*Trans*IT®-LT1 Transfection Reagent

Protocol for Product Nos. MIR 2300, 2304, 2305, 2306

Mirus
It All Begins at the Bench

INTRODUCTION

The easy to use *Trans*IT-LT1 (Low Toxicity) Transfection Reagent provides superior transfection efficiency, cell viability, and reproducibility in many primary cells and established cell lines. This reagent is serum-compatible, and its remarkably low cellular toxicity means that the observed post-transfection phenotype arises from the transfected DNA, not from a reagent artifact.

SPECIFICATIONS

Storage	Store *Trans*IT-LT1 Reagent at 4°C or -20°C
Stability	1 year from the date of purchase, when properly stored and handled
Number of reactions	One ml provides sufficient reagent for up to 500 transfections in 6-well plates

Some components of *Trans*IT-LT1 Reagent may form a precipitate during storage. **Before each use**, warm to room temperature and vortex gently.

MATERIALS

Materials supplied

The *Trans*IT-LT1 Transfection Reagent is supplied in ***one*** of the following formats.

Product No.	Quantity
MIR 2304	1 × 0.4 ml
MIR 2300	1 × 1.0 ml
MIR 2305	5 × 1.0 ml
MIR 2306	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified DNA (e.g., plasmid, cosmid, or linear DNA)
- Serum-free medium for reagent—DNA complex formation
- Sterile tube for reagent—DNA complex preparation
- Micropipettes
- Reporter or other assay as required

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BEFORE YOU START:

Important Tips for Optimal Transfections

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below generally yield highly efficient transfections. **Table 1** presents recommended starting conditions depending on culture vessel size.

- **Reduce pipetting errors.** When necessary, dilute the *Trans*IT-LT1 Reagent in 80% ethanol, rather than pipetting small volumes.
- **Complex formation conditions.** Form *Trans*IT-LT1 Reagent—DNA complexes in serum-free growth medium. Mirus recommends Opti-MEM® or RPMI 1640 medium. Use DNA stocks that range from 1-3 µg/µl. Use of more concentrated DNA may form insoluble precipitates when combined with *Trans*IT-LT1 Reagent.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfections. DNA that is endotoxin-free (bacterial lipopolysaccharide-free) is optimal. Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation.
- **DNA amount.** Determine the best DNA amount for transfection of each cell type. **Table 1** provides suggested starting conditions.
- **Ratio of *Trans*IT-LT1 reagent to DNA.** Determine the optimal *Trans*IT-LT1 Reagent-to-DNA ratio for each cell type. Start with 3 µL of *Trans*IT-LT1 Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-LT1 Reagent from 2-8 µl per 1 µg DNA to find the best ratio. **Table 1** provides recommended starting conditions based on cell culture vessel size.

Warm *Trans*IT-LT1 to room temperature and vortex before 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	T75 flask
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	75 cm^2
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
*Trans*IT-LT1 Reagent	0.28 µl	0.79 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
Complete growth medium	92 µl	263 µl	0.5 ml	1.0 ml	2.5 ml	15.5 ml	19.7 ml

Surface areas are based on Greiner tissue culture plates and Falcon 10-cm dishes and T75 flasks. All volumes given are per well (or per dish) for a given culture vessel.

- **Transfection conditions.** Culture cells in the appropriate medium, with or without serum. There is no need to perform a medium change to remove the transfection complexes.
- **Cell density (% confluence) at transfection.** Determine the optimal cell density for each cell type to maximize transfection efficiency. Divide the culture18-24 hours before transfection such that the actively growing cells reach optimal cell density (generally 50-70% confluence) at transfection.
- **Transfection incubation time.** Determine the optimal incubation time post-transfection for each cell type and experiment. Test a range of incubation times. The optimal incubation time is generally 24-72 hours, but will vary depending on the goal of the experiment and the transfected DNA.

PROCEDURE

The procedure below describes how to perform transfections in 6-well plates. The surface areas of other culture vessels are different and transfections must be scaled accordingly. Appropriately increase or decrease the amounts of serum-free medium, *Trans*IT-LT1 Reagent, DNA, and complete culture medium based on the surface area of the cell culture vessel (see **Table 1**).

Divide cultured cells 18-24 hours before transfection such that the cells reach optimal cell density at time of transfection.

A. Plate cells

1. Approximately 24 hours before transfection, plate cells in complete growth medium in a 6-well plate.

   For adherent cells:
   Plate cells at the optimal concentration (generally 1-3 × 10^5 cells/well).

   For suspension cells:
   Plate cells at 8-10 × 10^5 cells/well.

2. Incubate the cell cultures overnight.

If using highly concentrated DNA, dilute before adding to *Trans*IT-LT1 Reagent. For example, divide the serum-free medium into two aliquots. Add the DNA to one aliquot, the *Trans*IT-LT1 Reagent to the other, then gently pipet the diluted DNA into the diluted *Trans*IT-LT1 Reagent.

B. Prepare *Trans*IT-LT1 Reagent—DNA complex (immediately before transfection)

1. Warm *Trans*IT-LT1 Reagent to room temperature and vortex gently before using.
2. Place 250 µL of **serum-free** Opti-MEM or RPMI 1640 in a sterile tube.
3. Add 7.5 µL *Trans*IT-LT1 Reagent.
4. Pipet gently to mix completely.
5. Add 2.5 µg plasmid DNA to the diluted *Trans*IT-LT1 Reagent.
6. Pipette gently to mix completely.
7. Incubate at room temperature for 15-30 minutes.

