



## ***TransIT*<sup>®</sup>-LT1 Transfection Reagent**

### **INTENDED USE**

Mirus Bio's *TransIT*<sup>®</sup> Transfection Reagents provide superior transfection efficiency in a variety of primary cells and established cell lines. These reagents offer clear advantages over other gene delivery methods including ease of use, reproducibility and state-of-the-art transfection efficiencies while significantly reducing the amount of cellular toxicity normally associated with transfections. This unique formulation makes these reagents ideal for all gene expression studies where the post-transfection state of the cell is important.

Mirus offers a selection of application tested transfection kits and reagents for plasmid and siRNA delivery in a variety of cell types. To obtain the highest achievable transfection efficiency in specific cell types choose from our cell line specific *TransIT*<sup>®</sup> Transfection products (see Related Products Section).

### **FREQUENTLY ASKED QUESTIONS**

#### **General Questions and Answers:**

**Q1. What is the compositions of the *TransIT*<sup>®</sup>-LT1 Reagent?**

The *TransIT*<sup>®</sup>-LT1 Transfection Reagent is a broad spectrum protein/polyamine based reagent that contains histone and a unique lipid.

**Q2. Where can I find references in which other researchers have used *TransIT*<sup>®</sup> Reagents to successfully transfect their cells?**

Product Citations are available in the Technical Resources section of our website ([www.mirusbio.com](http://www.mirusbio.com)). From the homepage, simply go to "Research Reagent Products" then select "Technical Resources", then click "Product Citations". For your convenience, the citations are organized both by product name and cell type.

**Q3. Can I use *TransIT*<sup>®</sup>-LT1 Reagent to transfect pDNA in primary cells?**

Yes. We have successfully transfected the following primary cells: human astrocytes, human chondrocytes, mouse and rat hepatocytes and human keratinocytes. The *TransIT*<sup>®</sup>-LT1 protocol also contains an extensive list of cell types successfully transfected by other laboratories.

**Q4. Can the *TransIT*<sup>®</sup> Reagents be used to transfect suspension cells?**

Yes. Follow the *TransIT*<sup>®</sup>-LT1 protocol for adherent cells, and collect cells by centrifugation at the time of harvest/assay. It is important to note that the transfection efficiency is somewhat dependent on cell density. In general, suspension cells are plated at a higher density than adherent cells. We suggest plating 400,000 cells/ml the day prior to transfection or 800,000 cells/ml the day of transfection.

**Q5. Can I transfect proteins with *TransIT*<sup>®</sup>-LT1?**

Yes. While we have not specifically tested this application, our customers report successful transfection of proteins with *TransIT*<sup>®</sup>-LT1 transfection reagent.

**Q6. Can *TransIT*<sup>®</sup>-LT1 transfection reagent be used to transfect cells *in vivo*?**

No. *TransIT*<sup>®</sup>-LT1 is not recommended for use *in vivo*. We recommend choosing a kit that is designed for *in vivo* applications, such as the *TransIT*<sup>®</sup>-QR (Quick Recovery), *TransIT*<sup>®</sup>-EE (Enhanced Expression) or the *TransIT*<sup>®</sup> *In Vivo* Gene Delivery System. These systems have been specifically designed for *in vivo* gene transfer via a high pressure tail vein injection into mice and rats. Please see the Related Products Section for more information.

#### **TransIT Protocol Questions and Answers:**

**Q7. What is the best ratio of *TransIT*<sup>®</sup>-LT1 Reagent to DNA to use for my transfection?**

Our recommendation for most cell types is a ratio of 3:1 (7.5  $\mu$ l *TransIT*<sup>®</sup> Reagent: 2.5  $\mu$ g DNA) per well of a 6-well plate. Optimization may determine that the ratio may range from 1:1 to 8:1, depending on the cell type, passage number, cell density, and incubation time.

**Q8. Should I add the DNA complexes to cells in serum-containing media or serum-free media?**

*TransIT*<sup>®</sup> Reagents effectively deliver nucleic acids to cells in media with or without serum. In most instances, we obtain the best results by adding the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex directly to the cells in their complete growth media (containing serum), eliminating the need for a media change post-transfection. It is important that the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex is formed in serum-free media, as the complex will not form properly in the presence of serum.

