

**Label IT<sup>®</sup> Tracker<sup>™</sup>**  
**Intracellular Nucleic Acid Localization Kit**

Product Name	Label IT <sup>®</sup> Tracker <sup>™</sup> Reagent	Product No.
Label IT <sup>®</sup> Tracker <sup>™</sup> Intracellular Nucleic Acid Localization Kit*	Cy <sup>™</sup> 3	MIR 7010
	Cy <sup>™</sup> 5	MIR 7011
	CX-Rhodamine	MIR 7012
	TM-Rhodamine	MIR 7013
	Biotin	MIR 7014
	Fluorescein	MIR 7015

\* Each Kit contains Label IT<sup>®</sup> Tracker<sup>™</sup> Reagent, Tracker Reconstitution Solution, 10X Labeling Buffer A, and TransIT<sup>®</sup>-LT1 Transfection Reagent.

### 1.0 INTENDED USE

The ability to easily track fluorescently labeled DNA in the cell has the potential to greatly enhance the understanding of exogenous DNA biology. This is particularly important in the non-viral gene delivery field where tremendous efforts have focused on improving cytoplasmic delivery and nuclear uptake of DNA delivery vectors. The Label IT<sup>®</sup> Tracker<sup>™</sup> Intracellular Nucleic Acid Localization Kit provides a straightforward approach to directly label and deliver plasmid DNA, of any design, in an efficient yet non-destructive manner, for tracking experiments. Both subcellular localization and reporter transgene expression can be monitored simultaneously following introduction of the labeled plasmid into mammalian cells. This kit provides sufficient reagents to label 50 to 200 µg of plasmid DNA, and TransIT<sup>®</sup>-LT1 Transfection Reagent which is enough to perform at least 50 transfections (1 µg/well) in 35 mm wells.

### 2.0 DESCRIPTION

#### 2.1 General Information

The Label IT<sup>®</sup> Tracker<sup>™</sup> Kits are based on Mirus' proprietary nucleic acid labeling technology. The Label IT<sup>®</sup> Reagents were initially developed by Mirus' scientists to monitor the subcellular localization of plasmid DNA in cells following gene delivery. These reagents covalently attach marker molecules to intact nucleic acids in a simple one-step chemical reaction. The simplicity of the highly efficient and non-destructive labeling reaction, and the availability of a variety of labels, including the very sensitive and highly popular Cy<sup>™</sup> dyes, makes this the ideal kit to generate labeled DNA for *in vitro* tracking experiments.

Traditional non-radioactive labeling methods (random priming, nick translation) are enzyme mediated and thus inherently difficult to control. In addition, these types of reactions generate labeled products that are not representative of the starting DNA, but rather consist of a series of labeled species over a variable size range. Using the non-enzymatic Label IT<sup>®</sup> Tracker<sup>™</sup> Kits, any plasmid (linear or circular) can be custom labeled before introduction into mammalian cells. This allows for the ideal situation in which expression-competent fluorescently labeled DNA can be visually monitored during the transfection process.

**Table 1. Excitation and emission wavelengths of the Label IT<sup>®</sup> Tracker<sup>™</sup> Reagents**

Labeling Reagent	Excitation Wavelength (nm)	Emission Wavelength (nm)
Fluorescein	492	518
5-carboxy-X-rhodamine	576	597
Tetramethyl-rhodamine	546	576
Cy <sup>™</sup> 3	550	570
Cy <sup>™</sup> 5	649	670

## 2.2 Materials Supplied

Component	Volume	reagent cap color
<i>Label IT</i> <sup>®</sup> Tracker <sup>™</sup> Reagent	dried pellet	varies with label
Tracker Reconstitution Solution*	60 µl	clear
10X Labeling Buffer A	500 µl	lilac
<i>TransIT</i> <sup>®</sup> -LT1 Transfection Reagent	400 µl	yellow

\*An extra 10 µl of Tracker Reconstitution Solution is supplied with each kit to allow for slight variations in pipetting devices.

