in a cell population. HeLa cells at 50% and 90% confluency were transfected in duplicate using 3 µl of *TransIT®*-LT1 Reagent or 4 µl Lipofectamine<sup>TM</sup> 2000 (LF2K) per well of a 12-well plate. Twenty-four hours posttransfection the cells were harvested and cell viability assayed. Data from three independent experiments performed on different days were averaged then scaled to untransfected HeLa controls (cells alone) and are represented as a percentage of the untransfected control.

# **Cell Line Specific**

# **TransIT® CELL LINE SPECIFIC TRANSFECTION REAGENTS**

The *Trans*IT<sup>®</sup> Cell Line Specific DNA Transfection Reagents are formulated to maximize transfection efficiency while maintaining cellular health in many popular or hard-to-transfect cell lines types. **All of these reagents offer:** 

- Unique Formulations—Designed and optimized for each cell line or cell type to maximize transfection performance
- ▷ **Maximum Delivery Efficiency**—Enhanced transfection efficiency to ensure experimental success and improve experimental outcome
- ▷ Low Cellular Toxicity—Maintain cell density and reduce experimental biases due to toxicity induced cellular changes
- Delivery of Single or Multiple Plasmids—Suitable for many applications such as gene expression, siRNA/ shRNA cassette expression, viral production and promoter analysis
- Serum Compatible—No media changes necessary or extensive optimization required, saving valuable research time

Product Name*	Applicable Cell Line(s) or Cell Type(s)	Efficiency**	Product No.	Quantity
TransIT®-293 Tran	sfection Reagent			
			MIR 2704	0.4 ml
and the second	LIEK 202 LIEK 202T and valated	75 050/	MIR 2700	1.0 ml
	HER 293, HER 2931, and related	/0-00%	MIR 2705	$5 \times 1.0$ ml
A SHALL SHE			MIR 2706	$10 \times 1.0 \text{ ml}$
TransIT®-3T3 Tran	sfection Kit ( <i>Trans</i> IT <sup>®</sup> -3T3 Reagent and 3	T3 Authority Reagent)		
HE CONTRACTOR			MIR 2184	0.4 ml
N. N. B. M. A.	NIH 3T3, 3T3-L1 and related	55–65%	MIR 2180	1.0 ml
A THE REAL			MIR 2185	$5 \times 1.0$ ml
			MIR 2186	$10 \times 1.0 \text{ ml}$
TransIT®-CHO Tran	nsfection Kit (TransIT <sup>®</sup> -CHO Reagent and (	CHO Mojo Reagent)		
	CHO-K1 and related	50–60%	MIR 2174	0.4 ml
			MIR 2170	1.0 ml
			MIR 2175	$5 \times 1.0$ ml
			MIR 2176	$10 \times 1.0 \text{ ml}$
TransIT®-COS Tran	sfection Kit (TransIT <sup>®</sup> -COS Reagent and C	OS Boss Reagent)		
		90–95%	MIR 2194	0.4 ml
and the second	COS 1 COS 7 and related		MIR 2190	1.0 ml
and states	COS-1, COS-7 and related		MIR 2195	$5 \times 1.0$ ml
			MIR 2196	$10 \times 1.0$ ml

Product Name*	Applicable Cell Line(s) or Cell Type(s)	Efficiency**	Product No.	Quantity
TransIT-HeLaMONS	STER <sup>®</sup> Transfection Kit ( <i>Trans</i> IT <sup>®</sup> -HeLa Ro	eagent and MONSTER Reagent)		
			MIR 2904	0.4 ml
	Liel e and related		MIR 2900	1.0 ml
a la dana		30-60%	MIR 2905	5 × 1.0 ml
2020 AND ANY NOT LODGE			MIR 2906	$10 \times 1.0 \text{ ml}$
TransIT®-Jurkat Tra	nsfection Reagent			
			MIR 2124	0.4 ml
	Jurkat, Jurkat-E6, RAW 264.7,	10.25%	MIR 2120	1.0 ml
	cell lines	10-2378	MIR 2125	$5 \times 1.0$ ml
			MIR 2126	$10 \times 1.0 \text{ ml}$
TransIT®-Keratinoc	yte Transfection Reagent			
	NIKS (Near Diploid Immortalized Keratinocytes) and primary keratinocytes	20–30%	MIR 2804	0.4 ml
			MIR 2800	1.0 ml
			MIR 2805	$5 \times 1.0$ ml
			MIR 2806	$10 \times 1.0 \text{ ml}$
TransIT-Neural® Tra	ansfection Reagent			
11-10-10-10-10-10-10-10-10-10-10-10-10-1			MIR 2144	0.4 ml
	C6, Daoy, DB-TRG-05MG, DI- TNC1, HCN-1A, Neuro-2a, PC-12, SK-N-MC, SVGp12	≥75%	MIR 2140	1.0 ml
			MIR 2145	$5 \times 1.0$ ml
2010/2010/2010/04			MIR 2146	$10 \times 1.0 \text{ ml}$
TransIT®-Prostate 1	Transfection Kit (TransIT®-Prostate Reage	nt and Prostate Boost Reagent)		
		>50%	MIR 2134	0.4 ml
	DU 145 INCAP PC3		MIR 2130	1.0 ml
der the	DO 143, LINOAI, 1 03		MIR 2135	$5 \times 1.0 \text{ ml}$
			MIR 2136	$10 \times 1.0 \text{ ml}$

