Mirus
Gene Transfer Specialists™

Technical Report

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Nucleic Acid Labeling and Delivery Tools for Neurobiology

Aaron G. Loomis, Jennifer L. Duzeski, Laura K. Juckem, Kira J. Machnik, Shyla L. Tessmer, Mary-Anne V. Watt, James E. Hagstrom. Mirus Corporation, 2002.

Introduction

To address the needs of neuroscientists, Mirus has developed a variety of optimized reagents for labeling and transfection of nucleic acids. Mirus' ***Trans*IT®-Neural™ Reagent** is a plasmid transfection reagent specifically designed to achieve the highest transfection efficiency, while maintaining cell viability, in a wide range of neural cell lines. Mirus' popular ***Trans*IT-TKO® Transfection Reagent** efficiently delivers siRNA, enabling knock out of target gene expression in a wide variety of mammalian cell types, including those that are of neural origin. Mirus also provides ***Label* IT® Tracker™ Kits** and ***Label* IT® siRNA Tracker™ Kits** for one-step direct covalent labeling of nucleic acids in a non-destructive manner, allowing for intracellular tracking studies, where the original function of the nucleic acid is generally maintained. The *Label* IT® Tracker™ Kits and *Label* IT® siRNA Tracker™ Kits are available in a variety of labels (Cy™3, Cy™5, CX-Rhodamine, TM-Rhodamine, Fluorescein, and Biotin), which are ideal for tracking nucleic acids with one or more fluorophores. In this report, we demonstrate how these reagents, when used individually or in concert, facilitate effective neurobiological studies in gene delivery and regulation.

Methods

Preparation of Labeled pDNA

Plasmid DNA was labeled with the indicated *Label* IT® Tracker™ Reagent at a ratio of 1 µl reagent per 1 µg of DNA and incubated for 1 hour at 37ºC, as described in the *Label* IT® Tracker™ protocol. The sample was purified by ethanol precipitation, and the labeled DNA pellet was resuspended in sterile, molecular-biology grade water and quantified. Labeled DNA was stored at -20°C, protected from light.

Transfection of pDNA

Transfection complexes were prepared by adding 1-16 µl (depending on cell type) of the *Trans*IT®-Neural™ Transfection Reagent per 1 µg of plasmid DNA to 100 µl of serum-free media, vortexing and incubating at room temperature for 5-10 minutes. A firefly luciferase plasmid (1 µg per well of a 12-well plate) was added to the diluted *Trans*IT®-Neural™ Reagent, mixed by gentle pipetting and incubated at room temperature for an additional 5-10 minutes. Complexes were added dropwise to 50-70% confluent cells in their complete media and mixed by gentle rocking. Following the addition of the complexes, the cells were incubated at 37ºC for 24-48 hours and assayed for gene expression.

Preparation of siRNA Duplexes

An RNA oligonucleotide directed against the coding region for firefly luciferase and its reverse complement were synthesized and PAGE purified. Anti-firefly luciferase siRNA duplexes were formed by hybridizing the reverse complementary RNA oligonucleotides in 100 mM NaCl, 50 mM Tris pH 8 buffer. These were then diluted in the same buffer to a working concentration of 1 µM.

Preparation of Labeled siRNA

Duplex siRNA was labeled with the indicated *Label* IT® siRNA Tracker™ Reagent at a ratio of 1 µl reagent per 1 µg of siRNA and incubated for 1 hour at 37ºC, as described in the *Label* IT® siRNA Tracker™ protocol. The sample was purified by ethanol precipitation, and the labeled siRNA pellet was resuspended in the siRNA Dilution Buffer (provided in the kit) and quantified. Labeled siRNA was stored at -20°C, protected from light.

Transfection of siRNA Duplexes

Transfection complexes were prepared by adding 1-8 µl of *Trans*IT-TKO® Reagent to 50-200 µl (volumes depend on plate size) of serum-free media, vortexing and incubating the mixture at room temperature for 5-10 minutes. Anti-firefly luciferase siRNA duplex (5-50 nM final concentration in the well) was added to the mixture, mixed by gentle pipetting and incubated at room temperature for an additional 5-10 minutes. Complexes were added dropwise to 50-70% confluent cells in their complete media and mixed by gentle rocking. Cells were incubated at 37˚C for 24-48 hours and assayed for gene expression.