## 2.3 Storage and Stability

The *Label IT*<sup>®</sup> Tracker<sup>™</sup> Kit should be stored at –20°C. The *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent should be stored at –20°C in both its dried pellet and reconstituted form. The *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent is stable for 6 months after reconstitution. Unreconstituted *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent and all other reagents, including the *TransIT*<sup>®</sup>-LT1 Transfection Reagent, are stable for 1 year from the date of purchase. Warm the *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent to room temperature and quick spin before each use. Warm the *TransIT*<sup>®</sup>-LT1 Reagent to room temperature and vortex before each use.

## 3.0 PROCEDURE

### 3.1 Plasmid Labeling Reaction

1. Bring the tube containing the *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent to room temperature and quick spin to collect the pellet since it may have become dislodged during shipping. Add 50 µl of Tracker Reconstitution Solution to the pellet in the tube. To ensure complete reconstitution of the pellet, mix well by gently.
2. Prepare the labeling reaction according to the example shown below. Use molecular biology-grade (i.e. DNase and RNase-free) water. Add the *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent last.

**Example:\***

sterile H <sub>2</sub> O	37.5 µl
10X Labeling Buffer A	5 µl
1 mg/ml plasmid DNA	5 µl
<i>Label IT</i> <sup>®</sup> Tracker <sup>™</sup> Reagent	<u>2.5 µl</u>

**Total volume: 50 µl**

\* This example uses a 0.5:1 (v:w) ratio of *Label IT*<sup>®</sup> Tracker Reagent to DNA. The final concentration of DNA in the reaction is 0.1 mg/ml.

**For DNA tracking applications, we recommend a range of 0.25 to 1 µl of *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent per µg of DNA (i.e. 0.25:1 to 1:1 (v:w)).**

\* The labeling reaction may be scaled up or down, depending on the amount of DNA to be labeled. The amount of *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent should be less than 20% of the total reaction volume.

3. Incubate reaction at 37°C for 1 hour.  
**Note:** When performing the labeling reaction, perform a quick spin after 30 minutes of incubation, to minimize the effect of evaporation and keep the concentration of the reaction components at the appropriate levels.
4. Remove unreacted *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent from the labeled plasmid by ethanol precipitation. Add 0.1 volume of 5 M sodium chloride and 2 volumes of ice cold 100% ethanol to the reaction. Mix well and place in a –20°C (or colder) freezer for at least 30 minutes.  
**Note:** For smaller reaction volumes (<100 µl), bring the volume up to 200 µl with 1X Labeling Buffer A or sterile H<sub>2</sub>O before adding the sodium chloride and ethanol.
5. Centrifuge at full speed in a refrigerated microcentrifuge for 10 minutes to pellet the labeled DNA. Gently remove the ethanol with a pipet; do not disturb the pellet.  
**Note:** Orient the precipitate-containing tubes in the microcentrifuge in such a way that it is known where the pellet forms. Small DNA quantities can be invisible to the naked eye.
6. Wash the pellet once with 500 µl room temperature 70% ethanol. After an additional centrifugation at full speed for 10 minutes, remove all traces of ethanol with a micropipetter. **Do not allow the sample to air dry extensively**, as the pellet may become extremely difficult to resuspend.
7. Resuspend the labeled probe in 10 µl 1X Labeling Buffer A or sterile water.
8. Quantify the concentration of the purified, labeled DNA on a spectrophotometer. Dilute to a suitable working concentration, if necessary.
9. Store the purified, labeled DNA at –20°C. Store on ice if needed for immediate use. Protect from light.

