

**TransIT<sup>®</sup> Transfection Reagents**

Product # MIR 2300, MIR 2304, MIR 2305, MIR 2306, MIR 2400, MIR 2404, MIR 2405, MIR 2406

Product	Quantity	Product No.
<i>TransIT<sup>®</sup>-LT1</i>	0.4 mL	MIR 2304
	1 mL	MIR 2300
	5 mL (5 x 1 mL)	MIR 2305
	10 mL (10 x 1 mL)	MIR 2306
<i>TransIT<sup>®</sup>-LT2</i>	0.4 mL	MIR 2404
	1 mL	MIR 2400
	5 mL (5 x 1 mL)	MIR 2405
	10 mL (10 x 1 mL)	MIR 2406

**1.0 INTENDED USE**

Each milliliter of *TransIT<sup>®</sup>-LT* Transfection Reagent (MIR 2300 and MIR 2400) is sufficient quantity to perform up to 500 transfections in 35 mm dishes, depending on the specific cell type being used.

**2.0 DESCRIPTION**
**2.1 General Information**

The *TransIT<sup>®</sup>* Transfection Reagents were developed by the gene transfer specialists of Mirus Corporation. Although second generation cationic-liposome formulations yield increased transfection efficiencies, they often increase cellular toxicity. For the majority of applications and cell types, *TransIT<sup>®</sup>-LT* (Low Toxicity) Polyamine Transfection Reagents offer clear advantages for delivering DNA into cells via transfection, including minimal cellular toxicity, ease of use, and transfection reproducibility. These products provide state-of-the-art transfection efficiencies with significantly reduced levels of cell damage compared to other leading transfection reagents. In addition, transfections with the *TransIT<sup>®</sup>* LT Reagents do not require media changes and can be carried out in serum-containing media. This unique combination makes these reagents ideal for all gene expression studies where the post-transfection state of the cell is important.

**2.2 Cell Lines Successfully Tested by Mirus Corporation**

***TransIT<sup>®</sup>-LT1:*** NIH3T3, HeLa, COS-7, HepG2, C2C12, PC12, Rat-1, B50, CHO, HEK293, Jurkat, MEL, 10T1/2, A549, HUVEC, BHK-21, Huh-7, Chang liver, MCF-7, KB, SKOV3, OVCAR3, primary keratinocytes

***TransIT<sup>®</sup>-LT2:*** NIH3T3, HeLa, COS-7, HepG2, C2C12, PC12, Rat-1, B50, HEK293, CHO, A549, Chang liver

**2.3 Cell Lines Successfully Tested by Other Laboratories**

***TransIT<sup>®</sup>-LT1:*** 3T3-L1, A204, A-431, A-704, alpha TC1-6, AR 42J, As4.1, AtT-20, BC-1, BC-2, BC-3, BCBL-1, C3H/10T1/2, C6, Caco-2, Caki-1, Calpan-1, Calu-1, Calu-6, COS-1, DDTI MF-2,DT40, ECV304, EL4, ES-E14TG2a, EVSCC17M, FaDu, GP+E-86, H9c2, HaCaT, HCT-116, Hep 3B2.1-7, Hepa 1-6, Hs 766T, Ht-29, HT-1080, HTB-37, HTB-45, IC21, IEC-6, IIB-Me1-J, JEG-3, L57-3-11, L-6, L-929, MA-10, McA-RH7777, MCF-10-2A, MDA-MB-231, MDCK, Melanocyte, MG-63, Mv 1 Lu, Neuroblastoma, NRK, NT2/D1, OV-1063, P4, P19, PA317, PAM212, PG13, PK(15), PS-1, R2C, Rat chondrosarcoma, RAW 264.7, RBL-2H3, SC-1, Schneider line 2, SK-N-SH, SP2-0/0-Ag14, STO, SW-480, SW-837, T3M4, T-47D, TM4, U-87, U937, UCD, Vero, WE-38, WiDr, a variety of primary cell types.

**2.4 Specifications**

Concentration: *TransIT<sup>®</sup>-LT1* and *LT2*: 1.33 mg/mL in 80% ethanol

Storage:	4°C or –20°C. If the reagent is stored at –20°C, prior to use, warm to room temperature and gently vortex to redissolve any precipitate that may have formed.
Sterility:	No growth seen with Potato Dextrose Broth Assay or Tryptic Soy Broth Assay
Stability:	1 year when stored at 4°C or –20°C

### 3.0 PROCEDURE

#### 3.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each individual cell type. The transfection protocols described in Sections 3.2-3.4 should result in efficient transfection of most cell types; 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 usually falls within the range of 1-3 µg per 35 mm dish. As a starting point, we recommend using 2 µg per 35 mm dish.
- C. *TransIT*<sup>®</sup> Reagent to DNA ratio**—As a starting point, we recommend using 3 µL of *TransIT*<sup>®</sup> LT Reagent per 1 µg of DNA. The optimal *TransIT*<sup>®</sup> Reagent to DNA ratio can be determined by titrating the reagent starting at 2 µL/µg DNA up to 12 µL/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 a range of incubation times from 2-48 hours.

