Label IT® Plasmid Delivery Control

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

The *Label* IT® Plasmid Delivery Control is supplied at 0.5 µg /µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). MIR 7904 and MIR 7906 are formatted for small scale *in vitro* applications. For example, using 0.5 µg per well, 20 wells of a 24-well plate can be transfected. MIR 7905 and MIR 7907 are formatted for large scale *in vitro* transfection applications as well as *in vivo* delivery of the labeled plasmid DNA; they provide sufficient material to transfect 50 wells in 6-well plates (using 2 µg per well), or up to 10 mice using the *Trans*IT® *In Vivo* Delivery System.

1.0 DESCRIPTION

1.1 General Information

The *Label* IT® Plasmid Delivery Control consists of Cy™3- or fluorescein-labeled plasmid DNA (2.7 kb, double stranded, circular plasmid). Each kit is designed as a tool to facilitate visualization and optimization of plasmid DNA delivery, for both *in vitro* and *in vivo* applications.

*Trans*IT® Transfection Reagents (see Related Products Section) are specifically formulated for plasmid DNA delivery to cells. They enable highly efficient plasmid DNA transfection with significantly reduced cytotoxicity 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. *Trans*IT® Transfection Reagents are recommended for all studies involving plasmid DNA transfection including those using the *Label* IT® Plasmid Delivery Controls.

In mice, efficient *in vivo* delivery of plasmid DNA to parenchymal cells in the liver (and reduced expression in the spleen, lung, heart and kidney) can be obtained using a hydrodynamic injection protocol. The *Trans*IT® *In Vivo* Delivery System (see Related Products Section) involves a single tail vein injection of complexed DNA and is the recommended procedure for delivery of plasmid DNA, such as the *Label* IT® Plasmid Delivery Controls, to the liver.[1,2,3]

1.2 Specifications

Storage: Store the *Label* IT® Plasmid Delivery Control at -20ºC, protected from exposure to light.

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

2.0 PROCEDURE

2.1 *In Vivo* Delivery

In mice, efficient *in vivo* delivery of plasmid DNA to select tissues can be obtained using the *Trans*IT® *In Vivo* Delivery System (see Related Products Section). In this procedure, the *Label* IT® Plasmid Delivery Control is complexed with the *Trans*IT® *In Vivo* Polymer Solution and delivered via hydrodynamic injection into the tail vein of mice. Hydrodynamic tail vein injection results in efficient delivery to liver hepatocytes, with lower levels of delivery to other organs including the spleen, kidney, lungs, and heart. The *Label* IT® Plasmid Delivery Control may also be used with alternative methods of *in vivo* delivery.

2.2 *In Vitro* Transfection

A. Cell Plating

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

B. Optimal Transfection

The *Label* IT® Plasmid Delivery Controls can be directly substituted into standard *in vitro* transfection protocols. Mirus Bio recommends the broad-spectrum *Trans*IT®-LT1 Transfection Reagent to deliver the *Label* IT ® Plasmid Delivery Controls. Cell-line specific transfection reagents (see Related Products Section) that have been optimized for specific cell lines or types are also available to achieve the highest attainable transfection efficiencies. The key to successful transfection is careful optimization of reaction conditions for individual cell types. Please refer to the *Trans*IT®-LT1 Transfection Reagent protocol (available at www.mirusbio.com) for detailed instructions for *in vitro* plasmid delivery. If using another manufacturer's transfection reagent, follow their protocol. For a starting recommendation, use 0.5 µg of the *Label* IT® Plasmid Delivery Control per well of a 24-well plate.

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

NOTE: For suspension cells, fix and wash cells in a microcentrifuge 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® Plasmid Delivery Control in the transfected cells 4 to 24 hours post-transfection. Longer time points may be beneficial. The strength of the fluorescent signal may depend on several factors including transfection efficiency, amount of labeled plasmid used, growth rate of the cells, and incubation time post-transfection. To obtain a strong fluorescent signal, it may be necessary to titrate the amount of *Label* IT® Plasmid Delivery Control transfected.

B. Cell Fixation (For 24-well Plates)

NOTE: Protect cells from exposure to 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 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® Plasmid 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® Plasmid Delivery Control.

Table 1. Excitation and emission wavelengths of *Label* IT® Plasmid 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 the relevant *Trans*IT® Transfection Reagent protocol for troubleshooting advice. If using another transfection reagent, follow the manufacturer's recommendations.

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

- **Improper storage of the *Label* IT® Plasmid Delivery Control**
  Store at -20°C, protected from light.
- **Compromised quality of plasmid DNA**
  Avoid DNA degradation by using proper handling procedures and nuclease-free plasticware.
- **Excessive exposure to light**
  Protect samples and reagents from light to prevent photobleaching.
- **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 2.2.
- **Suboptimal amount of *Label* IT® Plasmid Delivery Control**
  Increase the amount of *Label* IT® Plasmid Delivery Control transfected. Mirus Bio recommends starting with 0.5 µg DNA per well of a 24-well plate.
- **Cells lost during fixation or mounting procedure**
  Perform all washing, fixing, and mounting 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. Lewis et al. Nature Genetics, **32:**107-107 (2002)
2. Zhang et al. Human Gene Therapy, **10:**1735-1737 (1999)
3. Liu et al. Gene Therapy, **6:**1258-1266 (1999)

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.