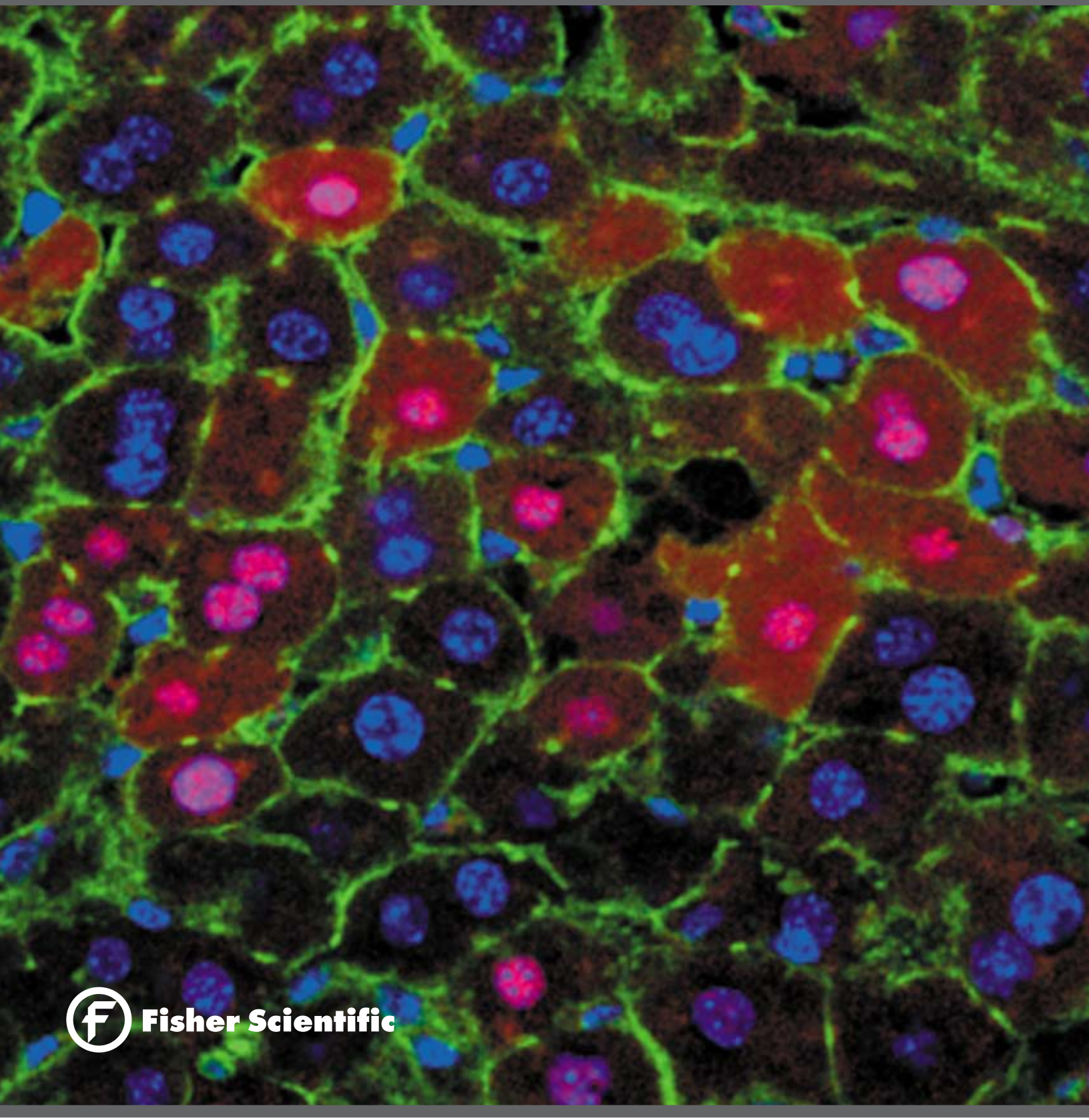
RNA Interference

- *Localization*
- *Expression Profiling*
- *Transfection*
- *Labeling*
- *In Vivo Delivery*