\* 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 quantification of the percentage of cells expressing EGFP.

### Electroporation

# **INGENIO™ ELECTROPORATION PRODUCTS**

- High Efficiency Electroporation of Hard-to-Transfect Cells— Conduct research in biologically relevant cells
- Compatible with All Electroporation Instruments—Use your existing system including an amaxa Nucleofector<sup>®</sup> or Bio-Rad Gene Pulser; no need to purchase additional specialized equipment
- Save Money—Replace your amaxa Nucleofector<sup>®</sup> Kits with the Ingenio<sup>™</sup> Electroporation Kit and realize significant savings without sacrificing performance
- Buy Only What You Need—Ingenio Electroporation Solution is available alone or as part of a complete kit with cuvettes and cell droppers
- Higher Cell Viability—Less harmful than other electroporation methods

### Description

The Ingenio<sup>™</sup> Electroporation Solution facilitates efficient and reliable delivery of nucleic acids to eukaryotic cells traditionally resistant to chemical transfection. Ingenio<sup>™</sup> is a broad spectrum solution that supports high efficiency electroporation with minimal toxicity. It replaces standard electroporation solutions including phosphate buffered saline and serum-free media. Ingenio<sup>™</sup> is compatible with multiple instruments and facilitates a wide range of applications requiring nucleic acid delivery to cells. The Ingenio Solution is available alone and as part of a kit with cuvettes and cell droppers.

### **PRODUCT SPECIFICATIONS**

Ingenio™	Electroporation	Kits
solution 0	4 cm cuvettes, c	ell dronnei

(solution, 0.4 cm cuvettes,	cell droppers)
PRODUCT NO.	SIZE
MIR 50113	25 RXNª
MIR 50116	50 RXNª
MIR 50119	100 RXN <sup>a</sup>

### Ingenio<sup>™</sup> Electroporation Kits

(solution, 0.2 cm cuvettes, cell droppers)		
SIZE		
25 RXNª		
50 RXNª		
100 RXNª		

### Ingenio<sup>™</sup> Electroporation Solution

PRODUCT NO.	SIZE	QUANTITY
MIR 50111	25 RXN⁵	6.25 ml
MIR 50114	50 RXN⁵	12.5 ml
MIR 50117	100 RXN <sup>ь</sup>	25 ml

### Ingenio<sup>™</sup> Electroporation Accessories

PRODUCT NO.	DESCRIPTION	SIZE
MIR 50120	0.2 cm Cuvettes	25 PK
MIR 50121	0.2 cm Cuvettes	50 PK
MIR 50122	0.4 cm Cuvettes	25 PK
MIR 50123	0.4 cm Cuvettes	50 PK
MIR 50124	Cell Droppers	25 PK
MIR 50125	Cell Droppers	50 PK

Electroporations per kit.

<sup>b</sup> Number of electroporations in 0.4 cm cuvette.

in 0.4 cm cuvette.





amaxa Nucleofector<sup>®</sup> Solution V on amaxa Nucleofector<sup>®</sup>

FIGURE 5. Ingenio<sup>™</sup> Solution Provides Comparable Efficiency on amaxa's Nucleofector<sup>®</sup> Device. Cells were electroporated in parallel with an EGFP reporter vector. Two electroporators were used with different electroporation kits: the Ingenio<sup>™</sup> Electroporation Kit was used in the Gene Pulser Xcell™ Eukaryotic System (Bio-Rad) and the amaxa Nucleofector<sup>™</sup> II Device (Lonza); the amaxa Nucleofector Kit V was used in the amaxa Nucleofector<sup>™</sup> II Device, all according to manufacturer's 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 6. Ingenio<sup>™</sup> Electroporation Kits were used to transfect representative cell types using amaxa Nucleofector<sup>®</sup> Device. Cells were assayed at 24 hours post-electroporation by flow cytometry and reported as percentage of live cell population. Visit www. TheTransfectionExperts.com for ideal pulse conditions.



