*Trans*IT®-Express Transfection Reagent

Product # MIR 2000, MIR 2004, MIR 2005, MIR 2006

Product	Quantity	Product No.
*Trans*IT®-Express	0.4 ml	MIR 2004
	1 ml	MIR 2000
	5 ml (5 x 1 ml)	MIR 2005
	10 ml (10 x 1 ml)	MIR 2006

1.0 INTENDED USE

*Trans*IT®-Express is developed for high-throughput transfection applications. The DNA/reagent complexes can be added directly to cells in their complete media, and these complexes do not need to be removed after transfection. Alternatively, the complexes may be formed directly in 96-well plates, and the cells in complete growth media are added subsequently. This can save significantly on both time and expense invested in the transfection process. Each milliliter of *Trans*IT®-Express Transfection Reagent is sufficient to perform 5,000 transfections in 96-well plates, depending on the specific cell type being used.

2.0 DESCRIPTION

2.1 General Information

The *Trans*IT® Transfection Reagents were developed by the gene transfer specialists of Mirus Bio Corporation in response to a need for high- efficiency and low-toxicity in vitro transfections. Although second-generation cationic-liposome formulations yield increased transfection efficiencies, they often increase cellular toxicity. For the majority of applications and cell types, *Trans*IT® transfection reagents offer clear advantages for delivering DNA into cells via transfection, including increased efficiency, 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. Due to the low toxic effects, the reagent may be added directly at the time of seeding cells. This results in a significant time savings, without sacrificing overall efficiency. In addition, transfections with the *Trans*IT®-Express Reagent can be carried out in serum-containing media and do not require media changes. This unique combination makes this reagent ideal for all gene expression studies, and it is especially suited for high-throughput applications.

For the 96-well format, cells can be seeded one day prior to transfection according to the standard protocol, but transfection time can be reduced even further by preparing the transfection complexes at the same time the cells are being plated. The complexes can be added to the cells immediately after they are plated, or the complex formation step can be performed directly in the plates and the cells can then be added to the same wells.

2.2 Cell Lines Successfully Tested by Mirus Bio Corporation

TransIT®-Express: COS-7, HEK293, HeLa, NIH3T3

2.3 Specifications

Concentration: *Trans*IT®-Express: 1.287 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: *Trans*IT®-Express is stored in ethanol to ensure sterility

Stability: 1 year from the date of purchase when stored at 4˚C or -20˚C

3.0 PROCEDURE

The protocols below are recommended for performing transfections with *Trans*IT®-Express Transfection Reagent in 96-well plates. When performing transfections in different sized dishes, the amounts of DNA, *Trans*IT® Reagent, and culture medium should be scaled in proportion to the surface area of the dish. The following chart provides suggestions for starting points toward optimizing transfection reactions.

Plate size	Surface area cm^2	DNA µg /well	*Trans*IT®-Express µl /well	Complex Formation Medium µl/well	Plating medium ml/well
96-well	0.32	0.05-0.1	0.15-0.3	7.5	0.1
24-well	1.9	0.5	1.5	37.5	0.5
12-well	3.8	1	3	75	1
6-well	9.4	2	6	150	2
35 mm	8.0	2	6	150	2
60 mm	21	5	15	375	5
100 mm	55	15	45	1125	15

3.1 *Trans*IT®-Express Protocol for 96-Well Format (for transient transfection of adherent or suspended cells)

*Trans*IT®-Express can be used for high-throughput transfections in 96-well plates. The *Trans*IT®/DNA complexes can be added directly to the cells in their complete medium, and these complexes do not need to be subsequently removed. For the 96-well format, cells can be seeded one day prior to transfection according to the standard protocol, but transfection time can be reduced even further by preparing the transfection complexes at the same time the cells are being plated.[a] The complexes can be added to the cells immediately after they are plated, or the complex formation step can be performed directly in the plates and then the cells can be added.

1. Add at least 7.5µl of serum-free media[b] to each well of a 96 well plate. If evaporation is a concern, the volume may be increased to as much as 50 µl. Immediately before transfection, add the *Trans*IT® Transfection Reagent (0.06-0.3 µl) dropwise directly into the wells.[c] To aid in pipetting, the necessary volume of reagent may be diluted 10-fold in water or ethanol. Mix thoroughly by pipetting and rocking the plate back and forth.

   NOTE: Dilute only the amount of reagent needed. Do not store diluted reagent. Discard after use. (The stated volume has been determined using a 3:1 reagent:DNA ratio, which we find optimal in most cell lines).

   NOTE: The cells may be plated first in the 96-well plates. In this case, simply prepare transfection complexes in another tube and transfer to the 96-well plates. For some cell lines, this will result in slightly higher efficiency.

2. Incubate at room temperature for 5 minutes.
3. Add DNA[e] (0.05-0.1µg) to the diluted *Trans*IT® Reagent. Mix thoroughly and rock from side to side.
4. Incubate at room temperature for 5 minutes (a range of 5-20 minutes is acceptable).
5. While the complexes are incubating, split the cells to a density of 2-4 x 10^4 cells/well in complete growth media. To seed the cells for a 24-hour incubation, scale back the seeding density two-fold (1-2 x 10^4 cells/well).

   NOTE: For several cell lines tested, we have found that *Trans*IT®-Express Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated.

6. Incubate for 8-48 hours.[f]
7. Assay.

3.2 Transfection Optimization

The key to successful transfection is careful optimization of the process for each individual cell type. The transfection protocol described in Section 4.1 should result in efficient transfection of most cell types; however, to ensure optimal results the following footnotes address variables that should be considered:

A. Cell density (confluence) at transfection – The recommended cell density for most cell types at transfection is 50%-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. *Trans*IT® Reagent to DNA ratio – As a general starting point, we recommend using a 3:1 (µl: µg) ratio of TransIT®-Express Reagent to DNA. The optimal *Trans*IT® 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. In our hands, a 2:1 ratio with HeLa Cells, and a 3:1 or 4:1 ratio with COS-7, HEK293, and NIH3T3 cells provides optimal results.

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

D. For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 25 µl.

E. 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 with the range of 0.02-0.1 µg per well of a 96-well plate. As a starting point, we recommend using 0.09µg per well.

F. Transfection incubation time – The optimal incubation time can be determined empirically by testing a range of incubation times from 8-48 hours. 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.

4.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT® Reagent to DNA ratio**
  Determine the optimal *Trans*IT® Reagent to DNA ratio by titrating the reagent from 2 µl/µg DNA up to 12 µl/µg DNA. As a general starting point, we recommend using a 3:1 (µl: µg) ratio of *Trans*IT®-Express Reagent to DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections.
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**
  Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT® 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 50-70% 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 *Trans*IT® reagent may form a precipitate during long term storage at –20˚C. Undetected, this could result in lowered efficiencies. Warm the reagent to room temperature and gently vortex to redissolve any precipitate.

High Cellular Toxicity

- ***Trans*IT®-Express Reagent/DNA complex mixture and cells were not mixed thoroughly after adding the complexes**
  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 *Trans*IT® Reagent/DNA complex mixture was used in transfection**
  Reduce the amount of *Trans*IT® 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.

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' products, please visit the Technical Resources section of our website. (www.mirusbio.com)

6.0 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®-mRNA Transfection 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)
*Trans*IT®-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
*Trans*IT®-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)
*Trans*IT®-QR Hydrodynamic Delivery Solution (Product # MIR 5240)
*Trans*IT®-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

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.