Simultaneous Transfection of pDNA and siRNA Duplexes

The transfection complexes were prepared in the specified order. One to two µl of *Trans*IT®-Neural™ Transfection Reagent were added to 50 µl of serum-free media, vortexed and incubated at room temperature for 5-10 minutes. Two plasmid DNAs, one encoding sea pansy luciferase (5 ng) and the other firefly luciferase (500 ng) were added to the diluted *Trans*IT®-Neural™ Transfection Reagent by gentle pipetting and incubated at room temperature for 5-10 minutes. To the same mixture, 0.5-2 µl of *Trans*IT-TKO® Reagent was added and mixed by gentle pipetting and incubated at room temperature for 5-10 minutes. Anti-firefly siRNA duplex (5-10nM) was then added and mixed by gentle pipetting and incubated at room temperature for 5-10 minutes. This entire mixture was added dropwise to 50-70% confluent cells (cultured in 24-well plates) in their complete media and mixed by gentle rocking. Cells were incubated at 37ºC for 24 hours and assayed for gene inhibition.

Results and Discussion

High Efficiency Neural Cell Transfection

*Trans*IT®-Neural™ Transfection Reagent has been optimized to successfully deliver plasmid DNA to many neural cell lines, including C6, Daoy, DBTRG-05MG, DI-TNC1, HCN-1A, Neuro-2a, PC-12, SK-N-MC, SVG p12 and human astrocytes. Neuro-2a cells, a common neuroblastoma cell line in neurobiology research, was used to demonstrate the ability of *Trans*IT®-Neural™ Transfection Reagent to deliver EGFP plasmid DNA. Forty-eight hours post-transfection, the cells were fixed and mounted for fluorescent microscopy (Figure 1). At least 75% of the Neuro-2a cells were successfully transfected, while cell viability was maintained.

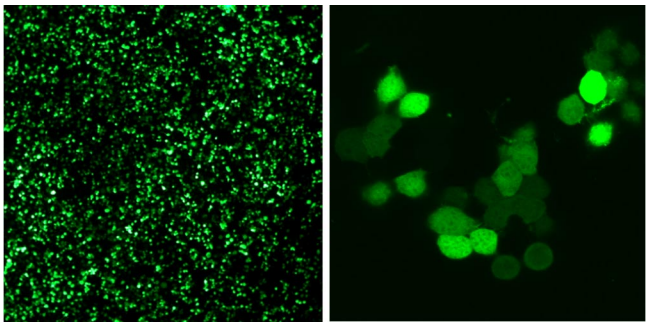

Figure 1. Expression of pEGFP in Neuro-2a Cells
Neuro-2a cells transfected with 4 µl *Trans*IT®-Neural™ Transfection Reagent and 2 µg pEGFP (Clontech) per well of a 6-well plate and incubated in complete media. The image on the left was acquired 48 hours post-transfection on a Zeiss Axiocam. The image on the right was acquired 48 hours post-transfection on a Zeiss LSM 510 Confocal Microscope.

Plasmid DNA Tracking and Expression in Neural Cells

Labeled plasmid DNA can be introduced into neural cells in culture by a variety of methods. Transfections using *Trans*IT®-Neural™ Transfection Reagent are characterized by low toxicity and high efficiency. As seen in Figure 2, *Label* IT® Tracker™ Cy™5 labeled EGFP plasmid DNA can be seen in all cells 24 hours post-transfection; EGFP expression can be visualized simultaneously. See Mirus' *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits Technical Report for more details on tracking and expression of plasmid DNA.

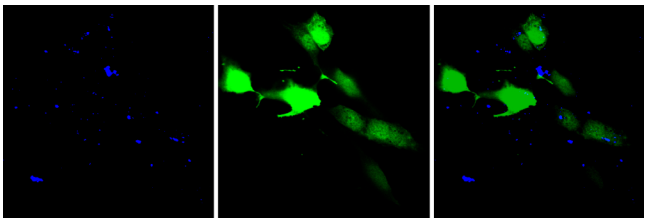

Figure 2. Tracking and Expression of Plasmid DNA in DI-TNC1 Cells
DI-TNC1 astrocytes were transfected with 1 µg *Label* IT® Tracker™ Cy™5 labeled pEGFP and 3 µl *Trans*IT®-Neural™ Transfection Reagent per well of a 6-well plate in complete media. The blue represents the Cy™5 fluorescence, and the green represents pEGFP expression. Images were acquired at 24 hours post-transfection using a Zeiss LSM 510 Confocal Microscope.

Knock Out of Target Gene Expression

siRNA complexed with *Trans*IT-TKO® Transfection Reagent has the ability to selectively knock out target gene

expression without affecting the expression of a similar non-targeted gene. To illustrate this, firefly luciferase and sea pansy luciferase expression vectors were co-transfected into a variety of cell types with *Trans*IT®-Neural™ Transfection Reagent. To the same tube, *Trans*IT-TKO® Reagent/anti-firefly luciferase siRNA complexes were added. Using 5-10 nM siRNA, greater than 85% knock out of firefly luciferase expression was observed in all cell lines tested at 24 hours when compared to the expression of the untargeted sea pansy luciferase (Figure 3).

