*Trans*IT®-293 Transfection Reagent

Product Name	Volume of *Trans*IT®-293 Reagent	Product No.
*Trans*IT®-293 Transfection Reagent	0.4 ml	MIR 2704
	1 ml	MIR 2700
	5 ml (5 × 1 ml)	MIR 2705
	10 ml (10 × 1 ml)	MIR 2706

Each milliliter of *Trans*IT®-293 Transfection Reagent (MIR 2700) provides sufficient reagent to perform up to 500 transfections in 6-well plates.

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT®-293 Transfection Reagent is designed by the nucleic acid delivery specialists at Mirus Bio Corporation. This novel kit was specifically optimized to provide superior transfection efficiency in HEK 293 cells without sacrificing cellular health. The specificity of the *Trans*IT®-293 Transfection Reagent makes this product a desirable alternative to broad spectrum transfection reagents. The kit provides all the attributes of the trusted *Trans*IT® Reagent line: high efficiency, low toxicity, simplicity of use and reproducibility. In addition, transfections with the *Trans*IT® Reagents do not require media changes and can be carried out in serum-containing media. The *Trans*IT®-293 Transfection Reagent is quality control tested on ATCC HEK 293 cells. These significant features establish the *Trans*IT®-293 Transfection Reagent as the product of choice for transfecting HEK 293 cells.

1.2 Specifications

Concentration: 1.2 mg/ml in 80% ethanol

Storage: Store tightly capped at 4˚C

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

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of transfection parameters. The transfection protocol described in Section 2.2 should result in efficient transfection of most 293 cell subtypes; however, to ensure optimal results consider the following variables:

A. Media conditions - The *Trans*IT®-293 Reagent yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection media change.

B. Cell density (% confluence) at the time of transfection - The recommended cell density for most cell types at transfection is 50-70% confluence. Determine the optimal cell density for each 293 cell subtype 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 pure, sterile, and free from contaminants such as endotoxin (lipopolysaccharides). Remove any traces of endotoxin using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal amount of DNA for transfection is usually within 1-3 µg per well of a 6-well plate. As a starting point, use 2 µg of DNA per well of a 6-well plate.

D. *Trans*IT®-293 Reagent to DNA ratio - As a starting point, use 3 µl of *Trans*IT®-293 Reagent per 1 µg of DNA. The optimal *Trans*IT®-293 Reagent to DNA ratio can be determined by titrating the reagent from 2 to 8 µl per 1 µg DNA. The volume of reagent that gives the highest transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

E. Transfection incubation time - The optimal incubation time can be determined empirically by testing a range from 24-48 hours.

The following protocol is recommended for performing transfections with *Trans*IT®-293 Transfection Reagent in 6-well plates. When performing transfections in different size plates, the amount of DNA, *Trans*IT®-293 Reagent, and serum-free medium should be scaled up or down in proportion to the surface area of the well.

Table 1. Recommend starting conditions for the *Trans*IT®-293 Transfection Reagent

Culture Vessel	24-well plate	12-well plate	6-well plate
Surface Area	1.9 cm^2	3.8 cm^2	9.6 cm^2
Serum-free Media	50 µl	100 µl	200 µl
*Trans*IT®-293 Transfection Reagent	1-2 µl	2-4 µl	4-8 µl
DNA (1 µg/µl stock)	0.5 µl	1 µl	2 µl
Complete Growth Media	500 µl	1000 µl	2500 µl

*All volumes in Table 1 are per well of the indicated size.

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

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10^5 cells in their complete growth medium per well of a 6-well plate) to obtain ~50-70% confluence the following day. [a]
2. Incubate the cells overnight.[b]

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

1. In a sterile plastic tube, add the *Trans*IT®-293 Reagent (2 to 8 µl per 1 µg DNA) directly into 200 µl of serum-free medium. [c,e] Mix thoroughly by pipetting or vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1 to 3 µg per well) to the *Trans*IT®-293 Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 15-30 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-293 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.

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 *Trans*IT®-293 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. Do not swirl the plate.
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 hour 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 gene expression.

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 (~1-3 x 10^5 cells in complete growth medium per well of a 6-well plate).
2. Incubate the cells overnight.[b]

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

1. In a sterile plastic tube, add the *Trans*IT®-293 Reagent (2 to 8 µl per 1 µg DNA) directly into 200 µl of serum-free medium[c,e] Mix thoroughly by pipetting or vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1-3 µg per well) to the diluted *Trans*IT®-293 Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 15-30 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-293 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.

1. If necessary, spin down the cells prepared in Step A, remove the medium from these cells, and replace it with 2 ml of fresh complete growth medium. Replate cells as described in Section 2.3A.
2. Add the *Trans*IT®-293 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. Do not swirl the plate.
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 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 gene expression.

a Since the optimal cell density (% confluence) for efficient transfection can vary between 293 subtypes this should be determined for each subtype. Maintain the optimal seeding protocol between experiments for each cell type.

b Standard incubation conditions for mammalian cells are 37˚C in 5% CO_2.

c The *Trans*IT®-293 Reagent/DNA complex may form improperly if the complex formation medium contains serum, resulting in poor transfection efficiencies. Any serum free media can be used for complex formation, provided it does not contain polyanions such as dextran sulfate and heparin.

d Determine the optimal incubation time empirically by testing a range of incubation times from 24-48 hours.

e For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 300-1000 µl.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-293 Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-293 Reagent to DNA ratio by titrating the reagent from 2 to 8 µl per 1 µg DNA. As a starting point, use 3 µl per 1 µg of DNA in 6-well plates. Use the amount that gives the best transfection efficiency and the lowest cellular toxicity for future transfections. Use 2 µg DNA per well of a 6-well plate.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, the transfection efficiencies may be adversely affected. Maintain a similar passage number between experiments to ensure reproducibility.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most 293 cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for different subtypes in order to maximize transfection efficiency. Maintain the optimal density in future experiments for reproducibility.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, then add to cells in complete growth media (containing serum). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media and a media change is eliminated.
- **Poor quality of transfecting DNA**
  Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (lipopolysaccharides) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT®-293 Reagent/DNA complex formation**
  Use serum-free media when forming the complexes.
- **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. If polyanions are necessary, the transfection media can be replaced with the optimal cell growth media 24 hours post transfection.

High Cellular Toxicity

- **Excessive amounts of *Trans*IT®-293 Reagent/DNA complex mixture was used in transfection**
  Reduce the amount of *Trans*IT®-293 Reagent in the transfection. See Table 1 for recommended starting conditions.
- **Media change or addition may be necessary**
  If incubating for 48-72 hours, it may be necessary to change the complete media 24 hours post-transfection. Alternatively, add additional complete media 4-24 hours post-transfection.
- **Cell density (% confluence) was not optimal at time of transfection**
  Allow cells to grow to a higher cell density and repeat the experiment.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media then add to cells in complete growth media (containing serum). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media and a media change is eliminated.

- **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.
- **Complexes were not mixed thoroughly**
  Thoroughly mix complexes before they are added to the cells. Ensure the complexes are added in a dropwise fashion. Rock the dish back and forth and from side to side. Do not swirl or rotate the dish, as this may result in uneven distribution.

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 Technical Resources 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 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®-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-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)
*Trans*IT-TKO® HTS-96 Plates (Product # MIR 2530, 2540, 2550, 2560, 2570)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7200,7201,7202,7203,7204,7205)

*These products are available in additional sizes.

The performance of this product is guaranteed for 1 year from the date of purchase if stored and handled properly.
Mirus Transfection Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811, 6,593,465 and patents pending.
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