### 3.2 Mammalian Cell Transfection

Mirus' *TransIT*<sup>®</sup>-LT1 (Low Toxicity) Polyamine Transfection Reagent (provided) offers clear advantages for delivering DNA into cells via transfection, including high efficiency gene transfer, minimal cellular toxicity, ease of use, and transfection reproducibility. This product provides state-of-the-art transfection efficiencies with significantly reduced levels of cell damage compared to other leading transfection reagents. It is a broad-spectrum reagent based on a proprietary protein/polyamine formulation. In addition, transfections using the *TransIT*<sup>®</sup>-LT1 Reagent do not require media changes and should be carried out in serum-containing media. This unique combination makes this reagent ideal for all gene expression studies where the post-transfection state of the cell is important. Please consult Mirus' entire product line for cell-line specific and oligonucleotide transfection reagents. (see Section 7.0)

#### 3.2.1 Transfection Optimization

The key to any successful transfection is careful optimization of reaction conditions for each individual cell type. This transfection protocol will result in efficient transfection of most cell types and requires minimal optimization; however, to ensure optimal results the following variables should be considered:

- A. Cell density (confluence) at transfection** — The recommended cell density for most cell types at transfection is 40-70% confluence. The optimal cell density should be determined for each cell type in order to maximize transfection efficiency. This density should be maintained in future experiments for reproducibility.
- B. DNA concentration for transfection** — DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxins. The optimal DNA concentration for transfection is 1-2 µg per 35 mm well.
- C. *TransIT*<sup>®</sup> Reagent to DNA ratio** —As a starting point, use 3 µl of *TransIT*<sup>®</sup>-LT1 per µg of DNA. The optimal *TransIT*<sup>®</sup> Reagent to DNA ratio can be determined by titrating the reagent from 2-12 µl per µg DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections.
- D. Transfection Incubation Time**— The optimal incubation time can be determined empirically by testing durations ranging from 4-48 hours. Following transfection in most cell lines, labeled DNA complexes begin adhering to individual cells and is taken up (endocytosed) shortly thereafter. Transgene expression can be observed within the first few hours after transfection.

The protocol below is recommended for performing transfections with the *TransIT*<sup>®</sup>-LT1 Transfection Reagent in 35 mm wells. When performing transfections in different sized wells, the amounts of DNA, *TransIT*<sup>®</sup> Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish. See Table 2 for recommended starting conditions.

**Table 2. Recommended starting conditions for transfections using *TransIT*<sup>®</sup>-LT1 Transfection Reagent**

Culture Vessel	100 mm dish	60 mm dish	6-well plate (35 mm)	12-well plate	24-well plate	96-well plate
Surface area	55 cm <sup>2</sup>	21 cm <sup>2</sup>	9.4 cm <sup>2</sup>	3.8 cm <sup>2</sup>	1.9 cm <sup>2</sup>	0.32 cm <sup>2</sup>
Opti-MEM <sup>®</sup> 1 or other serum-free media	1.125 ml	375 µl	150 µl	75 µl	37.5 µl	15 µl
<i>TransIT</i> <sup>®</sup> -LT1 Reagent	45 µl	15 µl	6 µl	3 µl	1.5 µl	0.15-3 µl
DNA (1 µg/µl)	15 µl	5 µl	2 µl	1 µl	0.5 µl	0.05-0.1 µl
Complete Growth Media	15 ml	5 ml	2 ml	1 ml	0.5 ml	0.1 ml

#### 3.2.2 Protocol for Adherent Cell Transfections in 35 mm wells

##### A. Cell Plating

**Note:** It may be advantageous to include an unlabeled DNA and a "no DNA" control in the transfection experimental design.

Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10<sup>5</sup> cells in complete growth medium per 35 mm well) to obtain ~40-70% confluency the following day.

**Note:** For DNA tracking studies, since cells tend to adhere poorly to glass, we recommend plating the cells on poly-D-lysine (PDL) coated coverslips, which can be mounted for microscopic observation. Autofluorescence from tissue culture plastic tends to interfere with direct microscopic viewing of transfected cells.