**Q9. Will antibiotics interfere with my transfection?**

Some antibiotics, such as Kanamycin, are cationic and can interfere with transfection. We recommend checking carefully for interference before using antibiotics or avoiding their use during transfection. We use 0.1X or 10 units/ml of both penicillin and streptomycin in the transfection media.

**Troubleshooting Questions:****Q10. I see a precipitate in my *TransIT*<sup>®</sup>-LT1 Reagent. Is this normal?**

Storing the *TransIT*<sup>®</sup>-LT1 at -20°C can sometimes cause a precipitate to form. This does not harm the reagent, and the precipitate will go back into solution by warming the reagent to room temperature and gently vortexing before each use. If you are performing transfections frequently, we recommend storing your *TransIT*<sup>®</sup>-LT1 at 4°C. Please check the product label for the appropriate storage temperature.

**Q11. Are cell density (% confluence) and passage number important factors in transfection reactions?**

Yes. Optimal cell density (50-70% confluence) is essential for successful transfections in most cell types. In general, we plate 60,000-225,000 cells/ml one day prior to transfection and 400,000-800,000 cells/ml the day of transfection. We recommend monitoring the passage number and growth time of your cells to determine which conditions yield optimal transfection efficiency.

**Q12. How long should I leave the complexes on my cells?**

For most cell types, we add the *TransIT*<sup>®</sup>-LT1 Reagent/DNA complex directly to the cells in serum-containing media, and harvest 24-48 hours later for transgene expression. Incubation times for efficient transfection may vary from 24-72 hours. We recommend leaving the complexes on the cells for at least 24 hours before performing any required media changes.

**Q13. How can I assess transfection efficiency for my cell type?**

Mirus has developed the Label IT<sup>®</sup> Tracker Intracellular Localization Kits (please see Related Products section) that provide the necessary reagents to label and transfect plasmid DNA. The protocol provides a straight forward approach to directly label and deliver DNA in an efficient yet non-destructive manner for tracking experiments. Mirus Bio also offers a prelabeled DNA control (please see Related Products section) for tracking applications. Both subcellular localization and functionality can be monitored simultaneously following the introduction of the labeled sample into mammalian cells.

**Q14. When I transfect my cells using the *TransIT*<sup>®</sup> Reagent, I see low transfection efficiency. What can I do?**

- **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 2-8 µl per 1 µg DNA. Choose the amount which gives the highest transfection efficiency and the lowest cellular toxicity. As a starting point, use 3 µl of *TransIT*<sup>®</sup>-LT1 Reagent per 1 µg of DNA.
- **Complexes were added to cells in serum-free media**  
Form complexes in serum-free media, and add to cells in complete growth media (serum-containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to the cells in complete growth media and the 4 hour media change is eliminated.
- **Cell density (% confluence) not optimal at time of transfection**  
The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments to ensure reproducibility.
- **Insufficient incubation time during complex formation**  
The recommended complex formation time is 15-30 minutes. It may be necessary to determine the optimal complex formation time for each cell type by testing various times within the range.

- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as endotoxin, may be present in the preparation)**  
DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (bacterial lipopolysaccharide, LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is 1-5 µg per well of a 6-well plate. As a starting point, use 2.5 µg per well of a 6-well plate.
- **Fetal calf serum present during *TransIT*®-LT1 Reagent/DNA complex formation**  
Use serum-free medium when forming the complexes. Transfections should be performed in the presence of serum.
- **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.
- **Cell morphology has changed**  
If the cell passage number is too high or too low the transfection efficiency may be adversely affected. Maintain a similar passage number between experiments to ensure reproducibility.