## **Protein Production**

# **TransIT-PRO™ TRANSFECTION KIT**

- High Performance—A cost-effective alternative for protein production
- ▷ Easy to Use—Compatible with multiple CHO media formulations
- ▷ **Reliable Scale-up**—Linear from 4 to 400 milliliters

### Description

Decrease time to produce usable protein by maximizing target protein yields through transient transfection. The *Trans*IT-PRO<sup>™</sup> 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. The *Trans*IT-PRO<sup>™</sup> outperforms linear PEI in protein yield, while providing a cost-effective alternative to FreeStyle<sup>™</sup> MAX.

### **PRODUCT SPECIFICATIONS**

### TransIT-PRO<sup>™</sup> Transfection Kit

PRODUCT NO.	SIZE
MIR 5700	1 ml
MIR 5760	10 ml

To inquire about bulk pricing, please call 888.530.0801.



# High Performance: A Cost-effective Alternative for Protein Production



**FIGURE 7.** Achieve High Antibody Titers Using the *Trans*IT-PRO<sup>TM</sup> Transfection Kit. Human IgG1 was produced by transient transfection using *Trans*IT-PRO and PRO Boost Reagent (1:1:1), 25kDa linear PEI (6:1) or FreeStyle<sup>TM</sup> MAX (1:1) 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  $0.5 \times 10^6$  cells/ml at the time of transfection. FreeStyle<sup>TM</sup> CHO-S cells were cultured in 20ml of FreeStyle<sup>TM</sup> CHO Expression medium 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 was included for quantification estimate (S1= 1.6 mg/L, S2= 3.2 mg/L, S3 = 6.3 mg/L).



The Transfection Experts

# Easy to Use: Compatible with Multiple Media Formulations

BD Select™ CD1000 Medium

FreeStyle™ CHO Expression Medium

BD Select™ CD Medium

PowerCHO®2 CD Medium

ProCHO™5 Medium

0.0E + 00 0 TransIT-PRO™ + 25kDa linear PEI FreeStyle™ MAX TransIT-PRO™ + FreeStyle™ MAX 25kDa linear PEI PRO Boost Reagent **PRO Boost Reagent** FIGURE 8. TransIT-PRO<sup>™</sup> Provides High Performance Across Varied Media Formulations. FreeStyle™ CHO-S cells were adapted to five representative growth media including: BD Select™ CD1000 medium (Becton, Dickinson and Company, Franklin Lakes, NJ), BD Select<sup>™</sup> CHO medium (Becton, Dickinson and Company, Franklin Lakes, NJ), FreeStyle<sup>™</sup> CHO Expression medium (Life Technologies Corporation, Carlsbad, CA), ProCHO™5 medium (Lonza Inc., Allendale, NJ) and PowerCHO®2 CD medium (Lonza Inc., Allendale, NJ). Cells were transfected with a plasmid using the TransIT-PRO and PRO Boost Reagent (1:1:1), 25kDa linear PEI (4:1) (Polysciences, Warrington, PA), or FreeStyle™ Max (1:1) (Life Technologies Corporation, Carlsbad, CA) transfection reagents according to the manufacturers or 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<sup>6</sup> cells/ml at the time of transfection, 2 ml total culture volume. (A) Luciferase expression was assessed 48 hours post-transfection using a conventional luciferase assay. Error bars represent the standard deviation of duplicate wells. (B) 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.

1600

1400

1200

1000

800 600

400 200

Human Fc (µg/L)



### Scale-up: No Problem

2.0E + 08

1.5E + 08

1.0E + 08

0.5E + 0.8

RLU (luciferase)

FIGURE 9. Scaling of Transient Transfection *Trans*IT-PRO™ Using Transfection Kit is Linear From 4 to 400 Milliliters. Human IgG1 was produced by transient transfection using the TransIT-PRO<sup>™</sup> Transfection Kit and a plasmid encoding a human IgG1 construct. A DNA concentration was 1 µg/ml of culture and a ratio of TransIT-PRO:PRO Boost Reagent:DNA ratio of 1:0.5:1. Cells were plated at a density of 0.5 x 10<sup>6</sup> cells/ml at the time of transfection. CHO-S cells were cultured in BD Select<sup>™</sup> CD1000 media using 4 ml per well of a 24-well deep well shake block, 40 ml in 125 ml Erlenmeyer shake flask, 40 ml in 125 ml 2 sidearm spinner flask and 400 ml in 500 ml 2 sidearm spinner flask. Day 3, 5 and 7 supernatants were clarified and analyzed by an anti-Fc sandwich ELISA. Error bars represent the standard deviation of triplicate technical replicates.