1. Place one sterile coverslip in each 35 mm well.
2. Cover each coverslip with 1 ml of 0.1 mg/ml PDL prepared in sterile water (high molecular weight, generally greater than 3000K, e.g. Sigma cat#P7280)
3. Incubate at room temperature for 20 minutes.
4. Aspirate the PDL solution with a pipet.
5. Wash the coverslips with sterile water three times (free polymer may be cytotoxic) and allow to dry in a sterile culture hood.

6. When coverslips are completely dry, plate cells.
7. Incubate the cells overnight.<sup>b</sup>

**B. Complex Formation (perform this procedure immediately prior to transfection)**

**Note:** Warm the *TransIT*<sup>®</sup>-LT1 Reagent to room temperature and vortex before each use.

1. In a sterile, plastic 12 x 75 mm tube, add the *TransIT*<sup>®</sup>-LT1 Reagent (2-12  $\mu$ l per  $\mu$ g of DNA) dropwise into 100-200  $\mu$ l of serum-free medium<sup>c,e</sup> (Opti-MEM<sup>®</sup>I or RPMI 1640 from Gibco BRL are recommended for mammalian cell types). Mix thoroughly by pipetting.
2. Incubate at room temperature for 5-20 minutes.
3. Add the labeled DNA (1-2  $\mu$ g) from Section 3.1 to the diluted *TransIT*<sup>®</sup>-LT1 Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

**C. Cell Preparation for Transfections in Complete Growth Medium**

**Note:** For all cell lines tested, the *TransIT*<sup>®</sup>-LT1 Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated. For transfections in serum-free medium, proceed to part D.

1. If necessary, remove the medium from the cells prepared in step A and replace it with 2 ml of fresh complete growth medium per well.
2. Add the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture prepared in step B dropwise to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 4-48 hours.<sup>b</sup>

**Note:** The above incubation is designed for transfections performed with no media change. To perform a media change, incubate the cells for 4-8 hours, replace the original medium with fresh complete growth medium, and continue incubation.<sup>b,d</sup>

4. Fix, mount and view as described in Sections 3.3 and 3.4.

**D. Cell Preparation for Transfections in Serum-Free Medium**

1. Remove the complete medium from the cells prepared in step A and wash cells once with 2 ml per well of sterile PBS or serum-free medium.
2. Remove the wash solution and add 2 ml per well of fresh serum-free medium to the cells.
3. Add the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture prepared in step B to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
4. Incubate for 4-8 hours.<sup>b,d</sup>
5. Remove the medium containing the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture and replace it with complete growth medium.
6. Incubate for 4-48 hours.<sup>b</sup>
7. Fix, mount and view as described in Sections 3.3 and 3.4.

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**3.2.3 Protocol for Suspension Cell Transfections in 35 mm wells**

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**A. Transfections in Complete Growth Medium**

1. Seed cells in a 35 mm well at a density of 1-2 X 10<sup>5</sup> cells per 1 ml of fresh complete growth medium.
2. Prepare the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture as described in Section 3.2.2, Part B.
3. Add the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture to the cell suspension and gently rock the dish back and forth and from side to side to distribute the complexes evenly.
4. Incubate for 4-48 hours.<sup>b,d</sup>
5. Fix, mount and view as described in Sections 3.3 and 3.4.

**B. Transfections in Serum-Free Medium**

1. Prepare the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture as described in Section 3.2.2, Part B.
2. Wash the suspension cells once with sterile PBS or serum-free medium.
3. Seed cells in a 35 mm well at a density of 1-2 X 10<sup>5</sup> cells per 1 ml of serum-free medium.
4. Add the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture to the cell suspension and gently rock the dish back and forth and from side to side to distribute the complexes evenly.
5. Incubate for 2-8 hours.<sup>b,d</sup>
6. Dilute cells to 3-4 ml per well with complete growth medium, or add 1 ml of complete media with 2X serum.
7. Incubate for 4-48 hours.<sup>b,d</sup>
8. Fix, mount and view as described in Sections 3.3 and 3.4.