Mirus®

It All Begins at the Bench



Fisher Scientific

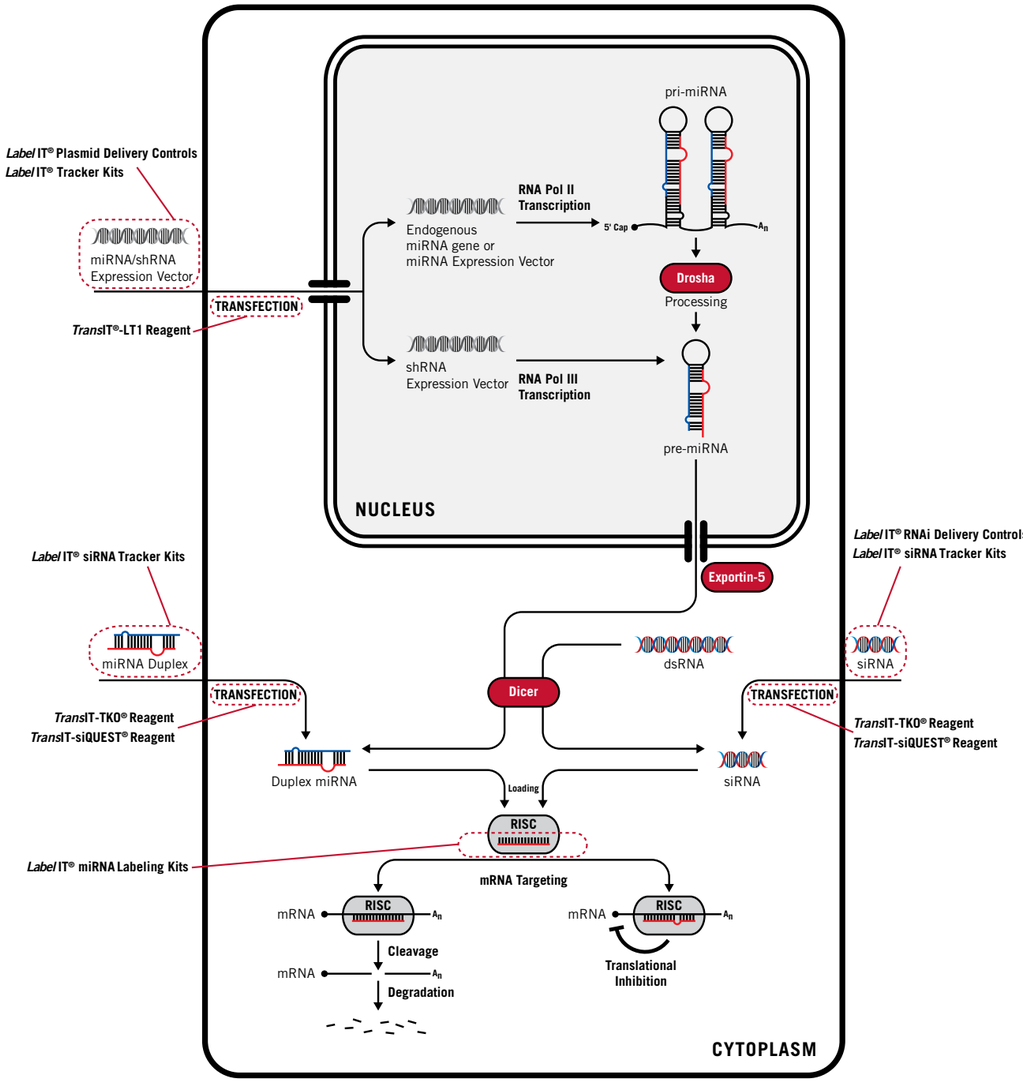


Figure 1. RNA interference (RNAi) is a powerful natural mechanism for post-transcriptionally inhibiting expression of a target gene. RNAi is triggered by small double-stranded RNAs (dsRNAs), either introduced into the cell by transfection (siRNAs), or arising from nuclear transcripts containing a stem-loop structure (pri-miRNAs). Pri-miRNAs are processed by the RNAse III-like enzymes DROSHA in the nucleus and DICER in the cytoplasm to yield mature miRNAs. siRNAs can also be generated by DICER-dependent cleavage of long dsRNA. Both siRNAs and miRNAs are capable of inhibiting expression of target mRNAs by promoting mRNA cleavage or inhibiting translation, depending on the degree of complementarity to the target sequence.

RNAi Tools

Application	Product	Page
siRNA and miRNA Transfection	*Trans*IT-TKO® Transfection Reagent *Trans*IT-siQUEST® Transfection Reagent	4 4
shRNA/siRNA Expression Vector Transfection	*Trans*IT®-LT1 Transfection Reagent	6
In Vivo Delivery of siRNA and/or shRNA/siRNA Expression Vector	*Trans*IT®-QR Hydrodynamic Delivery Kit	11
Delivery, Localization and Functional Knockdown Assessment	*Label* IT® siRNA Tracker Kits *Label* IT® Tracker Kits	8 10
Delivery and Localization Assessment	*Label* IT® RNAi Delivery Controls *Label* IT® Plasmid Delivery Controls	12 13
miRNA Expression Profiling	*Label* IT® miRNA Labeling Kits	14

Multiplexing Applications

Goal	Step 1	Step 2
Assess siRNA or Duplex miRNA Transfection Efficiency, Subcellular Localization and Target Knockdown	siRNA or Duplex miRNA Labeling *Label* IT® siRNA Tracker Kits	siRNA or Duplex miRNA Transfection *Trans*IT-TKO® Transfection Reagent *Trans*IT-siQUEST® Transfection Reagent
Assess shRNA/siRNA Expression Vector Transfection Efficiency, Subcellular Localization and Target Knockdown	shRNA/siRNA Expression Vector Labeling *Label* IT® Tracker Kits	shRNA/siRNA Expression Vector Transfection *Trans*IT®-LT1 Transfection Reagent
Assess *in vivo* siRNA or Duplex miRNA Delivery Efficiency, Subcellular Localization and Target Knockdown	siRNA or Duplex miRNA Labeling *Label* IT® siRNA Tracker	*In vivo* siRNA or Duplex miRNA Delivery *Trans*IT®-QR Hydrodynamic Delivery Kit
Assess *in vivo* shRNA/siRNA Expression Vector Transfection Efficiency, Subcellular Localization and Target Knockdown	shRNA/siRNA Expression Vector Labeling *Label* IT® Tracker Kits	*In vivo* shRNA/siRNA Expression Vector Delivery *Trans*IT®-QR Hydrodynamic Delivery Kit
Assess siRNA Transfection Efficiency and Subcellular Localization	Prelabeled siRNA *Label* IT® RNAi Delivery Controls	siRNA Transfection *Trans*IT-TKO® Transfection Reagent *Trans*IT-siQUEST® Transfection Reagent
Assess Plasmid Transfection Efficiency and Subcellular Localization	Prelabeled Plasmid DNA *Label* IT® Plasmid Delivery Controls	Plasmid Transfection *Trans*IT®-LT1 Transfection Reagent
Assess *in vivo* siRNA Delivery Efficiency and Subcellular Localization	Prelabeled siRNA *Label* IT® RNAi Delivery Controls	*In vivo* siRNA Delivery *Trans*IT®-QR Hydrodynamic Delivery Kit
Assess *in vivo* Plasmid Delivery and Subcellular Localization	Prelabeled Plasmid DNA *Label* IT® Plasmid Delivery Controls	*In vivo* Plasmid Delivery *Trans*IT®-QR Hydrodynamic Delivery Kit

TRANSFECTION ▷ siRNA and miRNA

*Trans*IT-TKO® AND *Trans*IT-siQUEST® RNAi TRANSFECTION REAGENTS

- ▷ **Broad Spectrum siRNA and Duplex miRNA Delivery**—Utilize each siRNA transfection reagent and protocol for a variety of cells
- ▷ **Two Different Reagent Formulations**—Two choices when identifying the best transfection reagent to maximize gene knockdowns 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

Description

*Trans*IT-TKO and *Trans*IT-siQUEST siRNA and duplex miRNA Transfection Reagents are both broad spectrum reagents that are easy to use and exhibit minimal cellular toxicity. Each reagent is uniquely formulated and exhibits distinct siRNA transfection profiles. These two reagents allow the user to identify the best siRNA transfection reagent for their particular cell line.

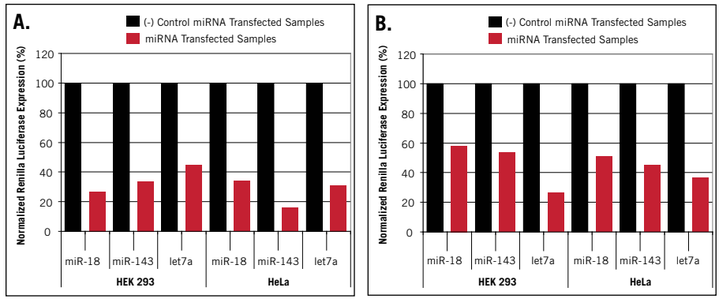


FIGURE 2. Highly Effective Delivery of miRNA Duplexes using Both the *Trans*IT-TKO® and *Trans*IT-siQUEST® Transfection Reagents. Three miRNA reporter constructs were created in the psiCHECK™-2 Vector (Promega) by cloning the target sequences of miR-18, miR-143, and let7a within the 3' untranslated region of expressed *Renilla* luciferase mRNA. The psiCHECK™-2 vector also expresses the internal control, firefly luciferase. In cells transfected with a psiCHECK™ construct, the presence of the specific miRNA would be indicated by a decrease in the level of *Renilla* luciferase activity relative to firefly luciferase activity. Each psiCHECK™ reporter plasmid was transfected into both HEK 293 and HeLa cells using the *Trans*IT®-LT1 Reagent. Approximately 6 hours post-transfection, the cells were transfected with 25 nM miR-18, miR-143 or let7a duplex miRNAs (Ambion) using either the *Trans*IT-TKO® (A) or *Trans*IT-siQUEST® (B) Transfection Reagents. Twenty-fours hours post-miRNA transfection, luciferase expression was determined and *Renilla* luciferase activity was normalized to firefly luciferase activity in each sample and then compared to cells transfected in parallel with a negative control miRNA duplex (Ambion Cat# AM17110).