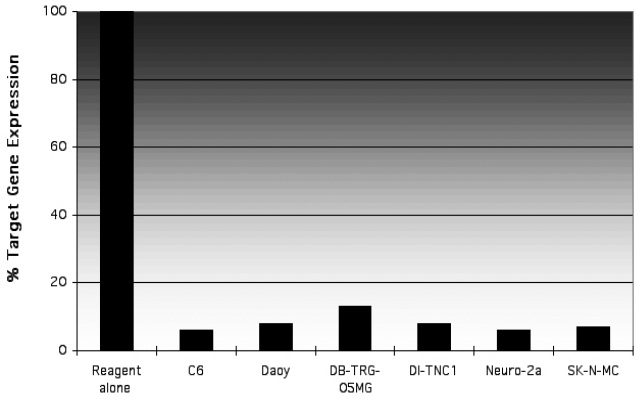

Figure 3. Target Gene Expression Knock Out in Neural Cell Lines
Efficiency of knock out in various neural lines using the *Trans*IT-TKO® Transfection Reagent with 5-10 nM anti-firefly luciferase siRNA duplexes (Dharmacon Research, Inc). Cell lines were first transfected with plasmids expressing firefly and sea pansy luciferase using the *Trans*IT®-Neural™ Transfection Reagent. Twenty-four hours post-transfection, cell lysates were assayed for both firefly and sea pansy luciferase expression using Promega's Dual-Luciferase® Reporter Assay System.

Tracking of siRNA Duplexes

In order to visualize the subcellular location of siRNA after delivery by *Trans*IT-TKO® Reagent, *Label* IT® siRNA Tracker™ Cy™3 labeled siRNA duplexes were transfected into human astrocytes (Figure 4) and C6 glioma cells (Figure 5) and then visualized using confocal microscopy. The majority of the siRNA is cytoplasmic. Both diffuse and punctate signals were observed, indicating that there are likely at least two populations of delivered labeled siRNA molecules; one which is free in the cytoplasm itself and the other which is contained within endosomes.

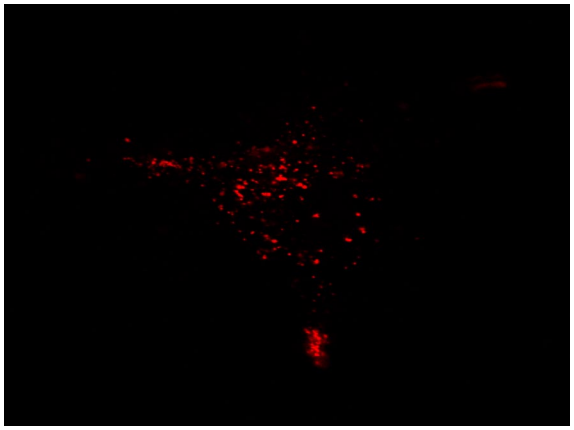

Figure 4. siRNA Tracking in Human Astrocytes
Human astrocytes (ScienCell Research Laboratories) were seeded on poly-D-lysine coated coverslips in a 24-well plate and incubated overnight. One µl of *Trans*IT-TKO® Transfection Reagent was diluted in 50 µl of serum-free media and 50 nM (final concentration in well) of *Label* IT® siRNA Tracker™ Cy™3 labeled siRNA duplex was added to the diluted *Trans*IT-TKO® Transfection Reagent for complex formation. Subsequently, these complexes were added to 30% confluent human astrocytes in their complete growth media. Confocal image of the single astrocyte was acquired 24 hours post-transfection on a Zeiss LSM 510 Confocal Microscope.

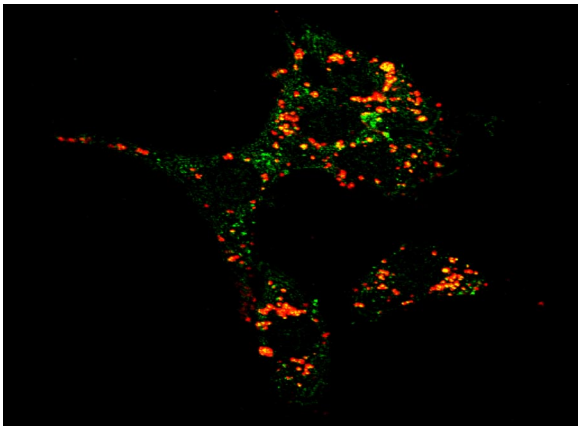

Figure 5. siRNA Tracking in C6 Cells
C6 glioma cells were seeded on poly-D-lysine coated coverslips in a 6-well plate and incubated overnight. Eight µl of *Trans*IT-TKO® Transfection Reagent were diluted in 200 µl of serum-free media and 50 nM (final concentration in well) of *Label* IT® siRNA Tracker™ Cy™3 labeled siRNA duplex was added to the diluted *Trans*IT-TKO® Transfection Reagent for complex formation. Subsequently, these complexes were added to 45% confluent C6 cells in their complete growth media. Confocal image was acquired 20 hours post-transfection on a Zeiss LSM 510 Confocal Microscope.