**Notes:**

<sup>a</sup> Since the optimal cell density (confluence) for efficient transfection can vary between cell types, maintain the same seeding protocol between experiments.

<sup>b</sup> Standard incubation conditions for mammalian cells are 37°C in 5% CO<sub>2</sub>. Other cell types, such as insect cells, require different temperatures and CO<sub>2</sub> concentrations. Use conditions appropriate for the cell type being transfected.

<sup>c</sup> The *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex may form improperly if the transfection medium contains serum, resulting in poor transfection efficiencies.

- <sup>d</sup> The optimal incubation time should be determined empirically by testing a range of incubation times from 4-48 hr.
- <sup>e</sup> For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 200-1,000  $\mu$ l.

### 3.3 Biotin Detection

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The tracking of Biotin-labeled DNA allows the use of a wide variety of commercially available detection reagents. Furthermore, the potential for multi-color tracking experiments is enhanced when the experimental design includes detection of a Biotin-labeled plasmid with a unique fluor conjugate and the direct detection of Cy<sup>TM</sup>3, Cy<sup>TM</sup>5 or Rhodamine-labeled plasmid(s).

1. Follow section 3.1 for nucleic acid labeling procedure and section 3.2.2 for transfection procedure for cells cultured on PDL treated coverslips.
2. After the transfection incubation period of 4-48 hours, aspirate the media.
3. Wash cells once with PBS.
4. Fix cells as described in Section 3.4, steps 1-4 only.
5. Dilute desired Streptavidin conjugate to ~20 ng/ $\mu$ l, or the best concentration determined for the reagent of choice, in PBS.
6. Gently add approximately 200  $\mu$ l Streptavidin conjugate dilution onto each coverslip.
7. Incubate at room temperature, shielded from light, for at least 1 hour.
8. After incubation, remove detection solution and wash 3 times with PBS.
9. To each well, add approximately 1 ml PBS to help with removal of the coverslips.
10. Mount 2 coverslips per glass slide as described in Section 3.4, step 6.

### 3.4 Cell Fixation and Mounting for Microscopy

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If desired, fix cells in 4% formaldehyde and mount coverslips on glass slides.

**Note:** It is best to keep exposure of the cells to light at a minimum to prevent loss of fluorescent signal.

1. Make a fresh dilution of 4% formaldehyde in PBS and store at 4°C until ready to use.
2. In a sterile culture hood, aspirate media from transfected cells, wash twice with PBS, and add approximately 1 ml 4% formaldehyde to each well.
3. Incubate cells at room temperature for 20 minutes.
4. Aspirate formaldehyde and gently wash wells 3 times with PBS.
5. Add approximately 1 ml PBS to each well to help with removal of the coverslips and to prevent drying.
6. Mount 2 coverslips per glass slide.
  - a. Using a small tip pap pen (Electron Microscopy Sciences) or nail polish, draw a complete circle on the glass slide. The diameter of the circle must be less than the height and width of the coverslip that will cover it. Two circles, with coverslips, will fit on a standard glass slide.
  - b. Place a small drop of antifade/mounting solution in the center of each marked circle.
  - c. Remove coverslips from wells with forceps and gently wipe off underside (non-cell side) of glass with a Kimwipe tissue.
  - d. Mount slowly, cell-side down onto antifade/mounting solution.
  - e. Use capillary action to drain excess mounting solution from under coverslip using a Kimwipe tissue.
  - f. Seal all edges of coverslip to glass slide with nail polish or rubber cement.
7. View on a fluorescent microscope using the appropriate filter sets (see Section 2.1, Table 1).

**For suspension cells**, fix and wash cells in solution. Spin and collect cells between washes. To visualize suspension cells by microscopy, apply cells to mounting area on a poly-lysine charged slide to aid in the adherence of the cells to the surface. Apply coverslip over cells, and seal as above.