PRODUCT SPECIFICATIONS

*Trans*IT-siQUEST® RNAi Transfection Reagent

PRODUCT NO.	SIZE*	QUANTITY
MIR 2114	400	0.4 ml
MIR 2110	1,000	1.0 ml
MIR 2115	5,000	5 × 1.0 ml
MIR 2116	10,000	10 × 1.0 ml

*Trans*IT-TKO® RNAi Transfection Reagent

PRODUCT NO.	SIZE*	QUANTITY
MIR 2154	400	0.4 ml
MIR 2150	1,000	1.0 ml
MIR 2155	5,000	5 × 1.0 ml
MIR 2156	10,000	10 × 1.0 ml

*Trans*IT®-siPAK Kit

Trial sizes of *Trans*IT-TKO® and *Trans*IT-siQUEST® Transfection Reagents.

PRODUCT NO.	SIZE*	QUANTITY
MIR 2260	100 Each	0.1 ml Each

*Trans*IT®-siPAK Plus Kit

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

PRODUCT NO.	SIZE*	QUANTITY
MIR 2270	100 Each	0.1 ml Each

* Number of transfections in 24-well plates.

COMPONENTS

*Trans*IT®-TKO and/or *Trans*IT®-siQUEST siRNA Transfection Reagents

STORAGE CONDITIONS

Store at 4°C, Do Not Freeze

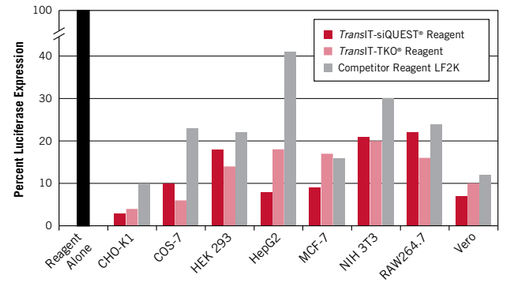


FIGURE 3. Knockdown Efficiencies Using *Trans*IT-siQUEST® and *Trans*IT-TKO® Reagents and Competitor Reagent LF2K. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines using the *Trans*IT®-LT1 Reagent. Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either *Trans*IT-siQUEST® (red), *Trans*IT-TKO® (pink) or Competitor 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	Knockdown Efficiency
A549-luc (human lung)	Luciferase*	77%
BNL CL.2 (mouse liver)	MAPK1	80%
	MAPK3	83%
CHO-luc (hamster ovary)	Luciferase*	86%
HEK 293-lux (human kidney)	Luciferase*	83%
HeLa (human cervix)	Lamin A/C	80%
	GAPDH	80%
HeLa-luc (human cervix)	Luciferase*	84%
HepG2 (human liver)	MAPK1	80%
NIH 3T3-lux (mouse fibroblast)	Luciferase*	85%
NIH 3T3-L1	MAPK1	70%
	MAPK3	70%
Secondary Human Astrocytes	Lamin A/C	80%
Primary Mouse Hepatocytes	ABC A1	70%
	Lamin A/C	81%

TABLE 1. Knockdown of Endogenous Genes Using *Trans*IT-TKO® Transfection Reagent. Cells were transfected with siRNAs targeting the indicated genes using the *Trans*IT-TKO® Reagent, 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.

Cell Line (Source)	Endogenous Transcript	Knockdown Efficiency
A549-luc (human lung)	Luciferase*	82%
CHO-luc (hamster ovary)	Luciferase*	91%
HEK 293-lux (human kidney)	Luciferase*	77%
HeLa-luc (human cervix)	Luciferase*	82%
Hepa-luc (mouse liver)	Luciferase*	92%
NIH 3T3-lux (mouse fibroblast)	Luciferase*	89%
Primary Mouse Hepatocytes	PPAR-alpha	82%

TABLE 2. Knockdown of Endogenous Genes Using *Trans*IT-siQUEST® Transfection Reagent. Cells were transfected with siRNAs targeting the indicated genes using the *Trans*IT-siQUEST® Reagent, and the knockdown percentage was determined using luciferase assays or quantitative RT-PCR.

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

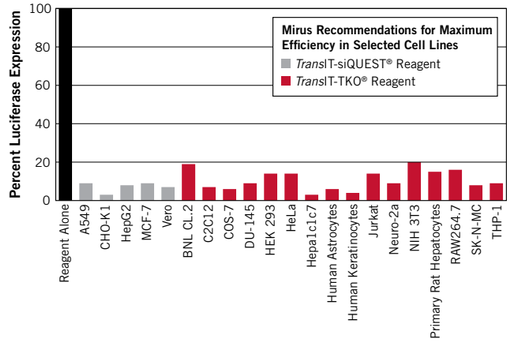


FIGURE 4. Highly Efficient Knockdown of Transiently Expressed Firefly Luciferase. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines. Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either the *Trans*IT-siQUEST® (gray) or *Trans*IT-TKO® (red) Transfection Reagent. Bars indicate the percent of normalized firefly luciferase expression as compared to the reagent alone control 24 hours post-transfection.

TRANSFECTION ▷ Broad Spectrum DNA

*Trans*IT®-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 with this Broad Spectrum DNA Delivery Reagent**—Utilize one transfection reagent and protocol for a variety of cells

Description

*Trans*IT-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.	SIZE*	QUANTITY
MIR 2304	200	0.4 ml
MIR 2300	500	1.0 ml
MIR 2305	2,500	5 × 1.0 ml
MIR 2306	5,000	10 × 1.0 ml

* Number of transfections in 6-well plates or 35 mm dishes.