# **Plasmid Controls**

# Label IT® PLASMID DELIVERY CONTROLS

- Sensitive—Easily visualize transfected cells and assess delivery efficiency using fluorescent microscopy
- Compatible—Suitable for co-delivery experiments with functional plasmids
- ▷ Easy to Use—Supplied as a ready to use 0.5 mg/ml solution for both *in vitro* and *in vivo* tracking studies

### Description

The *Label*  $IT^{\circledast}$  Plasmid Delivery Controls consist of either Cy<sup>TM</sup>3 or fluorescein labeled 2.7 kb plasmid for assessment of delivery efficiency in mammalian cells.

# 062

FIGURE 10. The Label IT<sup>®</sup> Cy™3 Plasmid Delivery Control Allows Quick Assessment Delivery of Efficiency. HeLa cells were transfected in complete media with the Label IT<sup>®</sup> Cy<sup>™</sup>3 Plasmid Delivery Control (red) using the TransIT®-LT1 Transfection Reagent. Twenty-four hours post-transfection, the cells were fixed, then counterstained to locate the nuclei (blue) and actin (green).



FIGURE 11. Label IT® Сү™З Plasmid Delivery Control Quick Assessment Allows of Electroporation Efficiency. CHO-K1 cells were electroporated in Ingenio Electroporation Solution with Label IT® Cy™3 Plasmid Delivery Control (red). Twenty-four hours post-electroporation, cells were washed, fixed and counterstained to identify the nuclei (blue) and actin (green). The image was acquired using a confocal microscope.

### **PRODUCT SPECIFICATIONS**

### Label IT® Plasmid Delivery Controls

LABEL	PRODUCT NO.	QUANTITY
Су™З	MIR 7904	10 µg
	MIR 7905	100 µg
Fluorescein	MIR 7906	10 µg
	MIR 7907	100 µg



### siRNA

# TransIT-TKO® & TransIT-siQUEST® TRANSFECTION REAGENTS

- Broad Spectrum siRNA and Duplex miRNA Delivery—Utilize each transfection reagent and protocol for a variety of cells
- Two Different Reagent Formulations—Two choices when identifying the best transfection reagent to maximize gene knockdown in a given cell line
- High Knockdown Efficiency—Achieve optimal gene silencing in a large percentage of cells to ensure experimental success
- Low Cellular Toxicity—Maintain cell density and reduce experimental biases due to alterations in cellular health
- Flexible Protocol—use with either standard or reverse transfections

### Description

*Trans*IT-TKO<sup>®</sup> and *Trans*IT-siQUEST<sup>®</sup> small RNA (siRNA and duplex 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 siRNA and duplex



**FIGURE 12.** Highly Efficient Knockdown of Transiently Expressed Firefly Luciferase. Firefly and sea pansy luciferase reporter vectors were cotransfected into various cell lines. Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either the *Trans*IT-siQUEST<sup>®</sup> (red) or *Trans*IT-TKO<sup>®</sup> (gray) Transfection Reagent. Bars indicate the percent of normalized firefly luciferase expression as compared to the reagent alone control 24 hours post-transfection. miRNA transfection reagent for their particular cell line. **PRODUCT SPECIFICATIONS** 

### TransIT-siQUEST<sup>®</sup> Transfection Reagent

PRODUCT NO.	QUANTITY
MIR 2114	0.4 ml
MIR 2110	1.0 ml
MIR 2115	$5 \times 1.0$ ml
MIR 2116	$10 \times 1.0$ ml

### TransIT-TKO<sup>®</sup> Transfection Reagent

PRODUCT NO.	QUANTITY
MIR 2154	0.4 ml
MIR 2150	1.0 ml
MIR 2155	$5 \times 1.0$ ml
MIR 2156	$10 \times 1.0 \text{ ml}$

### TransIT®-siPAK Kit

Trial sizes of *Trans*IT-TKO<sup>®</sup> and *Trans*IT-siQUEST<sup>®</sup> Transfection Reagents.

