Label IT® RNAi Delivery Control

Product Name	Label	Quantity	Product No.
Label IT® RNAi Delivery Control	Cy™3	10 µg (~0.75 nmol)	MIR 7900
	Cy™3	100 µg (~7.5 nmol)	MIR 7901
	Fluorescein	10 µg (~0.75 nmol)	MIR 7902
	Fluorescein	100 µg (~7.5 nmol)	MIR 7903

The *Label* IT® RNAi Delivery Control is provided as a 10 µM stock in RNAi Dilution Buffer (50 mM Tris pH 7.5, 100 mM NaCl). This kit is also supplied with 10X RNAi Dilution Buffer. MIR 7900 and MIR 7902 are formatted for small scale applications. For example, 100 wells of a 24-well plate, containing 300 µl of media, can be transfected with the *Label* IT® RNAi Delivery Control at 25 nM final concentration per well. MIR 7901 and MIR 7903 are formatted for large scale *in vitro* transfections as well as *in vivo* delivery of the *Label* IT® RNAi Delivery Controls.

1.0 DESCRIPTION

1.1 General Information

The introduction of short RNA duplexes into mammalian cells in culture leads to sequence-specific destruction of target mRNA without triggering an interferon response. These short double stranded (ds) RNAs, referred to as small interfering RNAs (siRNA), can act catalytically at sub-molar ratios to cleave greater than 95% of the target mRNA in the cell and destruction of the mRNA target can ultimately lead to decreased expression of the encoded protein. The RNA interference (RNAi) effect can be long-lasting and may be detectable after many cell divisions. These properties make siRNA extremely effective at inhibiting target gene expression once introduced into the cell.[1,2,3]

The *Label* IT® RNAi Delivery Control consists of Cy™3- or fluorescein-labeled double-stranded RNA duplexes that have the same length, charge, and configuration as standard siRNA used in RNAi studies. The sequence of the *Label* IT® RNAi Delivery Control is not homologous to any known mammalian gene and is not known to affect any cellular events. The *Label* IT® RNAi Delivery Control is designed as a tool to facilitate visualization and optimization of dsRNA oligonucleotide delivery during RNAi experiments, both *in vitro* and *in vivo*. It is also suitable for co-delivery with functional target-gene specific siRNA and should not affect the RNAi-mediated inhibition of the target gene.

The *Trans*IT-TKO® and *Trans*IT®-siQUEST™ Transfection Reagents (see Related Products Section) are specifically formulated for siRNA delivery to cells. These reagents enable highly efficient siRNA transfection with significantly reduced levels of cell damage when compared to cationic liposome-based transfection reagents. Transfections are most effective when carried out in complete growth media, with no media change or serum addition required. When siRNA is complexed with either of these reagents, reduced target mRNA levels in a variety of cell types can be observed. These unique features make the *Trans*IT-TKO® and *Trans*IT®-siQUEST™ Transfection Reagents ideal for all siRNA-mediated gene silencing studies, including those involving the *Label* IT® RNAi Delivery Controls.

In mice, efficient *in vivo* delivery to select tissues can be obtained using a hydrodynamic injection protocol. In this procedure, the *Label* IT® RNAi Delivery Control (in physiological saline) is rapidly injected into the tail vein of mice resulting in highly efficient delivery to liver hepatocytes.[4,5,6] An illustration of typical results that can be obtained by a tail-vein *in vivo* delivery procedure of Cy™3 labeled siRNA can be found at http://www.mirusbio.com/products/rnai/sitracker/index.asp.[7] Efficient siRNA delivery to limb skeletal muscle can also be achieved using an intravenous delivery injection procedure.[8]

1.2 Specifications

Storage: Store the *Label* IT® RNAi Delivery Control at -20ºC, protected from exposure to light. Store the 10X RNAi Dilution Buffer at 4ºC.

Stability: The *Label* IT® RNAi Delivery Controls and the 10X RNAi Dilution Buffer are stable for 6 months from the date of purchase, if used and stored properly. To ensure stability of the product, use RNase-free equipment and proper laboratory technique.