**Q15. After I transfect with the *TransIT*® Reagent, I see cellular toxicity. What can I do?**

- **Complexes were added to the cells in serum-free media**  
Form complexes in serum-free media, and add to cells in complete growth media (serum containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media and the media change is eliminated.
- **Cell density (% confluence) was not optimal at time of transfection**  
The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments to ensure reproducibility.
- **Excessive amount of *TransIT*®-LT1 Reagent/DNA complex mixture was in the transfection**  
Reduce the amount of *TransIT*®-LT1 Reagent or DNA added to the cells. See Table 1.
- ***TransIT*®-LT1 Reagent/DNA complex was not mixed thoroughly with the cells in the well**  
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.
- **Cell morphology has changed**  
If the passage number of the cells is too high or too low, they can be more sensitive to transfection reagents. Maintain a similar passage number between experiments to ensure reproducibility.

For specific questions or concerns, please contact Technical Support at 888.530.0801 or [techsupport@mirusbio.com](mailto:techsupport@mirusbio.com).

For product citations, please visit the Technical Resources section of our website ([www.mirusbio.com](http://www.mirusbio.com)).



## Related Products

### For endotoxin removal from DNA:\*

MiraCLEAN<sup>®</sup> Endotoxin Removal Kit (Product #5900)

### For DNA tracking studies:

Label IT<sup>®</sup> Tracker<sup>™</sup> 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)

### Additional Plasmid transfection reagents:\*

TransIT<sup>®</sup>-LT1 Transfection Reagent (Product # MIR 2300)

TransIT<sup>®</sup>-LT2 Transfection Reagent (Product # MIR 2400)

TransIT<sup>®</sup>-Express Transfection Reagent (Product # MIR 2000)

TransIT<sup>®</sup>-HeLaMONSTER<sup>®</sup> Transfection Kit (Product # MIR 2900)

TransIT<sup>®</sup>-Keratinocyte Transfection Reagent (Product # MIR 2800)

TransIT<sup>®</sup>-CHO Transfection Kit (Product # MIR 2170)

TransIT<sup>®</sup>-3T3 Transfection Kit (Product # MIR 2180)

TransIT<sup>®</sup>-293 Transfection Kit (Product # MIR 2700)

TransIT<sup>®</sup>-COS Transfection Kit (Product # MIR 2190)

TransIT<sup>®</sup>-Insecta Transfection Reagent (Product # MIR 2200)

TransIT<sup>®</sup>-Jurkat Transfection Reagent (Product # MIR 2120)

TransIT<sup>®</sup>-Prostate Transfection Kit (Product # MIR 2130)

TransIT<sup>®</sup>-mRNA Transfection Reagent (Product # MIR 2250)

TransIT-TKO<sup>®</sup> siRNA Transfection Reagent (Product # MIR 2150)

TransIT<sup>®</sup>-siQUEST<sup>™</sup> siRNA Transfection Reagent (Product # MIR 2110)

TransIT<sup>®</sup>-Oligo Transfection Reagent (Product # MIR 2160)

### In Vivo Gene Delivery Kits:\*

TransIT<sup>®</sup>-In Vivo Gene Delivery System (Product # MIR 5100)

TransIT<sup>®</sup>-EE Hydrodynamic Delivery Solution (Product # MIR 5340)

TransIT<sup>®</sup>-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)

TransIT<sup>®</sup>-QR Hydrodynamic Delivery Solution (Product # MIR 5240)

TransIT<sup>®</sup>-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

### RNA Interference Products:\*

TransIT-TKO<sup>®</sup> siRNA Transfection Reagent (Product # MIR 2150)

TransIT<sup>®</sup>-siQUEST<sup>™</sup> siRNA Transfection Reagent (Product # MIR 2110)

siXpress<sup>®</sup> PCR Vector Systems (Product # MIR 7300, 7301, 7302)

Label IT<sup>®</sup> siRNA Tracker Intracellular Localization Kit with TransIT-TKO<sup>®</sup> Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)

Label IT<sup>®</sup> siRNA Tracker Intracellular Localization Kit with TransIT<sup>®</sup>-siQUEST<sup>™</sup> Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)

Label IT<sup>®</sup> siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

\*These products are available in additional sizes.

Mirus Bio Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811; 6,593,465 and patents pending.

The performance of this product is guaranteed for one year from the date of purchase if stored and handled properly.

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