PRODUCT NO.	QUANTITY
MIR 2260	0.1 ml Each

### TransIT®-siPAK Plus Kit

The *Trans*IT<sup>®</sup>-siPAK with the addition of 10 µg the *Label* IT<sup>®</sup> RNAi Delivery Control, Fluorescein (MIR 7902).

PRODUCT NO.	QUANTITY
MIR 2270	0.1 ml Each

# TransIT-TKO® & TransIT-siQUEST® continued



FIGURE 13. Knockdown Efficiencies Using TransIT-siQUEST<sup>®</sup> and TransIT-TKO<sup>®</sup> Reagents Lipofectamine™ 2000 (LF2K). and Firefly and sea pansy luciferase reporter vectors were cotransfected into various cell lines using the TransIT®-Subsequently, firefly luciferase LT1 Reagent. expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either TransIT-siQUEST® (red), TransIT-TKO® (black) or LF2K (gray) 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	TransIT-TKO® Knockdown Efficiency	TransIT-siQUEST® Knockdown Efficiency
A549-luc (human lung)	Luciferase*	77%	82%
BNL CL.2	MAPK1	80%	
(mouse liver)	MAPK3	83%	
CHO-luc (hamster ovary)	Luciferase*	86%	91%
HEK 293-lux (human kidney)	Luciferase*	83%	77%
	Lamin A/C	80%	
HeLa (numan cervix)	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%
	MAPK1	70%	
NIT 313-L1	MAPK3	70%	
Secondary Human Astrocytes	Lamin A/C	80%	
Primary Mausa	ABC A1	70%	
	Lamin A/C	81%	
nepatocytes	PPAR-alpha		82%

**TABLE 1.** Knockdown of Endogenous Genes Using *Trans*IT-TKO<sup>®</sup> or *Trans*IT-siQUEST<sup>®</sup> Transfection Reagents. Cells were transfected with siRNAs targeting the indicated genes using the *Trans*IT-TKO<sup>®</sup> or *Trans*IT-siQUEST<sup>®</sup> Reagents, 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.

### siRNA

# **INGENIO™ ELECTROPORATION PRODUCTS**

- High Efficiency Electroporation of Hard to Transfect Cells— Conduct research in biologically relevant cells
- Compatible with All Electroporation Instruments—Use your existing system including an amaxa Nucleofector® or Bio-Rad Gene Pulser; no need to purchase additional specialized equipment
- Save Money—Replace your amaxa Nucleofector<sup>®</sup> Kits with the Ingenio<sup>™</sup> Electroporation Kit and realize significant savings without sacrificing performance
- Buy Only What You Need—Ingenio Electroporation Solution is available alone or as part of a complete kit with cuvettes and cell droppers
- Higher Cell Viability—Less harmful than other electroporation methods

### Description

The Ingenio<sup>™</sup> Electroporation Solution facilitates efficient and reliable delivery of nucleic acids to eukaryotic cells traditionally resistant to chemical transfection. Ingenio<sup>™</sup> is a broad spectrum solution that supports high efficiency electroporation with minimal toxicity. It replaces standard electroporation solutions including phosphate buffered saline and serum-free media. Ingenio<sup>™</sup> is compatible with multiple instruments and facilitates a wide range of applications requiring nucleic acid delivery to cells. The Ingenio Solution is available alone and as part of a kit with cuvettes and cell droppers.





### **PRODUCT SPECIFICATIONS**

### Ingenio<sup>™</sup> Electroporation Kits (solution, 0.4 cm cuvettes, cell droppers)

PRODUCT NO.	SIZE
MIR 50113	25 RXNª
MIR 50116	50 RXN <sup>a</sup>
MIR 50119	100 RXNª

### Ingenio<sup>™</sup> Electroporation Kits

### (solution, 0.2 cm cuvettes, cell droppers)

SIZE
25 RXNª
50 RXN <sup>a</sup>
100 RXNª

### Ingenio<sup>™</sup> Electroporation Solution

PRODUCT NO.	SIZE	QUANTITY
MIR 50111	25 RXN⁵	6.25 ml
MIR 50114	50 RXN⁵	12.5 ml
MIR 50117	100 RXN⁵	25 ml

### Ingenio<sup>™</sup> Electroporation Accessories

PRODUCT NO.	DESCRIPTION	SIZE
MIR 50120	0.2 cm Cuvettes	25 PK
MIR 50121	0.2 cm Cuvettes	50 PK
MIR 50122	0.4 cm Cuvettes	25 PK
MIR 50123	0.4 cm Cuvettes	50 PK
MIR 50124	Cell Droppers	25 PK
MIR 50125	Cell Droppers	50 PK

<sup>a</sup> Electroporations per kit.