2.0 PROCEDURE

2.1 *In Vivo* Delivery

The *Label* IT® RNAi Delivery Control can be used with a variety of *in vivo* siRNA delivery methods, including hydrodynamic injection of siRNA in the mouse tail vein for efficient delivery to liver hepatocytes[4,7] as well as intravenous delivery to limb skeletal muscle.[8] Please refer to literature references (Section 4.0) for delivery and visualization procedures.

2.2 *In Vitro* Transfection

A. Cell Plating

Mirus Bio recommends plating the cells, prior to transfection, on poly-D-lysine coated coverslips (for a detailed description, please refer to the *Label* IT® siRNA Tracker Kit protocol, available at www.mirusbio.com).

B. Sample Preparation

1. Immediately prior to use, thaw the vial of the *Label* IT® RNAi Delivery Control on ice.
2. Dilute the 10 µM stock *Label* IT® RNAi Delivery Control 10-fold using the 10X RNAi Dilution Buffer (provided) to make a 1 µM working solution. Dilute only as much of the stock *Label* IT® RNAi Delivery Control as required for the immediate experiment(s) and discard any remaining diluted Delivery Control.
   NOTE: For optimal visualization, a final concentration of 25 nM per well is recommended.
3. After use, return the stock solution to -20ºC for storage.

C. Optimal Transfection

The *Label* IT® RNAi Delivery Controls can be directly substituted into standard *in vitro* transfection protocols. Mirus Bio recommends the broad-spectrum *Trans*IT-TKO® or *Trans*IT®-siQUEST™ Transfection Reagents (see Related Products Section) to deliver the *Label* IT® RNAi Delivery Controls. The key to successful transfection is careful optimization of reaction conditions for individual cell type. Please refer to the *Trans*IT-TKO® or *Trans*IT®-siQUEST™ Transfection Reagent protocols (available at www.mirusbio.com) for detailed instructions for *in vitro* RNAi control delivery. If using another manufacturer's transfection reagent, follow their transfection protocol. For a starting recommendation, use 25nM RNAi control per well.

NOTE: If using electroporation, substitute the *Label* IT® RNAi Delivery Control into your optimized siRNA electroporation protocol. As a starting point, we recommend using 10 µl of the 10 µM stock per standard electroporation (using 1 x 10^5 to 5 x 10^6 cells). Prior to use in electroporation, Mirus Bio recommends ethanol precipitation purification of the required amount of the *Label* IT® siRNA Delivery Control to remove buffer salts which may adversely affect electroporation. Briefly, bring the required volume of *Label* IT® RNAi Delivery Control to at least 100 µl with MB-grade water, add glycogen to a final concentration of 100 µg/ml, and 2.5X volume of ice-cold ethanol. Incubate -20ºC (or colder) for at least 1 hour. Pellet the Delivery Control by centrifugation for 30 minutes at max speed at 4ºC. Wash the pellet with 70% ethanol, and resuspend the pellet in the required volume of molecular biology grade water.

2.3 Detection of *Label* IT® RNAi Delivery Control in Transfected Cells (on mounted coverslips)

NOTE: For suspension cells, fix and wash cells in a microfuge tube. Pellet cells by gentle centrifugation between washes. To visualize suspension cells by microscopy, apply cells to a Poly-d-lysine (PDL) coated slide to aid in the adherence of the cells to the surface. Apply a non-PDL treated coverslip over cells and seal as described below.

A. Detection Optimization

Assess the distribution of the fluorescent signal of the *Label* IT® RNAi Delivery Control in the transfected cells 4 to 24 hours post-transfection. The strength of the fluorescent signal may depend on several factors including transfection efficiency, amount of labeled control used, growth rate of the cells, and incubation time post-transfection. To obtain a strong fluorescent signal, it may be necessary to vary the final concentration of the *Label* IT® RNAi Delivery Control used in the transfection from 10 to 100 nM, depending on the cell line and transfection reagent used.

B. Cell Fixation (For 24-well Plates)

NOTE: Protect cells from light to prevent loss of fluorescent signal. These recommendations are for 24-well plates. If using a different well size, scale all volumes and amounts according to the surface area of the well.