### 3.5 Microinjection

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Microinjection involves the direct transfer of a dissolved substance into a living cell. It is a powerful technique that requires specialized equipment and technical expertise. The procedure is well tolerated, and can be successfully applied to a variety of cells in culture. It is an attractive strategy for studying primary cultures or cells which are recalcitrant to even the best transfection procedures. Since cells are processed one by one, a limited number of cells can be studied. Specific recommendations are difficult to provide since there are a variety of micromanipulation systems available, ranging from manual to fully automated.

#### Microinjection Tips to Consider:

- Cells should be plated on coverslips. Gridded coverslips (for example, Eppendorf's CELLocate coverslips) allow easy location of the microinjected cells.

- Cells should be cultured in a buffered medium to maintain the pH during prolonged exposure to ambient conditions during the microinjection process. For example, culture medium supplemented with 25 mM HEPES, pH 7.2 is suitable. Also, it is recommended that the microinjection procedure for each coverslip be restricted to less than 30 minutes. If it is anticipated that the microinjection procedure will take significantly longer than 30 minutes per coverslip, replace the media with PBS and use a microscope with a stage warmer, if possible.
- The labeled DNA sample can be ethanol precipitated twice to ensure quantitative removal of any remaining *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent. Suitable injection buffers for labeled DNA samples include TE (10 mM Tris, pH 7.2; 0.25 mM EDTA), PBS, and 25 mM HEPES, pH 7.2. Microinjection of a sample without labeled DNA (i.e. a buffer control) is recommended to assess its effect on the cells. Depending on the intended experiment, the DNA concentration in the sample can range from 0.1 to 2 µg/µl; for example, direct detection of labeled DNA (tracking experiments) would require higher concentrations, whereas tracking and expression experiments may be optimal at lower DNA concentrations. The microinjection sample must be centrifuged for 15 minutes at 4°C at a minimum of at least 10,000 x g immediately before loading the needle.
- Fluorescent injection markers, at a concentration of 0.05-0.1%, should be added to the microinjection sample to identify successfully injected cells (i.e. fluorescently labeled dextrans, antibodies, bovine serum albumin).
- After microinjection, replace the culture media and return the cells to the incubator. After the intended incubation time, fix and mount the coverslips as described in Sections 3.3 and 3.4.

#### 4.0 APPLICATION NOTES

##### A. Adjusting the Labeling Level

The labeling protocol in Section 3.1 readily allows detection of the labeled DNA in cultured mammalian cells. If there is a need to increase or decrease the number of labels in the final product, increase or decrease the ratio of labeling reagent to nucleic acid during the labeling reaction. Also, the labeling density can be controlled by adjusting the incubation time; the labeling reaction is linear over the first three hours of incubation at 37°C.

##### B. Maintaining Intact DNA during Labeling

By using the recommended reagent to nucleic acid ratio of 0.25:1 to 1:1 (v:w) during labeling, one can obtain labeled DNA in which the vast majority (>95%) remains intact. Using labeling reagent to DNA ratios significantly above 1:1 (v:w) may result in increased nicking of the DNA strands. To ensure stability of the labeled DNA, store at -20°C.

##### C. Transgene Expression using labeled DNA

The *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagents label intact plasmids (supercoiled or relaxed), linearized plasmid, or large linear DNA segments in a non-destructive manner, thus allowing the visualization of expression-competent DNA during the transfection process. Using the recommended range of *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent to DNA ratios will provide labeled plasmid DNA in which the expression of the transgene can be monitored simultaneously with plasmid localization. Reporter gene expression (for example, green fluorescent protein, β-galactosidase, luciferase, etc.), under the control of a strong CMV promoter, can be detected as early as 4 hours after transfection. Labeling of plasmid DNA at ratios significantly higher than the recommended range may result in decreased transgene expression.