*Trans*IT-LT1 is a low-toxicity reagent. There is no need to change to fresh culture medium after transfection, unless required by your cell culture.

C. Add complexes to cells in complete growth medium

1. Add the *Trans*IT-LT1 Reagent—DNA complex (prepared in Step B) dropwise to the cells. Gently rock the culture vessel back and forth and from side to side to evenly distribute the *Trans*IT-LT1 Reagent—DNA complexes.
2. Incubate for 24-72 hours.
3. Harvest cells and perform a reporter assay or other assay as required.

TROUBLESHOOTING

Problem	Solution
LOW TRANSFECTION EFFICIENCY	
Suboptimal *Trans*IT-LT1 Reagent-to-DNA ratio	Determine optimal *Trans*IT-LT1 Reagent-to-DNA ratio for each cell type.
	Titrate the *Trans*IT-LT1 Reagent from 2 to 8 µL per 1 µg DNA. Refer to "Before You Start."
*Trans*IT-LT1 Reagent was not accurately measured	Warm *Trans*IT-LT1 Reagent to room temperature and mix well before each use to ensure maximum activity every time.
	Dilute the *Trans*IT-LT1 Reagent in 80% ethanol immediately prior to each use when small volumes are required. Do not store diluted *Trans*IT-LT1 Reagent and reuse.
*Trans*IT-LT1 Reagent—DNA complexes did not form well	Prepare *Trans*IT-LT1 Reagent—DNA complexes in serum-free growth medium. We recommend either Opti-MEM or RPMI 1640 medium without serum.
	Highly concentrated DNA may form an insoluble precipitate during complex formation. Dilute DNA to 1-3 µg/µl before combining with *Trans*IT-LT1 Reagent.
*Trans*IT-LT1 Reagent—DNA complexes were added to cells not cultured in serum-containing medium	Allow *Trans*IT-LT1 Reagent—DNA complex to form in serum-free medium, then add the *Trans*IT-LT1 Reagent—DNA complex to cells cultured in complete growth medium.
	The presence of serum in the growth medium improves transfection efficiency and reduces cytotoxicity. No culture medium change is required.
Cell density (% confluence) not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this optimal density to ensure reproducibility. For most cell types, 50-70% confluence at transfection is recommended.
	Divide the culture 18-24 hours before transfection such that the cells reach optimal cell density at time of transfection.
Low-quality DNA (partially degraded or contaminated with an inhibitor, such as endotoxin)	Use highly purified, sterile, contaminant-free DNA for transfection. Do not use DNA prepared using miniprep kits.
	DNA that is endotoxin-free (bacterial lipopolysaccharide-free) is optimal. Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation.
Suboptimal DNA concentration	Determine the optimal DNA concentration for transfection. The optimal DNA concentration generally ranges between 1-3 µg/well of a 6-well plate. Highly concentrated DNA may produce a precipitate during complex formation.
	Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of reagent accordingly.
	Determine the optimal DNA concentration for transfection. The optimal DNA concentration generally ranges between 1 µg/well and 3 µg/well of a 6-well plate.

TROUBLESHOOTING (CONTINUED)

Problem	Solution
LOW TRANSFECTION EFFICIENCY	
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (for example, from 12 to 72 hours). The optimal incubation time is generally 24-48 hours.
Fetal calf serum present during *Trans*IT-LT1 Reagent—DNA complex formation	Use serum-free medium for complex formation. We recommend Opti-MEM or RPMI 1640 serum-free media.
Inhibitor present during transfection	Polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use culture medium that does not contain these polyanions.
Cell morphology has changed	A high or low cell passage number can reduce transfection efficiency. Maintain a similar passage number between experiments to ensure reproducibility.
HIGH CELLULAR TOXICITY	
*Trans*IT-LT1 Reagent—DNA complexes added to cells cultured in serum-free medium	*Trans*IT-LT1 Reagent does effectively transfect cells cultured in serum-free medium; however, toxicity may be higher if serum is absent. If toxicity is observed, consider adding serum to the culture medium.
Cell density (% confluence) not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this optimal density to ensure reproducibility. For most cell types, 50-70% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on cell type.
Excess *Trans*IT-LT1 Reagent—DNA complex mixture present during transfection	Reduce the amount of *Trans*IT-LT1 Reagent or DNA added to the cells. (See the "Before You Start" section for suggestions on optimization.)
*Trans*IT-LT1 Reagent—DNA complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-LT1 Reagent—DNA complex dropwise to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly. Do not swirl or rotate the dish, as this may cause uneven distribution
Cell morphology has changed	A high or low cell passage number can make cells more sensitive to transfection reagents. Maintain a similar passage number between experiments to ensure reproducibility.
Endotoxin contaminated DNA	Use highly purified, sterile, contaminant-free DNA for transfection. Use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells. Do not use DNA prepared using miniprep kits. DNA comtaminated with high levels of endotoxin (bacterial lipopolysaccharide) may cause high cell death depending on the cell line. Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation.

RELATED PRODUCTS

MiraCLEAN® Endotoxin Removal Kits
Label IT® Tracker Nucleic Acid Intracellular Localization Kits
*Trans*IT® Cell Line Specific Transfection Reagents and Kits
*Trans*IT® *In Vivo* Gene Delivery Kits
*Trans*IT®-QR and *Trans*IT®-EE Delivery Solutions and Kits
Label IT® Fluorescent Plasmid Delivery Controls

APPENDIX

Contact Mirus Bio for additional information.

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For publications citing the use of the *Trans*IT-LT1 Transfection Reagent, visit our website: www.mirusbio.com.

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