The protocols below are recommended for performing transfections with either of the *TransIT*<sup>®</sup> LT Transfection Reagents in 35 mm dishes. When performing transfections in different sized dishes, 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.

#### 3.2 Protocol for Transient Transfection (Adherent Cells)

##### A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10<sup>5</sup> cells in their complete growth medium per 35 mm well) so that they will be ~40-70% confluent the following day.<sup>a</sup>
2. Incubate the cells overnight.<sup>b</sup>

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

1. In a sterile, plastic 12 x 75 mm tube, add the *TransIT*<sup>®</sup> Transfection Reagent (2-12 µL/µg DNA) dropwise into ≥100 µL of serum-free medium<sup>c,e</sup> (Opti-MEM<sup>™</sup> or RPMI 1640 from Gibco BRL are recommended for mammalian cell types). Mix thoroughly by vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1-3 µg) to the diluted *TransIT*<sup>®</sup> Reagent. Mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

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

**NOTE:** For several cell lines tested, we have found that both of the *TransIT*<sup>®</sup>-LT Reagents yield 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 per well of fresh complete growth medium.
2. Add the *TransIT*<sup>®</sup> 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 24-72 hours.<sup>b</sup>

**Note:** The above incubation is designed for transfections performed with no media change. If you wish to perform a media change to remove the transfection complexes, incubate the cells for 2-8 hours, replace the original medium with fresh complete growth medium, and incubate for an additional 24-72 hours.<sup>b,d</sup>

4. Harvest cells and assay for reporter gene activity.

#### **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 Dulbecco's 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> 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 2-8 hours.<sup>b,d</sup>
5. Remove the medium containing the *TransIT*<sup>®</sup> Reagent/DNA complex mixture and replace it with complete growth medium.
6. Incubate for 24-72 hours.<sup>b</sup>
7. Harvest cells and assay for reporter gene activity.

### **3.3 Protocol for Stable Transfection (Adherent Cells)**

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1. Follow the protocol described in Section 3.2.
2. Subculture your cells at the desired dilution (at least 1:5) into selection medium.

### **3.4 Protocol for Transient Transfection (Suspension Cells)**

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#### **A. Transfections in Complete Growth Medium**

1. Seed cells in a 35 mm dish at a density of 1-2 X 10<sup>6</sup> cells per 1 mL of fresh complete growth medium.
2. Prepare the *TransIT*<sup>®</sup> Reagent/DNA complex mixture as described in Section 3.2, Part B.
3. Add the *TransIT*<sup>®</sup> 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 24-72 hours.<sup>b,d</sup>
5. Assay cells for reporter gene activity at 24-72 hours post-transfection.

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

1. Prepare the *TransIT*<sup>®</sup> Reagent/DNA complex mixture as described in Section 3.2, Part B.
2. Wash the suspension cells once with sterile Dulbecco's PBS or serum-free medium.
3. Seed cells in a 35 mm dish at a density of 1-2 X 10<sup>6</sup> cells per 1 mL of serum-free medium.
4. Add the *TransIT*<sup>®</sup> 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.
7. Assay cells for reporter gene activity at 24-72 hours post-transfection.

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<sup>a</sup> Since the optimal cell density (confluence) for efficient transfection can vary between cell types, we recommend that you 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> 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 2-8 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 µL.

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### **3.5 Troubleshooting**

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### **Low Transfection Efficiency**

- *Suboptimal TransIT<sup>®</sup> Reagent to DNA ratio*  
Determine the optimal TransIT<sup>®</sup> Reagent to DNA ratio by titrating the reagent from 2  $\mu\text{L}/\mu\text{g}$  DNA up to 12  $\mu\text{L}/\mu\text{g}$  DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, we recommend trying 3  $\mu\text{L}$  of TransIT<sup>®</sup> 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)*  
We recommend using double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (LPS) using one of the standard protocols.<sup>1-3</sup>
- *Fetal calf serum present during TransIT<sup>®</sup> Reagent/DNA complex formation*  
Be sure to 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. However, you should 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> reagent may form a precipitate during long term storage at  $-20^{\circ}\text{C}$ . Undetected, this could result in lowered efficiencies. Simply warm the reagent to room temperature and gently vortex to redissolve any precipitate.

### **High Cellular Toxicity**

- *TransIT<sup>®</sup> Reagent/DNA complex mixture and cells were not mixed thoroughly after adding the complex*  
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> Reagent/DNA complex mixture was used in transfection*  
Reduce the amount of TransIT<sup>®</sup> 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.

## **4.0 REFERENCES**

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## **5.0 BIBLIOGRAPHY**

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