COMPONENTS

*Trans*IT®-LT1 Transfection Reagent

STORAGE CONDITIONS

Store at 4°C or –20°C

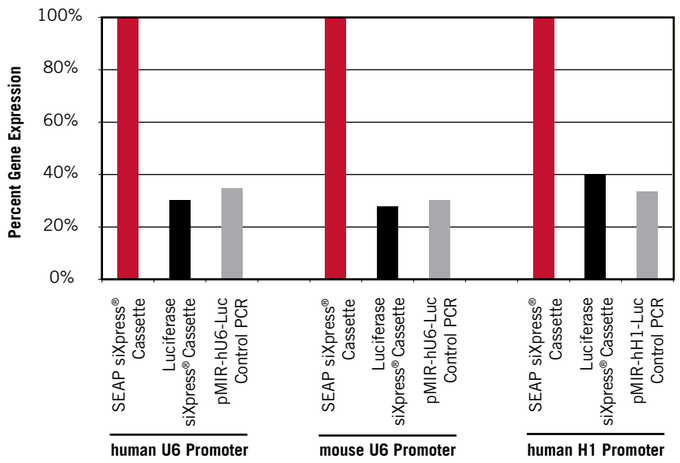


FIGURE 5. Efficient Target Gene Knockdown After Transfection of siXpress® PCR shRNA Expression Cassettes Using the *Trans*IT®-LT1 Transfection Reagent. Three shRNA siXpress® expression cassettes [nontargetting SEAP control (red), luciferase targeting (black) and a luciferase targeting control (gray)] using three different promoters (human U6, mouse U6, and human H1) were constructed. Each shRNA cassette was transfected using the *Trans*IT®-LT1 Transfection Reagent into CHO-K1 cells stably expressing firefly luciferase. Forty-eight hours post-transfection, cells were harvested and assayed for luciferase activity. Luciferase activity was normalized to that of the SEAP control cassette for each corresponding promoter to determine relative luciferase knockdown.

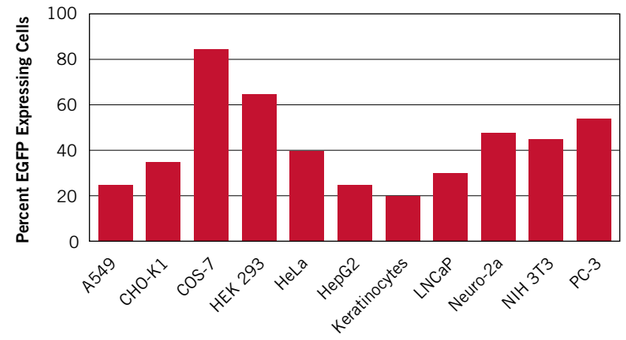


FIGURE 6. High Efficiency Transfection on a Broad Range of Cell Lines Using *Trans*IT®-LT1 Reagent. Various cell lines were transfected using *Trans*IT®-LT1 Transfection Reagent with an EGFP expression vector in complete growth media with no media changes and analyzed by flow cytometry.

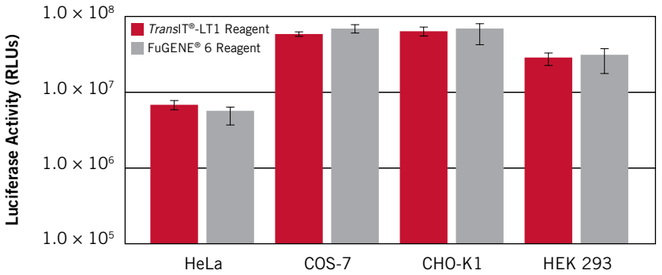


FIGURE 7. The *Trans*IT®-LT1 Reagent Performs Comparably to the FuGENE® 6 Reagent. The indicated cell lines were transfected in duplicate with a luciferase reporter vector (1 µg per well) using either *Trans*IT®-LT1 or FuGENE® 6 Transfection Reagents (3 µl each). Twenty-four hours post-transfection the cells were harvested and assayed for luciferase activity.

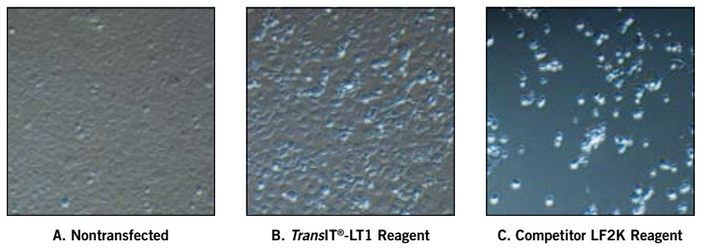


FIGURE 8. The *Trans*IT®-LT1 Reagent Exhibits Low Cellular Toxicity Compared to Another Leading Transfection Reagent. Compared to the nontransfected COS-7 cells (A), the LF2K Reagent transfected COS-7 cells (C) exhibited extreme cellular toxicity as evidenced by the rounded and missing cells in the sample. In contrast, the *Trans*IT®-LT1 Reagent transfected COS-7 (B) cells exhibited minimal cellular toxicity.

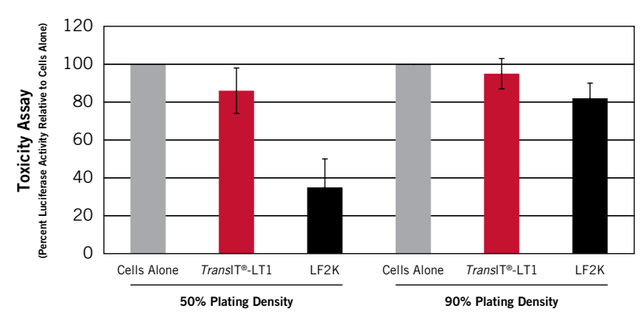


FIGURE 9. High Cell Viability with the *Trans*IT®-LT1 Reagent Assayed using the CellTiter-Glo® Cell Viability Assay. HeLa cells at 50% and 90% confluency were transfected in duplicate in three separate experiments using either *Trans*IT®-LT1 Reagent or Lipofectamine™ 2000 (LF2K). Cells were harvested 24 hours post-transfection, and cell viability measured using the CellTiter-Glo® Luminescent Cell Viability Assay. Data were scaled to untransfected HeLa controls (cells alone), averaged and presented as a percent of untransfected control.

Label IT® siRNA TRACKER INTRACELLULAR NUCLEIC ACID LOCALIZATION KITS

- ▷ **Superior Tracking and Knockdown—**Monitor both subcellular localization and functionality of your siRNA or duplex miRNA following transfection
- ▷ **High Efficiency Labeling—**Optimal visualization of siRNA or duplex miRNA in cells
- ▷ **One-step Chemical Method—**Easily and precisely control the labeling density
- ▷ **Save Money—**Significant savings on kits that include transfection reagents

Description

The *Label* IT siRNA Tracker Intracellular Nucleic Acid Localization Kits provide a straightforward approach to directly label and deliver siRNA in an efficient and non-destructive manner for *in vitro* or *in vivo* tracking experiments. Intracellular localization and functional inhibition of target gene expression can be monitored following introduction of the labeled siRNA or duplex miRNA into mammalian cells. *Label* IT siRNA Tracker Kits are also available with either *Trans*IT®–TKO or *Trans*IT®-siQUEST RNAi Transfection Reagents for delivery of labeled siRNA or duplex miRNA into mammalian cells.

