*Trans*IT®-Keratinocyte Transfection Reagent

Protocol for MIR 2800, 2804, 2805, 2806

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/2800

INTRODUCTION

*Trans*IT®-Keratinocyte Transfection Reagent is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in primary and immortalized keratinocytes. *Trans*IT-Keratinocyte Reagent provides all the attributes of the trusted *Trans*IT series of transfection reagents: high transfection efficiency, low toxicity, serum compatibility, simplicity of use and reproducibility. Transfection with *Trans*IT Keratinocyte Reagent does not require medium changes and can be carried out in serum-containing medium. *Trans*IT-Keratinocyte is suitable for both transient and stable transfection.

SPECIFICATIONS

Storage	Store *Trans*IT-Keratinocyte Reagent at 4°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

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

MATERIALS

Materials Supplied

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

Product No.	Quantity
MIR 2804	1 × 0.4 ml
MIR 2800	1 × 1.0 ml
MIR 2805	5 × 1.0 ml
MIR 2806	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified plasmid DNA
- Serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required
- *Optional:* Selection antibiotic (e.g., G418 or Hygromycin B) for stable transfection

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

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

- **Cell density (% confluence) at transfection.** Determine the optimal cell density for each keratinocyte subtype to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density (generally ≥80% confluence) 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-Keratinocyte Reagent to DNA.** Determine the best *Trans*IT-Keratinocyte Reagent:DNA ratio for each keratinocyte cell type. Start with 3 µl of *Trans*IT-Keratinocyte Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-Keratinocyte Reagent from 2–8 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-Keratinocyte Reagent:DNA complexes in serum-free growth medium. Mirus recommends Opti-MEM I Reduced-Serum 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 keratinocyte 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, and the half-life of the expressed protein.

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-Keratinocyte 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	1.0 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	0.25 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-Keratinocyte Reagent	0.3 µl	0.75 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 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-Keratinocyte need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-Keratinocyte Reagent.

PLASMID DNA TRANSFECTION PROTOCOL

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

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.
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-Keratinocyte Reagent:DNA complex (Immediately before transfection)

1. Warm *Trans*IT-Keratinocyte Reagent to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 2.5µg (2.5 µl of a 1 µg/µl stock) plasmid DNA. Pipet gently to mix completely.
4. Add 7.5µl *Trans*IT-Keratinocyte Reagent to the diluted DNA mixture. Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-Keratinocyte Reagent:DNA complexes drop-wise to different areas of the wells.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-Keratinocyte 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.

There is no need to change culture medium after transfection. If required, perform a medium change at least 4 hours post-transfection.

For generating stable cell transfectants, passage the cells 48-72 hours post-transfection in complete growth medium containing the appropriate selection antibiotic such as G418 or Hygromycin B. Maintain selection for 1–2 weeks, allowing selection of cells that have undergone stable integration of DNA.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
*Trans*IT-Keratinocyte Reagent was not mixed properly	Warm *Trans*IT-Keratinocyte to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-Keratinocyte Reagent:DNA ratio	Determine the best *Trans*IT-Keratinocyte Reagent:DNA ratio for each keratinocyte cell type. Titrate the *Trans*IT-Keratinocyte Reagent from 2–8 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Confirm DNA concentration and purity. 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-Keratinocyte Transfection Reagent accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. We recommend using Mirus Bio's 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 may contain high levels of endotoxin.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-Keratinocyte Reagent:DNA complexes in serum-free growth medium. We recommend Opti-MEM I Reduced-Serum Medium. Once transfection complexes are formed, they can be added directly to cells cultured in complete growth medium containing serum and 0.1–1X antibiotics. Polyanions such as dextran sulfate or heparin can inhibit transfection. Use culture medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hours post transfection.
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Transfection incubation time	Determine the optimal transfection incubation time for each keratinocyte cell type and experiment. Test a range of incubation times (e.g.12–72 hours). The best incubation time is generally 24–48 hours.
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.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents according to Table 1 on page 2 including serum-free medium, *Trans*IT-Keratinocyte and plasmid DNA. Precipitation may be 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 verify efficient transfection, use *Trans*IT-Keratinocyte Reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid. 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).

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-Keratinocyte Reagent:DNA complexes drop-wise to different areas of the wells containing 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	Allow *Trans*IT-Keratinocyte Reagent:DNA complexes to form in serum-free medium, then add these complexes 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 after the addition of transfection complexes to cells.
Endotoxin-contaminated plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. We recommend using Mirus Bio's MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of any traces 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 may contain high levels of endotoxin.
Expressed target gene is toxic to cells	Compare toxicity levels against a cells alone control and cells transfected with an empty vector to assess the cytotoxic effects of the target protein being expressed. If lower levels of target gene expression are desired in your transfection experiments, consider reducing the amount of target plasmid. Maintain the optimal *Trans*IT-Keratinocyte:DNA ratio by using carrier DNA such as an empty cloning vector.
Cell density not optimal at time of transfection	Determine the best cell density for each keratinocyte cell type to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most cell types, ≥80% confluence is recommended at 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.

RELATED PRODUCTS

- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- Cell Culture Antibiotic Solutions
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT-PRO® Transfection Kit
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits

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

Reagent Agent®

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