 Number of electroporations in 0.4 cm cuvette.

### siRNA

# Label IT® RNAi DELIVERY CONTROLS

- Sensitive—Easily visualize transfected cells and assess delivery efficiency using fluorescent microscopy
- Inert—Does not target any known mammalian genes or cause off-target effects
- Compatible—Suitable for co-delivery experiments with functional siRNA
- ▷ Easy to Use—Supplied as a ready to use 10 µM stock with a 10X RNAi Dilution Buffer

### PRODUCT SPECIFICATIONS

### Label IT® RNAi Delivery Controls

LABEL	PRODUCT NO.	QUANTITY
Су™З	MIR 7900	10 µg
	MIR 7901	100 µg
Fluorescein	MIR 7902	10 µg
	MIR 7903	100 µg

To inquire about bulk pricing, please call 888.530.0801.

### Description

The *Label* IT RNAi Delivery Controls consist of either  $Cy^{TM}3$  or fluorescein labeled RNA duplex that has the same length, charge and configuration as standard siRNA. The sequence of the *Label* IT RNAi duplex is inert and is not known to affect any cellular events. These controls are designed to facilitate assessment of delivery efficiency of dsRNA oligonucleotides in both *in vitro* and *in vivo* applications and can be co-transfected with a functional target gene-specific siRNA.



**FIGURE 15.** Visualization of *Label* IT<sup>®</sup> RNAi Delivery Control. HeLa cells were transfected in complete media with the *Label* IT<sup>®</sup> Fluorescein RNAi Delivery Control (green) using the *Trans*IT<sup>®</sup>-TKO Transfection Reagent. Twenty-four hours post-transfection, the cells were fixed, then counterstained to locate the nuclei (blue) and actin (red).

# Large RNA (Viral RNA and mRNA)

# TransIT®-mRNA TRANSFECTION KIT

- High Efficiency RNA Delivery—Ensures experimental success by effectively transfecting RNA into a large percentage of the cell population
- ▷ Serum Compatible—
  - ► Simplifies the transfection protocol
  - Saves valuable time
  - Increases cell viability thereby minimizing experimental bias due to alterations in cell physiology
- Transfects Various RNA Types—Ideal for multiple applications such as viral production and replication studies and short-term protein expression from mRNA

### Description

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



Transfection Control

lacZ mRNA Transfection

**FIGURE 16.** The *Trans*IT<sup>®</sup>-mRNA Transfection Kit Efficiently Delivers *lacZ* mRNA to CHO-K1 Cells. CHO-K1 cells were transfected with an *in vitro* synthesized *lacZ* mRNA using the *Trans*IT<sup>®</sup>-mRNA Transfection Kit. Cells were stained 18 hours post-transfection with the Beta-Gal Staining Kit to detect *lacZ* expressing cells.

### **FIGURE 17.** The *Trans*IT®-mRNA Transfection Kit Efficiently Delivers mRNA to a Variety of Cell Lines. Cells were transfected with a capped and polyadenylated EGFP encoding mRNA using the *Trans*IT®-mRNA Transfection Kit and analyzed by flow cytometry 18 hours post-transfection to identify the EGFP expressing cells.

### **PRODUCT SPECIFICATIONS**

PRODUCT NO.	QUANTITY
MIR 2225	0.25 ml
MIR 2250	0.5 ml
MIR 2255	5 × 0.5 ml
MIR 2256	$10 \times 0.5$ ml

To inquire about bulk pricing, please call 888.530.0801.



100

80

60

40

20

0

CHO-K1

COS-7

HeLa

Percent EGFP Expressing Cells



### TransIT<sup>®</sup>-mRNA continued



No RNA Control

**MHV RNA Transfected** 

**FIGURE 18.** The *Trans*IT<sup>®</sup>-mRNA Transfection Kit Successfully Delivers Viral RNAs at Least 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. Successful transfection was assessed by the formation of syncytia 24-48 hours post-transfection. Syncytia were visualized by phase contrast microscopy.

Data courtesy of Mark Clementz, Loyola University of Chicago.

TABLE 2	. Viral	RNAs	Successfully
Transfected	d using	the T	<i>ans</i> IT®-mRNA
Transfectio	on Kit.		