COMPONENTS

Label IT® siRNA Tracker Reagent

Reconstitution Solution

Labeling Buffer A

siRNA Dilution Buffer

May also contain *Trans*IT®-TKO OR *Trans*IT®-siQUEST Transfection Reagent

STORAGE CONDITIONS

Store *Label* IT® reagent as a dry pellet or as a reconstituted solution at -20°C

All other components store at 4°C

PRODUCT SPECIFICATIONS

Label IT® siRNA Tracker Intracellular Localization Kits

LABEL	PRODUCT NO.	SIZE
Cy™3	MIR 7212	50 µg
Cy™5	MIR 7213	50 µg
Fluorescein	MIR 7216	50 µg
CX-Rhodamine	MIR 7214	50 µg
TM-Rhodamine	MIR 7215	50 µg
Biotin	MIR 7217	50 µg

Label IT® siRNA Tracker Intracellular Localization Kits with *Trans*IT®-TKO Transfection Reagent

LABEL	PRODUCT NO.	SIZE*
Cy™3	MIR 7200	50 µg
Cy™5	MIR 7201	50 µg
Fluorescein	MIR 7205	50 µg
CX-Rhodamine	MIR 7202	50 µg
TM-Rhodamine	MIR 7203	50 µg
Biotin	MIR 7204	50 µg

Label IT® siRNA Tracker Intracellular Localization Kits with *Trans*IT®-siQUEST Transfection Reagent

LABEL	PRODUCT NO.	SIZE*
Cy™3	MIR 7206	50 µg
Cy™5	MIR 7207	50 µg
Fluorescein	MIR 7210	50 µg
CX-Rhodamine	MIR 7208	50 µg
TM-Rhodamine	MIR 7209	50 µg
Biotin	MIR 7211	50 µg

* Each kit contains sufficient quantities to label 50 µg of siRNA and perform at least 500 transfections in 24-well plates.

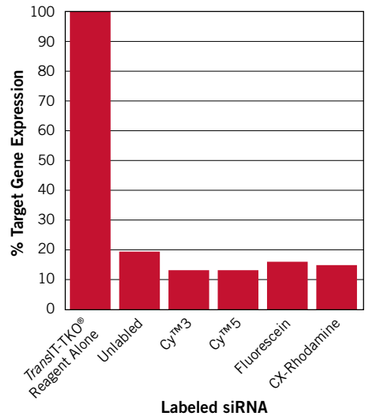


FIGURE 10. siRNA Labeled with *Label* IT® siRNA Tracker Maintain Knockdown Activity While Monitoring Subcellular Localization. *Trans*IT®-TKO Transfection Reagent was used to transfect anti-firefly luciferase siRNA into CHO-luc cells that stably express the firefly luciferase protein. The siRNA was either unlabeled or labeled with *Label* IT® siRNA Tracker Cy™3, Cy™5, Fluorescein, or CX-Rhodamine reagent. Bars indicate the percent firefly luciferase expression 24 hours after delivery of 5 nM anti-firefly luciferase siRNA.

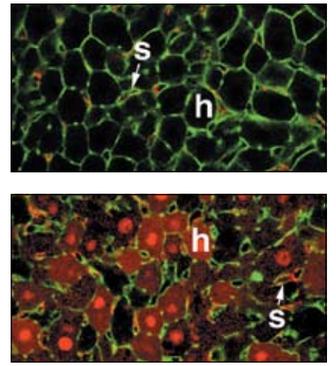


FIGURE 11. Visualization of *Label* IT® siRNA Tracker Cy™3 Labeled siRNA in Tissue Sections. Twenty-five µg siRNA was labeled post-synthetically with the *Label* IT® siRNA Tracker Cy™3 Kit and delivered into mice (25 µg/mouse; 25 µg/x mice) through the tail vein using either low volume injection (100 µl, top panel) or hydrodynamic injection conditions (2 ml over 6–8 seconds, bottom panel). Livers were harvested 30 minutes after injection, fixed, sectioned and analyzed via confocal microscopy. Representative hepatocytes (h) and sinusoid cells (s) are indicated. The hydrodynamic injection of siRNA results in siRNA uptake (red) by a majority of the liver hepatocytes

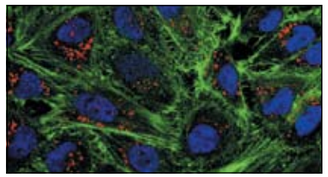


FIGURE 12. Visualization of *Label* IT® siRNA Tracker Cy™3 Labeled siRNA in Tissue Culture. HeLa cells were transfected with *Label* IT® siRNA Tracker Cy™3 labeled siRNA and *Trans*IT®-siQUEST Transfection Reagent in complete media for 24 hours. Cells were then fixed and counterstained to locate the nuclei (blue) and actin (green). The image was acquired on a confocal microscope.

Label IT® TRACKER INTRACELLULAR NUCLEIC ACID LOCALIZATION KITS

- ▷ **Superior Visualization and shRNA/siRNA Expression**—Monitor both subcellular localization and shRNA/siRNA expression following transfection of labeled plasmid DNA
- ▷ **Versatile Labeling**—Efficiently label and visualize any shRNA/siRNA expression plasmid
- ▷ **One-step Chemical Method**—Easily and precisely control the labeling reactions
- ▷ **High Efficiency Labeling**—Optimal visualization of plasmid in cells

Description

The *Label* IT Tracker Intracellular Nucleic Acid Localization Kits provide a straight forward approach to directly label and deliver shRNA/siRNA plasmid DNA in an efficient and non-destructive manner for *in vitro* or *in vivo* experiments. Both subcellular localization and shRNA/siRNA expression can be monitored simultaneously following introduction of the labeled plasmid into mammalian cells.

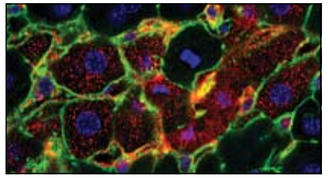


FIGURE 13. Visualization of *Label* IT® Tracker Cy™3 Labeled Plasmid in Liver Section Following Tail Vein Injection. Plasmid DNA (red) was labeled with the *Label* IT® Tracker Cy™3 Intracellular Nucleic Acid Localization Kit and delivered to mouse liver using the hydrodynamic tail vein injection procedure. One hour post-injection the liver was harvested, fixed, sectioned and counterstained to locate the nuclei (blue) and actin (green).

PRODUCT SPECIFICATIONS

Label IT® Tracker Intracellular Nucleic Acid Localization Kits

LABEL	PRODUCT NO.	SIZE*
Cy™3	MIR 7010	50–200 µg
Cy™5	MIR 7011	50–200 µg
Fluorescein	MIR 7015	50–200 µg
CX-Rhodamine	MIR 7012	50–200 µg
TM-Rhodamine	MIR 7013	50–200 µg
Biotin	MIR 7014	50–200 µg

* Total amount of DNA labeled.

