*Trans*IT®-LT1 Transfection Reagent

Product Name	Volume of *Trans*IT®-LT1 Reagent	Product No.
*Trans*IT®-LT1 Transfection Reagent	0.4 ml	MIR 2304
	1 ml	MIR 2300
	5 ml (5 × 1 ml)	MIR 2305
	10 ml (10 × 1 ml)	MIR 2306

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT®-LT1 (Low Toxicity) Transfection Reagent is specifically designed by the nucleic acid delivery specialists at Mirus Bio Corporation. This novel formulation provides superior transfection efficiency and cell viability in various primary cells and established cell lines. *Trans*IT®-LT1 Transfection Reagent provides all the attributes of the trusted *Trans*IT® Reagent line: high efficiency, low toxicity, simplicity of use, and reproducibility. Transfections with the *Trans*IT®-LT1 Reagent do not require media changes and can be carried out in serum-containing media. This unique combination makes the *Trans*IT®-LT1 Reagent ideal for all gene expression studies where the post-transfection state of the cell is important. One milliliter (MIR 2300) provides sufficient reagent to perform up to 500 transfections per well in 6-well plates, depending on the specific cell type being used.

1.2 Cell Lines Successfully Transfected by Mirus Corporation

A549, BHK-21, BNL.CL2, BRL-3A, C2C12, *C6, *CHO-K1, Clone 9, *COS-1, *COS-7, *Daoy, *DBTRG-05MG, *DI-TNC1, *DU 145, *HEK 293, *HeLa, Hepa 1-6, Hepa 1cLc7, HepG2, HLF-a, Huh-7, *HUVEC, *Jurkat, *K562, KB, KLN 205, *LL/2 (LLC1), *LNCaP-FGC, MCF-7, MEL, *Neuro-2a, *NIH3T3, OVCAR3, *PC3, *PC-12, *primary human astrocytes, *primary human chondrocytes, primary mouse hepatocytes, primary rat hepatocytes, *primary human keratinocytes, *RAW 264.7, *SK-N-MC, SKOV3, *SVGp12, SW900, *THP-1, Vero, and WRL-68.

*Higher transfection efficiencies can be achieved using Mirus *Trans*IT® Cell Line Specific Reagents. Please see the Related Products Section or contact Technical Support at 888.530.0801 or techsupport@mirusbio.com for assistance.

1.3 Cell Lines Successfully Tested by Other Laboratories

3T3-L1, A1847, AR42ce4, ARP1, C2C12, CHO-K1, corneal endothelium, COS-1, COS-7, chicken embryo fibroblast, CV-1, CVEC, ECV 304, HCT-116, HEC-1A, HEC-1B, HEK 293, HeLa, HepG2, H1299, Hs578T, human bladder carcinoma, HUVEC, LLC-PK, M19, MA-10, MC3T3-E1, MCF-7, MDA-MB-231, MDA-MB-435, MDCK, melanocyte, Mv 1 Lu, myometrial, neuroepithelial, NIH3T3, OV-1063, ovarian cancer, OVCAR3, OVCAR4, OVCAR7, OVCAR8, OVCAR10, PC3, PC-12, PE01, PE04, primary human astrocytes, primary human melanoma cells, primary human skin fibroblasts, primary mouse embryonic cells, primary mouse fibroblasts, primary mouse hepatocytes, primary mouse myotubes, primary mouse thymocytes, primary rat osteoblasts, rat smooth muscle cells, RAW 264.7, RBL-2H3, RBL, SJPL, SK-BR-3, SKOV3, U266, U20S, UPN251, Vero, WB rat liver epithelial cells, and WiDr.

1.4 Specifications

Concentration: 1.33 mg/ml in 80% ethanol

Storage: Store the *Trans*IT®-LT1 Reagent at 4˚C or -20˚C. Warm to room temperature and gently vortex before each use to dissolve any precipitate that may have formed.

Stability: 1 year from the date of purchase when stored properly

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each individual cell type. The transfection protocols described in Sections 2.2-2.3 should result in highly efficient transfections. However, to ensure optimal results, consider the following variables:

A. **Media conditions -** *Trans*IT® Reagents yield improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated.

B. **Cell density (% confluence) at transfection -** The recommended cell density for most cell types at transfection is 50-70% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.

C. **DNA purity and concentration for transfection -** DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (bacterial lipopolysaccharide) using the MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is 1-3 µg per well of a 6-well plate. As a starting point, use 2.5 µg per well of a 6-well plate.

D. ***Trans*IT®-LT1 Reagent to DNA ratio -** As a starting point, use 3 µl of *Trans*IT®-LT1 Reagent per 1 µg of DNA. Titrate the *Trans*IT®-LT1 Reagent from 2-8 µl per 1 µg DNA, depending on the specific cell type. For future transfections, use the ratio that gives the best transfection efficiency with the lowest cellular toxicity, on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

E. **Transfection incubation time -** Determine the optimal incubation time empirically by testing a range from 4-48 hours. Mirus recommends an incubation time of 24-48 hours for most applications.

The protocols below are recommended for performing transfections in 6-well plates. When performing transfections in different sized wells, the amount of DNA, *Trans*IT®-LT1 Reagent, and culture medium should be scaled up or down in proportion to the surface area of the well. To minimize pipetting small volumes, dilute the *Trans*IT®-LT1 Reagent in 80% ethanol immediately prior to each use.