##### D. *In Vivo* Tracking Experiments

The ability to simultaneously track and monitor transgene expression *in vivo* is another attractive application of this technology; especially when aspects of gene expression can be studied in their proper biological context. Plasmid DNA, labeled with *Label IT*<sup>®</sup> Tracker<sup>™</sup> (as described in Section C above) can also be used to monitor transgene expression and plasmid localization after delivery to tissues *in vivo*. Efficient *in vivo* gene delivery can be obtained using Mirus' *TransIT*<sup>®</sup> *In Vivo* Gene Delivery System. This kit is specifically designed for the efficient delivery of plasmid DNA into laboratory animals via tail vein injection. The plasmid is delivered using a highly efficient, nonviral method. Following gene delivery, the highest levels of transgene expression are found in the liver, with lower levels of expression found in many other organs including the spleen, kidneys, lungs and heart.

#### 5.0 TROUBLESHOOTING

##### 5.1 Labeling Reaction - Poor Efficiency

###### • Poor quality DNA

Use clean, intact DNA in labeling reactions.

###### • Labeling reaction was not scaled properly

Perform reaction in minimal volume, keeping the amount of *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagent less than 20% of the total reaction volume and the Labeling Buffer A at 1X final concentration in the reaction.

###### • Improper storage of reagents

Store both reconstituted and unreconstituted *Label IT*<sup>®</sup> Tracker<sup>™</sup> Reagents tightly capped at -20°C. Protect from exposure to light and moisture.

**Note: The relative density of fluorescent labels on purified, labeled DNA can be assessed by:**

1. **Agarose gel electrophoresis without ethidium bromide.** The DNA will appear faint under UV illumination because the transilluminator emits at approximately 300 nm, which is not optimal for the fluorescent labels (see Table 1).
2. **Spectrophotometric absorbance at  $\lambda_{\max}$ .** Several  $\mu\text{g}$  of labeled DNA may be required to generate significant  $\lambda_{\max}$  absorbance readings.
3. **Fluorescent microscopy.** Spot dilutions of labeled DNA onto a glass slide and view with fluorescent microscope.
4. **In Vitro Tracking.** Transfect labeled DNA into a common cell line, such as COS-7, HeLa, or NIH 3T3 to ascertain detection efficiency and optimal parameters.

## 5.2 Transfection - Low Transfection Efficiency

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- **Suboptimal *TransIT*<sup>®</sup>-LT1 Reagent to DNA ratio**  
Determine the optimal *TransIT*<sup>®</sup>-LT1 Reagent to DNA ratio by titrating the reagent from 2-12  $\mu\text{l}$  per  $\mu\text{g}$  DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 3  $\mu\text{l}$  of *TransIT*<sup>®</sup>-LT1 Reagent per 1  $\mu\text{g}$  of DNA (in a 35 mm well).
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**  
Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (LPS) using Mirus' MiraCLEAN™ Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex formation**  
Use serum-free medium when forming the complexes.
- **Cell density (% confluence) not optimal at time of transfection**  
The recommended cell density for most cell types at the time of transfection is 40-70% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Inhibitor present during transfection**  
The presence of polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use transfection medium that does not contain these polyanions.
- **Reagent formed precipitate during storage**  
The *TransIT*<sup>®</sup>-LT1 Reagent may form a precipitate during long term storage at  $-20^{\circ}\text{C}$ . Warm the reagent to room temperature and gently vortex to dissolve any precipitate.

## 5.3 Transfection - High Cellular Toxicity

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- ***TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture and cells were not mixed thoroughly after adding the complexes**  
Mix thoroughly to evenly distribute the complexes to all cells. Rocking the dish back and forth and from side to side is recommended. Do not swirl or rotate the dish, as this may result in uneven distribution.
- **Excessive amount of *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture was used in transfection**  
Reduce the amount of *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex mixture in the transfection.
- **Cell density was too low at time of transfection**  
Grow cells to a higher cell density and repeat the experiment.