COMPONENTS

Label IT® Tracker Reagent
*Trans*IT®-LT1 Transfection Reagent
Reconstitution Solution
Labeling Buffer A

STORAGE CONDITIONS

Store *Label* IT® reagent as a dry pellet or as a reconstituted solution at –20°C

All other components store at 4°C

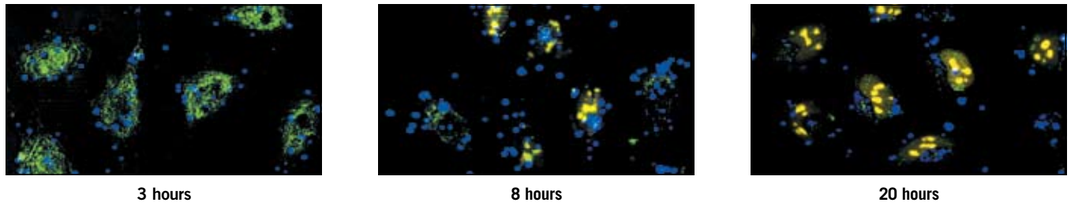


FIGURE 14. Tracking of Plasmid Localization and Expression. COS-7 cells were transfected with *Label* IT® Tracker Cy™5 labeled EYFP-nuc and *Trans*IT®-LT1 Transfection Reagent in complete media. Images were acquired at 3, 8, and 20 hours post-transfection. The blue staining indicates the cellular localization of the labeled plasmid while the yellow signal is the expression of the nuclear yellow fluorescent protein (EYFP) reporter.

*Trans*IT®-QR DELIVERY SOLUTION AND STARTER KIT

- ▷ **Potent Gene Knockdown**—High efficiency delivery of siRNA or shRNA/siRNA expression plasmid promoting target gene knockdown
- ▷ **Versatile Platform**—Delivers siRNA or shRNA/siRNA expression plasmids via hydrodynamic tail vein injection to the mouse strain of your choice
- ▷ **Low Toxicity**—Minimized loss of cardiac output compared to saline injections, which allows for quick recovery (within minutes) of the mouse post-injection

Description

A non-toxic, "Quick Recovery" *in vivo* delivery solution for the delivery of DNA or siRNA to the livers of laboratory mice using hydrodynamic tail vein injection.

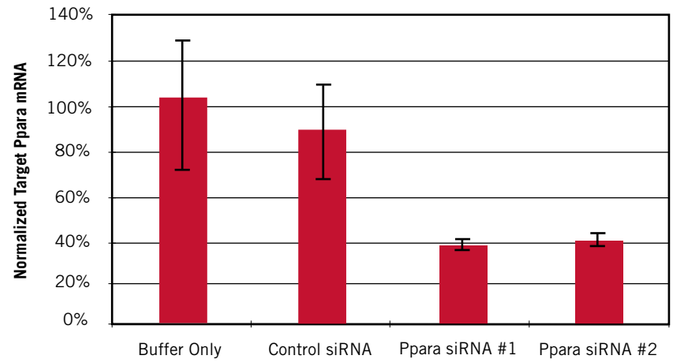


FIGURE 15. Endogenous Target Knockdowns Are Achievable in the Liver Using Hydrodynamic Delivery of siRNA. Two different siRNAs directed against PPAR-alpha or a non-targeting control siRNA (40 µg each siRNA per mouse) were delivered via the hydrodynamic tail vein injection procedure to mice using the *Trans*IT®-QR Hydrodynamic Delivery Solution. Twenty-four hours post-injection, the livers were harvested and the PPAR-alpha levels were determined using qRT-PCR and averaged for each group.

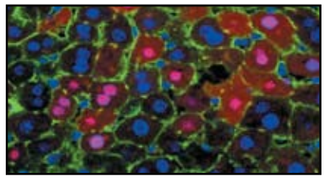


FIGURE 16. *Trans*IT®-QR Hydrodynamic Delivery Solution Effectively Delivers the *Label* IT® Cy™3 RNAi Delivery Control to Hepatocytes. The *Trans*IT®-QR Hydrodynamic Delivery Solution was used to deliver 25 µg of *Label* IT® Cy™3 RNAi Delivery Control (red) to a mouse liver using hydrodynamic tail vein injection. Forty-five minutes post-injection, the liver was harvested, sections were fixed and counterstained for nuclei (blue) and actin (green).

PRODUCT SPECIFICATIONS

_Trans_IT®-QR Starter Kit

PRODUCT NO.	QUANTITY
MIR 5210	10 Injections

_Trans_IT®-QR Delivery Solution

PRODUCT NO.	QUANTITY
MIR 5240	40 Injections

Certified RNase-, DNase- and endotoxin-free

COMPONENTS

The Starter Kit contains:

*Trans*IT®-QR Delivery Solution
10 Needles
10 Syringes
10 Alcohol Swabs
Mouse Restraint Device

STORAGE CONDITIONS

Store at room temperature

DNA & siRNA LABELING ▷ Prelabeled Delivery Controls

Label IT® RNAi DELIVERY CONTROLS

- ▷ **Sensitive**—Easy visualization to 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

Description

The *Label* IT RNAi Delivery Controls consist of either Cy3 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 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.

PRODUCT SPECIFICATIONS

***Label* IT® RNAi Delivery Controls**

LABEL	PRODUCT NO.	QUANTITY
Cy™3	MIR 7900	10 µg
	MIR 7901	100 µg
Fluorescein	MIR 7902	10 µg
	MIR 7903	100 µg

COMPONENTS

Label IT® RNAi Delivery Control

STORAGE CONDITIONS

Store at –20°C

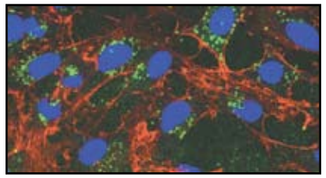


FIGURE 17. Visualization of *Label* IT® RNAi Delivery Control. HeLa cells were transfected in complete media with the *Label* IT® Fluorescein RNAi Delivery Control (green) using the *Trans*IT®-TKO Transfection Reagent. Twenty-four hours post-transfection, the cells were fixed, then counterstained to locate the nuclei (blue) and actin (red).

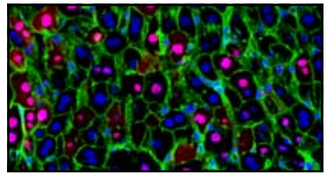


FIGURE 18. Visualization of *Label* IT® Cy™3 RNAi Delivery Control in Liver Sections Following Tail Vein Injection. *Trans*IT®-QR Hydrodynamic Delivery Solution was used to deliver 25 µg of *Label* IT® Cy™3 RNAi Delivery Control (red) to a mouse using hydrodynamic tail vein injection. Forty-five minutes post-injection the liver was harvested. Sections were fixed and counterstained to locate the nuclei (blue) and actin (green). The image was acquired using a confocal microscope.

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 concentration solution for both *in vitro* and *in vivo* tracking studies

Description

The *Label* IT Plasmid Delivery Controls consist of either Cy3 or fluorescein labeled 2.7 kb plasmid. These controls facilitate assessment of delivery efficiency of plasmid DNA in both *in vitro* and *in vivo* applications.

