*Trans*IT®-Keratinocyte Transfection Reagent

Product # MIR 2800, MIR 2804, 2805, 2806

Product	Quantity	Product No.
*Trans*IT®-Keratinocyte Transfection Reagent	1 ml	MIR 2800
	0.4 ml	MIR 2804
	5 x 1 ml	MIR 2805
	10 x 1 ml	MIR 2806

1.0 INTENDED USE

Each milliliter of *Trans*IT®-Keratinocyte Transfection Reagent (MIR 2800) is a sufficient quantity to perform up to 500 transfections in primary keratinocytes in 35 mm dishes, 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. Although second generation cationic-liposome formulations yield increased transfection efficiencies, they often increase cellular toxicity. *Trans*IT®-Keratinocyte Transfection Reagent offers clear advantages for delivering DNA into primary keratinocytes via transfection, including minimal cellular toxicity, ease of use and transfection reproducibility. This product provides state-of-the-art transfection efficiencies with significantly reduced levels of cell damage compared to other leading transfection reagents. In addition, transfections with the *Trans*IT®-Keratinocyte Reagent do not require media changes and can be carried out in serum-containing media. This unique combination makes this reagent ideal for all gene expression studies where the post-transfection state of the cell is important.

2.2 Specifications

Concentration: *Trans*IT®-Keratinocyte: 1.37 mg/ml in 80% ethanol

Storage: 4˚C

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

3.0 PROCEDURE

3.1 Transfection Optimization

The key to successful transfection always involves careful optimization of reaction conditions for each individual cell type. The transfection protocols described in Sections 4.2-4.3 should result in efficient transfection of most types of keratinocytes; however, to ensure optimal results the following variables 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 keratinocyte subtype in order to maximize transfection efficiency. This density should be maintained in future experiments for reproducibility.

B. 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 (LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is within the range of 1-3 µg per 35 mm well. As a starting point, use 2 µg per 35 mm well.

C. *Trans*IT®-Keratinocyte Reagent to DNA ratio - As a starting point, we recommend using 3-4 µl of *Trans*IT®-Keratinocyte Reagent per 1 µg of DNA. The optimal *Trans*IT®-Keratinocyte Reagent to DNA ratio can be determined by titrating the reagent starting at 2 µl/µg DNA up to 10 µl/µg DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections.

D. Transfection Incubation Time - The optimal incubation time can be determined empirically by testing a range of incubation times from 2-48 hours.

The protocols below are recommended for performing transfections with TransIT®-Keratinocyte Transfection Reagent in 35 mm dishes. When performing transfections in different sized dishes, the amounts of DNA TransIT®-Keratinocyte and culture medium should be scaled up or down in proportion to the surface area of the dish. For your convenience, we have supplied the following chart of surface areas:

3.2 Protocol for Transient Transfection (Adherent Cells)

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 35 mm well) so that they will be ~50-70% confluent the following day. [a]
2. Incubate the cells overnight.[b]

B. Complex Formation (perform this procedure just prior to transfection)

1. In a sterile, plastic tube, add the *Trans*IT®-Keratinocyte Transfection Reagent (2-10 µl/µg DNA) dropwise into ≥100 µl of serum-free medium.[c,e] Mix thoroughly by vortexing.
2. Incubate at room temperature for 5 minutes.
3. Add DNA (1-3 µg) to the diluted *Trans*IT®-Keratinocyte Reagent. Mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: Transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated. For transfections in serum-free medium, proceed to Part D.

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®-Keratinocyte 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]
4. Harvest cells and assay as needed.

D. Cell Preparation for Transfections in Serum-Free Medium

1. Remove the complete medium from the cells prepared in Step A and wash cells once with 2 ml per well of sterile Dulbecco's PBS or serum-free medium.
2. Remove the wash solution and add 2 mL per well of fresh serum-free medium to the cells.
3. Add the *Trans*IT®-Keratinocyte Reagent/DNA complex mixture prepared in Step B to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
4. Incubate for 2-8 hours.[b,d]
5. Remove the medium containing the *Trans*IT®-Keratinocyte Reagent/DNA complex mixture and replace it with complete growth medium.
6. Incubate for 24-48 hours.[b]
7. Harvest cells and assay for reporter gene activity.

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

[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® Reagent/DNA complex may form improperly if the transfection medium contains serum, resulting in poor transfection efficiencies.

[d] The optimal incubation time should be determined empirically by testing a range of incubation times from 2-8 hr.

[e] For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 200-1,000 µl.

3.3 Protocol for Stable Transfection (Adherent Cells)

1. Follow the protocol described in Section 4.2.
2. Subculture your cells at the desired dilution (at least 1:5) into selection medium.

4.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal TransIT®-Keratinocyte Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-Keratinocyte Reagent to DNA ratio by titrating the reagent from 2 µl/µg DNA up to 10 µl/µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, we recommend trying 3-4 µl of *Trans*IT®-Keratinocyte Reagent per 1 µg of DNA (in a 35 mm well).
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an 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 (LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is within the range of 1-3 µg per 35 mm well. As a starting point, use 2 µg per 35 mm well.
- **Fetal calf serum present during *Trans*IT®-Keratinocyte 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.

High Cellular Toxicity

- ***Trans*IT®-Keratinocyte Reagent/DNA complex mixture and cells were not mixed thoroughly after adding the complex**
  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®-Keratinocyte Reagent/DNA complex mixture was used in transfection**
  Reduce the amount of *Trans*IT®-Keratinocyte 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 Bio products, please visit the Technical Resources section of our website. (www.mirusbio.com)

5.0 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.