Virus	Application	RNA Size	Laboratory
Feline Calicivirus	Virus Production	8 kb	Hardy Lab Montana State University
Poliovirus	Virus Production	7.5 kb	Ehrenfeld Lab LID, NAIAD, NIH
Hepatitis C Virus	Replicon	9 kb	Striker Lab University of Wisconsin
Yellow	Virus Production	9 kb	Striker Lab University of Wisconsin
Fever Virus	Replicon	6 kb	Striker Lab University of Wisconsin
Murine Coronavirus	Virus Production	32 kb	Baker Lab Loyola University of Chicago

**FIGURE 19.** The *Trans*IT<sup>®</sup>-mRNA Transfection Kit Outperforms Electroporation and Saves Valuable Time. 10X Better Performance Using 2.5X Less RNA. Huh7 cells were transfected with 2 µg of a yellow fever virus-luciferase expressing RNA replicon (YFVluc) using the *Trans*IT<sup>®</sup>-mRNA Transfection Kit or electroporated in parallel with 5 µg of the YFV-luc RNA replicon using an ECM630 Electroporator (BTX) in PBS. Twenty-four hours later, cells were harvested and assayed for luciferase activity and total protein content. Luciferase activity was normalized to total protein content to correct for differences in cell number between samples.\*

\* Gonzalez G et al. Selection of an Optimal RNA Transfection Reagent and Comparison to Electroporation for the Delivery of viral RNA. J Virol Meth 2007; 145:14.



# NOTES

 <u> </u>

NOTES			

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### Mirus Bio LLC

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### PATENT AND LICENSING INFORMATION

Mirus Bio *Trans*IT<sup>®</sup> polyamine transfection reagents are covered by U.S. Patent No. 5,744,335, No. 6,180,784, and No. 6,458,382. Mirus Bio *Label* IT<sup>®</sup> nucleic acid labeling and modifying reagents are covered by U.S. Patent No. 6,262,252, and No. 6,593,465. Cy<sup>TM</sup>3 and Cy<sup>TM</sup>5 products or portions thereof are manufactured under license from Carnegie Mellon University and are covered by U.S. Patent No. 5,268,486.

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# **DNA TRANSFECTION**

### **Broad Spectrum**

Product Name	Product No.	Quantity	
TransIT®-2020	MIR 5400	1 ml	
Transfection Reagent	MIR 5404	0.4 ml	
-	MIR 5405	5 x 1 ml	
	MIR 5406	10 x 1 ml	
TransIT <sup>®</sup> -LT1	MIR 2300	1 ml	
Transfection Reagent	MIR 2304	0.4 ml	
	MIR 2305	5 x 1 ml	
	MIR 2306	10 x 1 ml	

### **Cell Line Specific DNA Transfection**

Product Name	Product No.	Quantity	
TransIT®-293	MIR 2700	1ml	
Transfection Reagent	MIR 2704	0.4 ml	
	MIR 2705	5 x 1 ml	
	MIR 2706	10 x 1 ml	
TransIT®-3T3	MIR 2180	1 ml	
Transfection Kit*	MIR 2184	0.4 ml	
	MIR 2185	5 x 1 ml	
	MIR 2186	10 x 1 ml	
TransIT®-CHO	MIR 2170	1 ml	
Transfection Kit*	MIR 2174	0.4 ml	
	MIR 2175	5 x 1 ml	
	MIR 2176	10 x 1 ml	
TransIT®-COS	MIR 2190	1 ml	
Transfection Kit*	MIR 2194	0.4 ml	
	MIR 2195	5 x 1 ml	
	MIR 2196	10 x 1 ml	
TransIT-HeLaMONSTER®	MIR 2900	1 ml	
Transfection Kit*	MIR 2904	0.4 ml	
	MIR 2905	5 x 1 ml	
	MIR 2906	10 x 1 ml	
<i>Trans</i> IT <sup>®</sup> -Jurkat	MIR 2120	1 ml	
Transfection Reagent	MIR 2124	0.4 ml	
-	MIR 2125	5 x 1 ml	
	MIR 2126	10 x 1 ml	
TransIT <sup>®</sup> -Keratinocyte	MIR 2800	1 ml	
Transfection Reagent	MIR 2804	0.4 ml	
	MIR 2805	5 x 1 ml	
	MIR 2806	10 x 1 ml	
TransIT-Neural®	MIR 2140	1 ml	
Transfection Reagent	MIR 2144	0.4 ml	
-	MIR 2145	5 x 1 ml	
	MIR 2146	10 x 1 ml	
TransIT <sup>®</sup> -Prostate	MIR 2130	1 ml	
Transfection Kit*	MIR 2134	0.4 ml	
	MIR 2135	5 x 1 ml	
	MIR 2136	10 x 1 ml	

