*Trans*IT®-Insect Transfection Reagent

Mirus | The Transfection Experts

Protocol for MIR 6100, 6104, 6105, 6106

INTRODUCTION

*Trans*IT®-Insect Transfection Reagent is a novel transfection formulation specifically developed for high-performance transfection of plasmid DNA into insect cells such as: Sf9, High Five™ (BTI-TN-5B1-4), and *Drosophila melanogaster* Schneider's 2 (S2 or D. Mel. (2)). *Trans*IT-Insect can also be used to transfect insect cells with baculovirus DNA to make recombinant virus. *Trans*IT-Insect is composed of animal-origin free components and is serum compatible, which eliminates the need for any culture medium change after transfection.

SPECIFICATIONS

Storage	Store *Trans*IT-Insect Reagent at –20°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	6 months from the date of purchase, when properly stored and handled.

Warm *Trans*IT-Insect to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

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

Product No.	Quantity
MIR 6104	1 × 0.4 ml
MIR 6100	1 × 1.0 ml
MIR 6105	5 × 1.0 ml
MIR 6106	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified plasmid DNA
- Serum-free medium (e.g. Grace's Insect Basal Medium, Cat. No. 13-200-CV, Cellgro®)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency transfection using *Trans*IT-Insect Transfection Reagent. **Table 1** presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Divide the cells 18-24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate density at the time of transfection.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are desirable. DNA prepared using miniprep kits is not recommended as it might contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove endotoxin from your DNA preparation.
- **Ratio of *Trans*IT-Insect Reagent to DNA.** Determine the best *Trans*IT-Insect Reagent:DNA ratio for each cell type. Start with 2 µl of *Trans*IT-Insect Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-Insect Reagent from 1–4 µl per 1 µg DNA to find the optimal ratio. **Table 1** provides recommended starting conditions based on cell culture vessel size.
- **Complex formation conditions.** Prepare *Trans*IT-Insect Reagent:DNA complexes in serum-free growth medium. Mirus recommends Grace's Insect Basal Medium.
- **Cell culture 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.
- **Presence of antibiotics:** Antibiotics may inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added directly to cells grown in complete culture medium containing serum and low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time.

Do not use DNA prepared using miniprep kits for transfection.

Do not use serum or antibiotics in the medium during transfection complex formation.

Table 1. Recommended starting conditions for DNA transfections with *Trans*IT-Insect 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
Complete growth medium	92 µl	263 µl	0.5 ml	1 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-Insect Reagent	0.2 µl	0.52 µl	1 µl	2 µl	5 µl	30 µl	38 µl

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.

If small volumes of *Trans*IT-Insect need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-Insect Reagent.

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections using *Trans*IT-Insect Transfection Reagent 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-Insect Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 1** on Page 2).

Reverse transfection protocol for high throughput screening available at: http://www.mirusbio.com/hts

Transient plasmid DNA transfection protocol per well of a 6-well plate

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells in 2.5 ml complete growth medium per well in a 6-well plate. Ideally cells should be ≥ 80% confluent prior to transfection.

   For adherent cells: Plate cells at a density of 4–8 × 10^5 cells/well.

   For suspension cells: Plate cells at a density of 8–12 × 10^5 cells/well.
2. Incubate cell cultures overnight.

Divide cultured cells 18–24 hours before transfection to ensure active cell division at the time of transfection.

B. Prepare *Trans*IT-Insect Reagent:DNA complex (Immediately before transfection)

1. Warm *Trans*IT-Insect Reagent to room temperature and vortex gently before using.
2. Place 250 µl of Grace's Insect Basal Medium in a sterile tube.
3. Add 2.5 µg (2.5 µl of a 1 µg/µl stock) plasmid DNA.
4. Pipet gently to mix completely.
5. Add 5 µl *Trans*IT-Insect Reagent to the diluted DNA mixture.
6. Pipet gently to mix completely.
7. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

During complex formation the *Trans*IT-Insect: DNA solution may have an opaque appearance. This is normal and does not negatively impact transfection performance.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-Insect Reagent:DNA complexes (prepared in Step B) drop-wise to different areas of the wells. For suspension cultures, slowly add reagent:DNA complexes into the flask containing cells while gently swirling the flask to evenly distribute the complexes.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-Insect Reagent:DNA complexes.
3. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay as required.

*Trans*IT-Insect is a low-toxicity reagent. There is no need to change fresh culture medium after transfection. If required, perform a medium change at least 4 hours post-transfection.

Transfection complexes, visualized as small particles, are sometimes observed following transfection. The complexes are not toxic to cells and do not impact transfection efficiency or transgene expression.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA. We recommend transfecting plasmids where the gene of interest is codon optimized and under the control of insect-specific promoter and enhancer elements (e.g. hr5/ie1).
Suboptimal *Trans*IT-Insect:DNA ratio	Determine the best *Trans*IT-Insect: DNA ratio for each cell type. Titrate the *Trans*IT-Insect Reagent from 1–4 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Determine the DNA concentration accurately. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8–2.0. The optimal DNA concentration generally ranges between 1–3 µg/well of a 6-well plate. Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of *Trans*IT-Insect Reagent accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin- and contaminant-free DNA for transfection. We recommend using Mirus MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation. Alternatively, 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 as it might contain high levels of endotoxin.
Cells not actively dividing at the time of transfection	Divide the culture at least 18–24 hours before transfection to ensure that the cells are actively dividing and reach optimal cell density at time of transfection.
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g.12–72 hours). The best incubation time is generally 24–48 hours.
*Trans*IT-Insect was not mixed properly	Warm *Trans*IT-Insect Reagent to room temperature and vortex gently before each use.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents according to **Table 1** on Page 2 including serum-free media, *Trans*IT-Insect Reagent, and plasmid DNA. Precipitation maybe observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold.
Proper experimental controls were not included	To assess delivery efficiency of plasmid DNA, use Mirus *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid or Mirus prelabeled *Label* IT Plasmid Delivery Controls (please refer to Related Products on Page 6). To verify efficient transfection, use *Trans*IT-Insect Reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Cell density not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this density to ensure reproducibility. For most cell types, ≥ 80% confluence is recommended at the time of transfection, but use of higher or lower densities may increase cell viability depending on cell type.
Cell morphology has changed	Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check your cells for mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate mycoplasma. A high or low cell passage number can make cells more sensitive and refractory to transfection. Maintain a similar passage number between experiments to ensure reproducibility.
Medium change or addition may be necessary	If incubating for 48–72 hours, it may be necessary to change the complete medium 24 hours post-transfection.
Transfection complexes and cells not mixed thoroughly after complex addition	Add transfection complexes drop-wise 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.
Transfection complexes added to cells cultured in serum-free medium	*Trans*IT-Insect Reagent efficiently transfects cells cultured in serum-free medium; however, toxicity may be higher if serum is not present. If toxicity is a problem, consider adding serum to the culture medium.

RELATED PRODUCTS

- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- *Trans*IT-X2™ Dynamic Delivery System
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT-PRO® Transfection Kit
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- *Trans*IT-siQUEST® Transfection Reagent
- *Trans*IT-TKO® Transfection Reagent

For details on our products, visit www.mirusbio.com or www.TheTransfectionExperts.com.

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.TheTransfectionExperts.com/reagentagent

Contact Mirus Bio for additional information.

Mirus Bio LLC
545 Science Drive
Madison, WI 53711
Toll-free: 888.530.0801
Direct: 608.441.2852
Fax: 608.441.2849