1. Make fresh 4% (wt:vol) formaldehyde in PBS (commercial stocks are usually 37% (wt:vol)) and store at 4°C until ready to use.
2. Wash the transfected cells twice with PBS.
3. Fix cells in 0.25 ml per well 4% formaldehyde/PBS at room temperature for 20 minutes.
4. Aspirate formaldehyde and gently wash cells 3 times with PBS.
5. Add 0.25 ml PBS to each well.
6. For each well, mount the coverslip onto a glass slide (see Step C).

C. Slide Preparation

1. Using a small tip pap pen or nail polish, draw a complete circle on the glass slide. The diameter of the circle must be less than the diameter of the coverslip that will cover it.
2. Place a small drop of mounting solution in the center of each marked circle. Mirus Bio recommends antifade mounting solutions when using the fluorescein-labeled *Label* IT® RNAi Delivery Controls.
3. Remove a coverslip with forceps and gently wipe off the underside (non-cell side) with a Kimwipe® tissue.
4. Carefully mount the coverslip, cell-side down, onto the mounting solution.
5. Use capillary action to drain excess mounting solution from under the coverslip using a Kimwipe® tissue.
6. Seal all edges of the coverslip to the glass slide with nail polish or rubber cement.

D. Cell Visualization

View mounted coverslips on a fluorescent microscope using the appropriate filter sets. See Table 1 for fluorescent excitation and emission wavelengths for the *Label* IT® RNAi Delivery Controls.

Table 1. Excitation and emission wavelengths of *Label* IT® RNAi Delivery Controls

Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
Cy™3	549	570
Fluorescein	495	518

3.0 TROUBLESHOOTING

Transfection - Low Transfection Efficiency or High Cellular Toxicity

Please see *Trans*IT-TKO® or *Trans*IT®-siQUEST™ Transfection Reagent protocols for troubleshooting advice. If using another transfection reagent, refer to manufacturer's recommendations.

Tracking - Poor Visualization of the Label IT® RNAi Delivery Control in Cells

- **Improper storage of *Label* IT® RNAi Delivery Control**
  Store at -20°C, protected from light.
- **Compromised quality of *Label* IT® RNAi Delivery Control**
  Avoid RNA degradation by using RNase-free handling procedures and plasticware.
- **Excessive exposure to light**
  Protect samples and reagents from light.
- **Trouble detecting fluorescent signal**
  Use proper filter sets for microscopic detection. See Table 1. Confocal microscopy may distinguish signal that is inside the cells from that adhering to the outside of the cells.
- **Suboptimal transfection efficiency**
  See Section 2.1.
- **Suboptimal levels of *Label* IT® RNAi Delivery Control used**
  For *in vitro* transfection, use up to 100 nM (final concentration per well).
- **Cells lost during fixation or mounting procedure**
  Perform all washing, fixing, and m ounting steps gently. Check for presence of cells following each step using a light microscope.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801 or techsupport@mirusbio.com.
For a list of citations using Mirus Bio products, please visit the Technical Resources section of our website at www.mirusbio.com.

4.0 REFERENCES

1. Elbashir, S.M. et al. (2001) Nature **411:**494-498
2. Caplen, N.J. et al (2001) Prot. Natl. Acad. Sci. **98:**9742-9747
3. Sharp, P.A. (2001) Genes and Development **15:**485-490
4. Lewis et al. (2002) Nature Genetics, **32**:107-107
5. Zhang et al. (1999) Human Gene Therapy, **10**:1735-1737
6. Liu et al. (1999) Gene Therapy, **6**:1258-1266
7. http://www.mirusbio.com/products/rnai/sitracker/index.asp
8. Hagstrom et al. (2004) Molecular Therapy, **10**:386-398

5.0 RELATED PRODUCTS

RNA Interference Products: *

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

For endotoxin removal from DNA:*

MiraCLEAN® Endotoxin Removal Kit (Product MIR #5900)

For custom plasmid DNA tracking studies:

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

Transfection reagents:*

*Trans*IT®-293 Transfection Reagent (Product # MIR 2700)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)

*These products are available in additional sizes.