Table 1. Recommended starting conditions for the *Trans*IT®-LT1 Transfection Reagent

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10 cm dish
Surface Area*	0.35 cm^2	1.0 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2
Serum-free Media	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml
*Trans*IT®-LT1 Reagent	0.28 µl	0.79 µl	1.5 µl	3 µl	7.5 µl	45 µl
DNA (1µg/µl stock)	0.1 µl	0.26µl	0.5 µl	1 µl	2.5 µl	15 µl
Complete Growth Media	0.092 ml	0.263 ml	0.500 ml	1.0 ml	2.5 ml	15.5 ml

*Surface areas are based on Greiner tissue culture plates and Falcon 10 cm dishes. All volumes in Table 1 are per one well of indicated size.

2.2 Protocol for Transient Transfection (Adherent Cells in 6-Well Plates)

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at a cell density of 1-3 x 10^5 cells in complete growth medium per well of a 6-well plate to obtain 50-70% confluence the following day. [a]
2. Culture the cells over night.[b]

B. Complex Formation (Perform This Procedure Immediately Prior To Transfection)

1. In a sterile plastic tube, add the *Trans*IT®-LT1 Reagent (2-8 µl per 1 µg DNA; see Table 1 and Section 2.1D) directly into 250 µl of serum-free medium.[c] Mix completely by gentle pipetting.
2. Incubate at room temperature for 5-20 minutes.

3. Add plasmid DNA (1-3 µg per well) to the diluted *Trans*IT®-LT1 Reagent and mix by gentle pipetting. [e]
4. Incubate at room temperature for 15-30 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-LT1 Reagent yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated.

1. If necessary, remove the medium from the cells prepared in step A and replace with 2 ml of fresh complete growth medium per well of a 6-well plate.
2. Add the *Trans*IT®-LT1 Reagent/DNA complex mixture, prepared in Step B, dropwise to the cells in complete growth medium. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 24-48 hours. [b]
   NOTE: The above incubation is designed for transfections performed with no media change. To perform a media change after a 4-24 hours incubation with the complexes, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.[b,d]
4. Harvest cells and assay for reporter gene activity.

2.3 Protocol for Transient Transfection (Suspension Cells in 6-Well Plates)

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at a cell density of 8-10 x 10^5 cells in complete growth medium per well of a 6-well plate.
2. Culture the cells overnight.[b]

B. Complex Formation (Perform This Procedure Immediately Prior To Transfection)

1. In a sterile plastic tube, add the *Trans*IT®-LT1 Reagent (2-8 µl per 1 µg DNA; see Table 1 and section 2.1D) directly into 250 µl of serum-free medium.[c] Mix by gentle pipetting.
2. Incubate at room temperature for 5-20 minutes.
3. Add plasmid DNA (1-3 µg per well) to the diluted *Trans*IT®-LT1 Reagent and mix completely by gentle pipetting. [e]
4. Incubate at room temperature for 15-30 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-LT1 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.

1. If necessary, spin down the cells prepared in Step A, remove the medium from these cells, and replace it with 2.5 ml per well of a 6-well plate (see Table 1) of fresh complete growth medium. Replate cells as described in Section 2.3A.
2. Add the *Trans*IT®-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 24-48 hours.[b,d]
   NOTE: The above incubation is designed for transfections performed without a media change. To perform a media change after a 4-24 hours incubation with complexes, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.[b,d]
4. Harvest cells and assay for reporter gene activity.

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[a] Since the optimal cell density (% confluence) for efficient transfection can vary between cell types, maintain the same seeding protocol between experiments.

[b] Standard incubation conditions for mammalian cells are 37˚C in 5% CO_2. Other cell types, such as insect cells, require different temperatures and CO_2 concentrations. Use conditions appropriate for the cell type being transfected.

[c] The *Trans*IT®-LT1 Reagent/DNA complex may form improperly if the complex formation medium contains serum, resulting in poor transfection efficiencies.

[d] The optimal post transfection incubation time can be determined empirically by testing a range of incubation times from 4-48 hours.

[e] For transfecting larger amounts of DNA, or if a precipitate forms upon adding the DNA, increase the volume of serum-free medium up to 1 ml.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-LT1 Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-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 *Trans*IT®-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 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.
- **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) using the 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 µg per well of a 6-well plate.
- **Fetal calf serum present during *Trans*IT®-LT1 Reagent/DNA complex formation**
  Use serum-free medium when forming the complexes. Add these complexes to cells growing in complete growth serum. 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.

High Cellular Toxicity

- **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 *Trans*IT®-LT1 Reagent/DNA complex mixture was in the transfection**
  Reduce the amount of *Trans*IT®-LT1 Reagent or DNA added to the cells. See Table 1.
- ***Trans*IT®-LT1 Reagent/DNA complex was not mixed thoroughly with the cells in the well plat**e
  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 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 RELATED PRODUCTS

For endotoxin removal from DNA:*

MiraCLEAN® Endotoxin Removal Kit (Product #5900)

For 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)

Additional Plasmid transfection reagents:*

*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-293 Transfection Kit (Product # MIR 2700)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-mRNATransfection Reagent (Product # MIR 2250)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo* Gene Delivery Kits:

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

RNA Interference Products:*

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
siXpress® PCR Vector Systems (Product # MIR 7300, 7301, 7302)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT-TKO® Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®-siQUEST™ Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

*These products are available in additional sizes.