PRODUCT SPECIFICATIONS

***Label* IT® Plasmid Delivery Controls**

LABEL	PRODUCT NO.	QUANTITY
Cy™3	MIR 7904	10 µg
	MIR 7905	100 µg
Fluorescein	MIR 7906	10 µg
	MIR 7907	100 µg

COMPONENTS

Label IT® Plasmid Delivery Control

STORAGE CONDITIONS

Store at –20°C

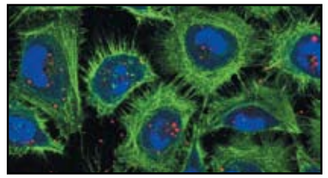


FIGURE 19. The *Label* IT® Cy™3 Plasmid Delivery Control Allows Quick Assessment of Delivery Efficiency. HeLa cells were transfected in complete media with the *Label* IT® Cy™3 Plasmid Delivery Control (red) using the *Trans*IT®-LT1 Transfection Reagent. Twenty-four hours post-transfection, the cells were fixed, then counterstained to locate the nuclei (blue) and actin (green).

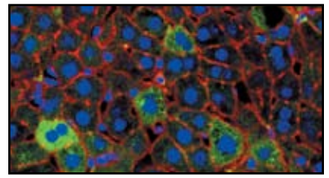


FIGURE 20. Visualization of *Label* IT® Fluorescein Plasmid Delivery Control in Liver Sections Following Tail Vein Injection. *Trans*IT®-QR Hydrodynamic Delivery Solution was used to deliver 25 µg of *Label* IT® Fluorescein Plasmid Delivery Control (green) to a mouse using hydrodynamic delivery via the tail vein. One hour post-injection the liver was harvested. Sections were fixed and counterstained to locate the nuclei (blue) and actin (red). The image was acquired using a confocal microscope.

miRNA LABELING ▷ miRNA Expression Profiling

Label IT® miRNA LABELING KITS

- ▷ **Accurate**—Labels all miRNAs present in the sample
- ▷ **Universal**—Labels miRNAs from all organisms, including plants
- ▷ **Sequence Independent Labeling**—Labels all nucleotides with equal efficiency
- ▷ **Sensitive**—Detects subfemtamole amounts of miRNA species
- ▷ **Saves Time**—Simple, one-step, one hour protocol
- ▷ **Reproducible**—Generate consistent, high quality miRNA microarray data
- ▷ **Validated For Use With The Following Arrays:**
  - ◇ **Species specific MicroRNA 4 × 2K Microarrays**—CombiMatrix
  - ◇ **miRMAX miRNA Microarrays X-Species Arrays**—Rutgers University
  - ◇ **NCode™ Multi-Species miRNA Microarrays**—Invitrogen
  - ◇ ***mir*Vana™ miRNA Bioarrays**—Ambion
  - ◇ **FlexmiR Human Panel**—Luminex

Description

The *Label* IT miRNA Labeling Kits enable rapid and efficient covalent labeling of microRNA (miRNA) from mammalian and plant cells for expression profiling. These kits have been optimized for the accurate detection of miRNAs on microarrays.

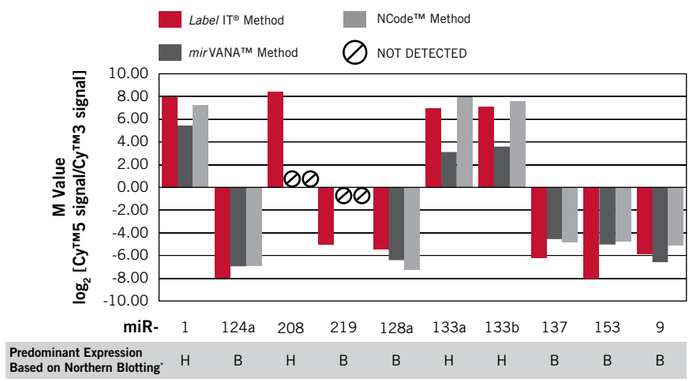


FIGURE 21. Accurately Detect All miRNAs in Your Sample. Discrepant microarray miRNA profiles obtained from chemical and enzymatic labeling methods. miRNA-enriched mouse heart and brain samples were hybridized to miRNA microarrays after labeling with either *Label* IT® alkylation or *mir*Vana™ (Ambion) or NCode™ (Invitrogen) enzymatic methods. Positive relative expression values expressed as the $\log_2$ transformed ratio of heart/brain signal represent mouse miRNAs differentially expressed in heart tissue, while negative values correspond to miRNAs differentially expressed in brain. The tissue specificity of each miRNA, as determined by northern blot analysis* is also presented.

* Sempere, L.F., S. Freemantle, I. Pitha-Rowe, E. Moss, E. Dmitrovsky, and V. Ambros. 2004 *Genome Biology* **5(3):**R13.

PRODUCT SPECIFICATIONS

***Label* IT® miRNA Labeling Kits**

LABEL	PRODUCT NO.	SIZE*
Cy™3/Cy™5	MIR 8305	2 × 5 µg
	MIR 8325	2 × 25 µg
Cy™3	MIR 8510	10 µg
	MIR 8550	50 µg
Cy™5	MIR 8610	10 µg
	MIR 8650	50 µg
Biotin	MIR 8410	10 µg
	MIR 8450	50 µg

* Total amount of miRNA labeled

COMPONENTS

Label IT® Reagent(s)
Reconstitution Solution
10X Labeling Buffer M
10X Stop Solution
Precipitation Enhancer Solution
1X Hybridization Solution

STORAGE CONDITIONS

Label IT® reagent as a dry pellet, or as a reconstituted solution, store at –20°C

All other components store at 4°C

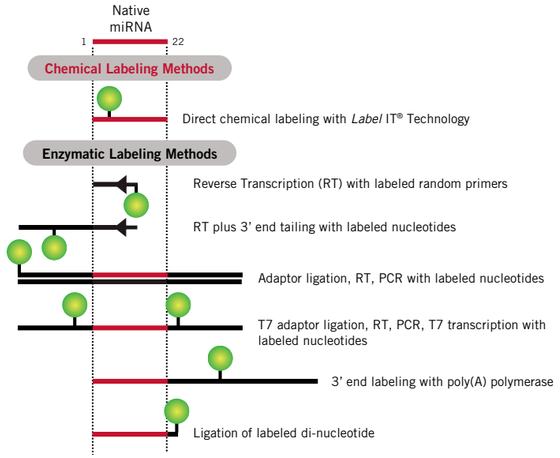


FIGURE 22. Schematic Representation of Chemical and Enzymatic miRNA Labeling Methods. Direct chemical labeling using the *Label* IT® miRNA Labeling Kit maintains the integrity of the original miRNA species. Extraneous nucleotides added by enzymatic labeling or truncated reverse transcription products may decrease hybridization performance and increase non-specific hybridization.