Summary

Mirus' *Trans*IT®-Neural™ and *Trans*IT-TKO® Transfection Reagents allow for highly efficient transfection of plasmid DNA and siRNA, respectively, in a variety of neural cell lines. These reagents include optimized protocols which result in high cellular viability and are most effective when carried out in serum containing media, with no media changes or additions. The *Label* IT® Tracker™ and *Label* IT® siRNA Tracker™ Kits allow for the direct covalent labeling of plasmid DNA and siRNA. These tools give researchers the opportunity to assess transfection efficiencies on their particular neural cell line, and perform intracellular localization studies, where the original function of the nucleic acid is generally maintained. The combination of these products provides neurobiologists with simple methods to reproducibly study gene transfer and expression as well as target gene knock out in neural cell lines.

Product Protocols (available at www.genetransfer.com)

- *Trans*IT®-Neural™ Transfection Reagent Protocol #ML022
- *Trans*IT-TKO® Transfection Reagent Protocol #ML015
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits Protocol #ML018
- *Label* IT® siRNA Tracker™ Intracellular Localization Kits Protocol #ML023

Technical Reports (available at www.genetransfer.com)

- *Trans*IT-TKO® Short Interfering RNA (siRNA) Transfection Reagent (Technical Report #MTR001)
- Intracellular Localization and Expression of Labeled Plasmid DNA using *Label* IT® Tracker™ Nucleic Acid Labeling Kits (Technical Report #MTR002)

Mirus Product References are available at www.genetransfer.com.

For Customer and Technical Support contact Mirus at:
888.530.0801 or 608.441.2852
www.genetransfer.com

Ordering Information

*Trans*IT®-Neural™ Transfection Reagent

Product Name	Quantity	Cat. No.
*Trans*IT®-Neural™ Transfection Reagent	1 ml	MIR 2140
*Trans*IT®-Neural™ Transfection Reagent	0.4 ml	MIR 2144
*Trans*IT®-Neural™ Transfection Reagent	5 x 1 ml	MIR 2145
*Trans*IT®-Neural™ Transfection Reagent	10 x 1 ml	MIR 2146

*Trans*IT-TKO® Transfection Reagent

Product Name	Quantity	Cat. No.
*Trans*IT-TKO® Transfection Reagent	1 ml	MIR 2150
*Trans*IT-TKO® Transfection Reagent	0.4 ml	MIR 2154
*Trans*IT-TKO® Transfection Reagent	5 x 1 ml	MIR 2155
*Trans*IT-TKO® Transfection Reagent	10 x 1 ml	MIR 2156

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kits

Product Name	Quantity	Cat. No.
Label IT® Tracker™ Cy™3	*	MIR 7010
Label IT® Tracker™ Cy™5	*	MIR 7011
Label IT® Tracker™ CX-Rhodamine	*	MIR 7012
Label IT® Tracker™ TM-Rhodamine	*	MIR 7013
Label IT® Tracker™ Fluorescein	*	MIR 7015
Label IT® Tracker™ Biotin	*	MIR 7014

*Each kit contains *Label* IT® Tracker™ Reagent, Tracker Reconstitution Solution, 10X Labeling Buffer A, and *Trans*IT®-LT1 Transfection Reagent. Each kit provides sufficient reagents to label 50 to 200 µg of plasmid DNA and perform at least 50 transfections

Label IT® siRNA Tracker™ Intracellular Localization Kits

Product Name	Quantity	Cat. No.
Label IT® siRNA Tracker™ Cy™3	**	MIR 7200
Label IT® siRNA Tracker™ Cy™5	**	MIR 7201
Label IT® siRNA Tracker™ CX-Rhodamine	**	MIR 7202
Label IT® siRNA Tracker™ TM-Rhodamine	**	MIR 7203
Label IT® siRNA Tracker™ Fluorescein	**	MIR 7205
Label IT® siRNA Tracker™ Biotin	**	MIR 7204

**Each kit contains *Label* IT® siRNA Tracker™ Reagent, *Label* IT® Reconstitution Solution, 10X Labeling Buffer A, siRNA Dilution Buffer, and *Trans*IT-TKO® Transfection Reagent. Each kit provides sufficient reagents to label 50 µg of siRNA and perform up to 500 transfections in 24-well plates.