## 5.4 Tracking - Poor Visualization of Labeled DNA in Cells

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- **Poor quality labeled DNA**  
Use purified, intact DNA in transfections.
- **Low labeling ratio**  
Increase the labeling ratio (volume of *Label IT*<sup>®</sup> Tracker™ Reagent to weight of DNA). See Section 3.1.
- **Excessive exposure to light**  
Protect samples and reagents from light.
- **Trouble detecting fluorescent signal**  
Use proper filter sets for microscopic detection. See Table 1.
- **Sub-optimal transfection**  
See Section 5.2.
- **Sub-optimal levels of DNA transfected**  
Use 1-2  $\mu\text{g}$  of labeled DNA per 35 mm well, and 3  $\mu\text{l}$  of *TransIT*<sup>®</sup>-LT1 per  $\mu\text{g}$  DNA.
- **Cells lost during fixation or mounting procedure**  
Perform all washing, fixing, and mounting steps gently. Check for presence of cells following each step on a visible light microscope.
- **Improper storage of labeled DNA**  
Labeled DNA must be kept at  $-20^{\circ}\text{C}$ .

### 5.5 Little or No Transgene Expression Observed

- **Labeling density is too high**  
Label DNA at a lower ratio. See Section 3.1 for recommendations.
- **Reporter protein is not expressed**  
Ensure that reporter protein is being expressed and detected by transfecting an unlabeled plasmid control.
- **Poor transfection efficiency**  
See Low Transfection Efficiency section of Troubleshooting section.
- **Observation time is not optimal**  
Perform time course to determine kinetics of expression.

### 6.0 GENERAL REFERENCES

#### *In Vitro* References:

1. Budker, V. *et al.* (1996) *Nature Biotechnology* Vol. **14**: 760-764.
2. Hagstrom, J. *et al.* (1996) *Biochim. Biophys. Acta* **1284**: 47-55.
3. Fritz, J. D. *et al.* (1996) *Human Gene Therapy* **7**: 1395-1404.

#### *In Vivo* References:

1. Budker, V. *et al.* (2000) *J. Gene Med.* **2**: 76-88.
2. Budker, V. *et al.* (1998) *Gene Therapy* **5**: 272-6.
3. Lui, F. *et al.* (1999) *Gene Therapy* **6**: 1258-1266.
4. Zhang, G. *et al.* (1999) *Human Gene Therapy* **10**: 1735-7.
5. Zhang, G. *et al.* (1997) *Human Gene Therapy* **8**: 1763-72.

### 7.0 RELATED PRODUCTS

#### **For determination of gene expression efficiency:**

Beta-Gal Staining Kit (Product # MIR 2600)

#### **For endotoxin removal from DNA:**

MiraCLEAN™ Endotoxin Removal Kit (Product #5900)

#### **Additional transfection reagents:**

TransIT®-LT1 and -LT2 Transfection Reagents (Product # MIR 2300, 2400)

TransIT®-Express Transfection Reagent (Product # MIR 2000)

TransIT®-HeLaMONSTER™ Transfection Reagent (Product # MIR 2900)

TransIT®-293 Transfection Reagent (Product # MIR 2700)

TransIT®-Keratinocyte Transfection Reagent (Product # MIR 2800)

TransIT®-CHO Transfection Reagent (Product # MIR 2170)

TransIT®-3T3 Transfection Reagent (Product # MIR 2180)

TransIT®-COS Transfection Reagent (Product # MIR 2190)

TransIT®-Insecta™ Transfection Reagent (Product # MIR 2200)

TransIT-TKO® siRNA Transfection Reagent (Product # MIR 2150)

TransIT®-Oligo Transfection Reagent (Product # MIR 2160)

#### ***In Vivo* Gene Delivery Kits:**

TransIT®-*In Vivo* Gene Delivery System (Product # MIR 5100)

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