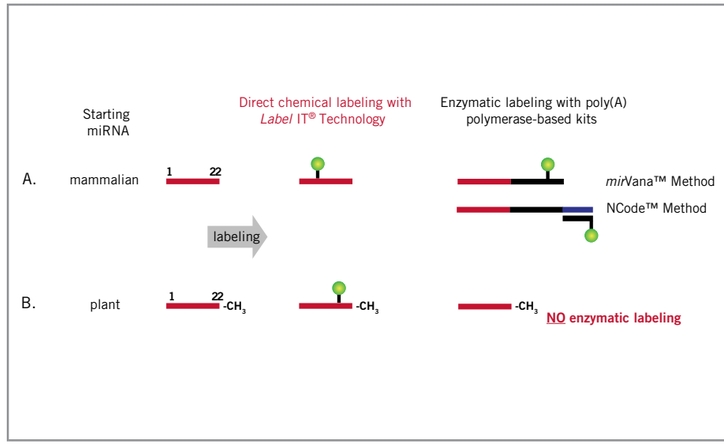


FIGURE 23. Efficiently Label Plant miRNAs Independent of 3' Methylation. Mammalian miRNAs can be labeled using direct chemical labeling (*Label* IT® miRNA Labeling Kits) or enzymatic methods. The poly(A) polymerase-based methods generate long 3' tails which dramatically extend the length of the miRNA species. The *Label* IT® reagents covalently modify plant and mammalian miRNAs at internal sites. However, due to their endogenous 3' methylation, plant miRNAs can NOT be efficiently labeled using poly(A) polymerase-based methods.

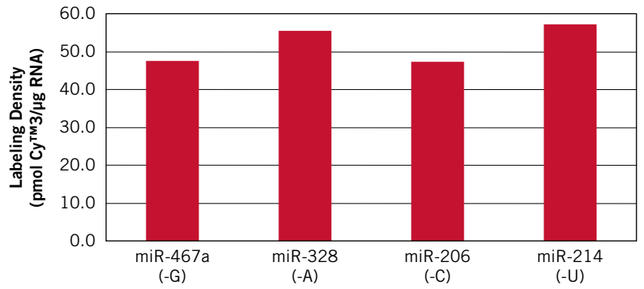


FIGURE 24. Sequence Independent Labeling of miRNA. Synthetic RNA representing miRNAs with no Gs (miR-467a), no As (miR328), no Us (miR-214) and no Cs (miR-206) were labeled in triplicate using *Label* IT® miRNA Labeling Kit, Cy™3, purified and spectophotometrically measured to estimate labeling density (pmol Cy™3/µg RNA). Average labeling densities are plotted. Similar results were observed with the *Label* IT® miRNA Labeling Kit, Cy™5.

siRNA and miRNA Transfection

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

Broad Spectrum DNA Transfection

Product Name	Product No.	Quantity
*Trans*IT®-LT1 Transfection Reagent	MIR 2300	1 ml
	MIR 2304	0.4 ml
	MIR 2305	5 X 1 ml
	MIR 2306	10 X 1 ml
*Trans*IT®-LT2 Transfection Reagent	MIR 2400	1 ml
	MIR 2404	0.4 ml
	MIR 2405	5 X 1 ml
	MIR 2406	10 X 1 ml
*Trans*IT®-Express Transfection Reagent	MIR 2000	1 ml
	MIR 2004	0.4 ml
	MIR 2005	5 X 1 ml
	MIR 2006	10 X 1 ml

Cellular Imaging and siRNA Localization

Kits Without Transfection Reagent

Product Name	Product No.	Quantity
Label IT® siRNA Tracker Cy™3 Kit	MIR 7212	Labels 50 µg
Label IT® siRNA Tracker Cy™5 Kit	MIR 7213	Labels 50 µg
Label IT® siRNA Tracker CX-Rhodamine Kit	MIR 7214	Labels 50 µg
Label IT® siRNA Tracker TM-Rhodamine Kit	MIR 7215	Labels 50 µg
Label IT® siRNA Tracker Fluorescein Kit	MIR 7216	Labels 50 µg
Label IT® siRNA Tracker Biotin Kit	MIR 7217	Labels 50 µg

Kits Including *Trans*IT-TKO® Transfection Reagent

Product Name	Product No.	Quantity
Label IT® siRNA Tracker Cy™3 Kit	MIR 7200	Labels 50 µg
Label IT® siRNA Tracker Cy™5 Kit	MIR 7201	Labels 50 µg
Label IT® siRNA Tracker CX-Rhodamine Kit	MIR 7202	Labels 50 µg
Label IT® siRNA Tracker TM-Rhodamine Kit	MIR 7203	Labels 50 µg
Label IT® siRNA Tracker Fluorescein Kit	MIR 7205	Labels 50 µg
Label IT® siRNA Tracker Biotin Kit	MIR 7204	Labels 50 µg

Each kit contains sufficient quantities to perform at least 500 transfections in 24-well plates.

Kits Including *Trans*IT-siQUEST® Transfection Reagent

Product Name	Product No.	Quantity
Label IT® siRNA Tracker Cy™3 Kit	MIR 7206	Labels 50 µg
Label IT® siRNA Tracker Cy™5 Kit	MIR 7207	Labels 50 µg
Label IT® siRNA Tracker CX-Rhodamine Kit Reagent	MIR 7208	Labels 50 µg
Label IT® siRNA Tracker TM-Rhodamine Kit	MIR 7209	Labels 50 µg
Label IT® siRNA Tracker Fluorescein Kit	MIR 7210	Labels 50 µg
Label IT® siRNA Tracker Biotin Kit	MIR 7211	Labels 50 µg

Each kit contains sufficient quantities to perform at least 500 transfections in 24-well plates.

Cellular Imaging and DNA Localization

Product Name	Product No.	Quantity
Label IT® Tracker™ Cy™3 Kit	MIR 7010	Labels 50-200 µg
Label IT® Tracker™ Cy™5 Kit	MIR 7011	Labels 50-200 µg
Label IT® Tracker™ Fluorescein Kit	MIR 7015	Labels 50-200 µg
Label IT® Tracker™ CX-Rhodamine Kit	MIR 7012	Labels 50-200 µg
Label IT® Tracker™ TM-Rhodamine Kit	MIR 7013	Labels 50-200 µg
Label IT® Tracker™ Biotin Kit	MIR 7014	Labels 50-200 µg

Labeled RNAi Delivery Controls

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

Labeled Plasmid Delivery Controls

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

miRNA Expression Profiling

Product Name	Product No.	Quantity
Label IT® miRNA Labeling Kit, Cy3™/Cy5™	MIR 8305	Labels 2 x 5 µg
	MIR 8325	Labels 2 x 25 µg
Label IT® miRNA Labeling Kit, Cy3™	MIR 8510	Labels 10 µg
	MIR 8550	Labels 50 µg
Label IT® miRNA Labeling Kit, Cy5™	MIR 8610	Labels 10 µg
	MIR 8650	Labels 50 µg
Label IT® miRNA Labeling Kit, Biotin	MIR 8410	Labels 10 µg
	MIR 8450	Labels 50 µg

IN VIVO DELIVERY

siRNA or shRNA/siRNA Expression Plasmid

Product Name	Product No.	Quantity
*Trans*IT® QR Delivery Solution	MIR 5240	40 Injections
*Trans*IT® QR Delivery Starter Kit	MIR 5210	10 Injections

Mirus
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