### Electroporation

Product Name	Product No.	Size
Ingenio™ Electroporation Kits	MIR 50113	25 RXN
(solution, 0.4 cm cuvettes	MIR 50116	50 RXN
cell droppers)	MIR 50119	10 RXN
Ingenio <sup>™</sup> Electroporation Kits	MIR 50112	25 RXN
(solution, 0.2 cm cuvettes	MIR 50115	50 RXN
cell droppers)	MIR 50118	10 RXN
Ingenio <sup>™</sup> Electroporation	MIR 50111	25 RXN (6.25 ml)
Solution	MIR 50114	50 RXN (12.5 ml)
	MIR 50117	10 RXN (25 ml)
Ingenio <sup>™</sup> Electroporation	MIR 50120	0.2 cm cuvettes (25 PK)
Accessories	MIR 50121	0.2 cm cuvettes (50 PK)
	MIR 50122	0.4 cm cuvettes (25 PK)
	MIR 50123	0.4 cm cuvettes (50 PK)
	MIR 50124	Cell Droppers (25 PK)
	MIR 50125	Cell Droppers (50 PK)

### **NEW!** Protein Production

Product Name	Product No.	Quantity
<i>Trans</i> IT-PRO™	MIR 5700	1 ml
Transfection Kit	MIR 5760	10 ml

### Labeled Plasmid Delivery Controls

Product Name	Product No.	Quantity
Label IT <sup>®</sup> Plasmid	MIR 7904	10 µg
Delivery Control, Cy™3	MIR 7905	100 µg
Label IT <sup>®</sup> Plasmid	MIR 7906	10 µg
Delivery Control, Fluorescein	MIR 7907	100 µg

# **RNA TRANSFECTION**

### siRNA & miRNA Transfection

Product Name	Product No.	Quantity
TransIT-TKO <sup>®</sup> Transfection	MIR 2150	1 ml
Reagent	MIR 2154	0.4 ml
	MIR 2155	5 x 1 ml
	MIR 2156	10 x 1 ml
TransIT-siQUEST® Reagent	MIR 2110	1 ml
	MIR 2114	0.4 ml
	MIR 2115	5 x 1 ml
	MIR 2116	10 x 1 ml
<i>Trans</i> IT <sup>®</sup> -siPAK Kit		0.1 ml of each
		TransIT-siQUEST® and
	MIR 2260	TransIT-TKO <sup>®</sup> Reagents
TransIT <sup>®</sup> -siPAK Plus Kit		0.1 ml of each
		TransIT-siQUEST <sup>®</sup> and
		TransIT-TKO <sup>®</sup> Reagents
		and 10 µg Label IT®
		RNAi Delivery
	MIR 2270	Control, Fluorescein

### Electroporation

Product Name	Product No.	Size
Ingenio <sup>™</sup> Electroporation Kits	MIR 50113	25 RXN
(solution, 0.4 cm cuvettes	MIR 50116	50 RXN
cell droppers)	MIR 50119	10 RXN
Ingenio <sup>™</sup> Electroporation Kits	MIR 50112	25 RXN
(solution, 0.2 cm cuvettes	MIR 50115	50 RXN
cell droppers)	MIR 50118	10 RXN
Ingenio <sup>™</sup> Electroporation	MIR 50111	25 RXN (6.25 ml)
Solution	MIR 50114	50 RXN (12.5 ml)
	MIR 50117	10 RXN (25 ml)
Ingenio <sup>™</sup> Electroporation	MIR 50120	0.2 cm cuvettes (25 PK)
Accessories	MIR 50121	0.2 cm cuvettes (50 PK)
	MIR 50122	0.4 cm cuvettes (25 PK)
	MIR 50123	0.4 cm cuvettes (50 PK)
	MIR 50124	Cell Droppers (25 PK)
	MIR 50125	Cell Droppers (50 PK)

### Labeled RNAi Delivery Controls

Product Name	Product No.	Quantity	
Label IT® RNAi	MIR 7900	10 µg	
Delivery Control, Cy™3	MIR 7901	100 µg	
Label IT® RNAi	MIR 7902	10 µg	
Delivery Control, Fluorescein	MIR 7903	100 µg	

### **mRNA** Transfection

Product Name	Product No.	Quantity	
<i>Trans</i> IT <sup>®</sup> -mRNA	MIR 2225	0.25 ml	
Transfection Kit	MIR 2250	0.5 ml	
	MIR 2255	5 x 0.5 ml	
	MIR 2256	10 x 0.5 ml	

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