*Trans*IT®-mRNA Transfection Kit

Protocol for MIR 2225, 2250, 2255, 2256

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/2225

INTRODUCTION

*Trans*IT®-mRNA Transfection Kit is designed to transfect RNA into a broad range of cell types with minimal cellular toxicity. RNA delivery avoids transcriptional regulation effects by directly delivering the RNA to the cytoplasm for expression. The *Trans*IT-mRNA Transfection Kit can be used to deliver a variety of RNA molecules including mRNAs and viral RNAs (2–10 kb). It can be used for multiple applications such as short-term protein expression, viral production and replication studies.

*Trans*IT-mRNA Transfection Kit contains two components, namely: *Trans*IT-mRNA Reagent and the mRNA Boost Reagent. This kit is compatible with serum; transfection efficiency is optimal when transfections are performed in the presence of serum, with no medium change required.

SPECIFICATIONS

Storage	Store both *Trans*IT-mRNA Reagent and mRNA Boost 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-mRNA and mRNA Boost Reagent to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

*Trans*IT-mRNA Transfection Kit is supplied in ***one*** of the following formats.

Product No.	Volume of *Trans*IT-mRNA Reagent	Volume of mRNA Boost Reagent
MIR 2225	1 × 0.4 ml	1 × 0.4 ml
MIR 2250	1 × 1.0 ml	1 × 1.0 ml
MIR 2255	5 × 1.0 ml	5 × 1.0 ml
MIR 2256	10 × 1.0 ml	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified RNA
- Serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal RNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency RNA transfection using the *Trans*IT-mRNA Transfection Kit. **Table 1** on page 3 presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** Determine the optimal cell density for each cell type to maximize transfection efficiency. Passage 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.
- **RNA features.** The structure of the transfected RNA substrate can have a profound effect on transfection results. For example, an *in vitro* transcribed RNA substrate that is designed to express a protein product should be capped and polyadenylated, simulating a wild type mammalian messenger RNA (mRNA). The presence of the 5′ cap structure and 3′ polyA tail will both stabilize the mRNA after transfection and improve translation efficiency. Many *in vitro* transcription kits include cap analogs that can be incorporated into the 5′ end of the *in vitro* transcript. The transcript can also be polyadenylated using polyA polymerase or by the presence of a run of dA bases in the DNA transcription template. In some cases, the *in vitro* transcript can be produced with an internal ribosome entry site (IRES) in the 5′ untranslated region of the mRNA. The presence of the IRES can increase transcription in the absence of a 5′ cap.
- **RNA purity.** RNA used for transfection should be highly purified and sterile. We recommend mMESSAGE mMACHINE® T7 Ultra kit (Ambion) for *in vitro* transcription followed by purification of RNA transcripts using RNeasy® spin columns (Qiagen®). However, other methods of purification can also produce RNA of sufficient quality for highly efficient transfection. Avoid RNase contamination to maintain the integrity of the RNA molecules. RNA integrity can be verified using agarose gel electrophoresis.
- **RNA quantity.** As a starting point, use 1 µg of RNA per well of a 12-well plate. The optimal RNA amount can be determined by titrating from 1–3 µg of RNA per well of a 12-well plate. For certain applications, the optimal amount may be outside the recommended range of this protocol.
- ***Trans*IT-mRNA Reagent.** As a starting point, use 2 µl of *Trans*IT-mRNA Reagent per µg of RNA. The optimal *Trans*IT-mRNA Reagent volume can be determined by titrating the reagent from 1–3 µl per well of a 12-well plate. Please refer to Table 1 on Page 3 for recommended starting conditions.
- **mRNA Boost Reagent.** As a starting point, use 2 µl of mRNA Boost Reagent per µg of RNA. The optimal mRNA Boost volume should be determined by titrating the reagent from 1–3 µl per well of a 12-well plate. Refer to Table 1 on Page 3 for recommended starting conditions.
- **Complex formation conditions.** Prepare *Trans*IT-mRNA Reagent:mRNA Boost:RNA complexes in serum-free growth medium. Mirus recommends Opti-MEM I Reduced-Serum Medium. Use RNA stocks that range in concentration from 1–3 µg/µl.
- **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. The *Trans*IT-mRNA Transfection Kit yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change.

Depending on the goal of the experiment, it may not be necessary to cap and polyadenylate the RNA transcript. For example, some viral transcripts do not need to be capped and polyadenylated in order to start replicating and/or produce infectious virus after transfection.

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

- **Presence of antibiotics:** Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** The best post-transfection incubation time can be determined empirically by testing a range of incubation times from 4–48 hours. Optimal incubation times will depend on the characteristics of the RNA being transfected. For transfection of an mRNA encoding a protein, the recommended starting incubation range is from 12–18 hours. This recommendation was determined using a capped, polyadenylated *in vitro* transcript expressing firefly luciferase and may be shorter if the transfected RNA is not capped or polyadenylated. However, depending on the goal of the transfection experiment, longer incubation times may also be required. For instance, viral rescue by RNA transfection may require more time to generate infectious virus.

Table 1. Recommended starting conditions for RNA transfections with the *Trans*IT-mRNA Transfection Kit.

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 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
RNA (1µg/µl stock)	0.09 µl	0.25 µl	0.5 µl	1 µl	2.5 µl	15.5 µl	19.7 µl
*Trans*IT-mRNA Reagent	0.18 µl	0.5 µl	1 µl	2 µl	5 µl	31 µl	39.4 µl
mRNA Boost Reagent	0.18 µl	0.5 µl	1 µl	2 µl	5 µl	31 µl	39.4 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-mRNA and mRNA Boost need to be pipetted, dilute the reagents in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted reagents.

RNA TRANSFECTION PROTOCOL

The following procedure describes how to perform RNA transfections in 12-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-mRNA Reagent, mRNA Boost Reagent, RNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 1** on Page 3).

Transient RNA transfection protocol per well of a 12-well plate

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells in 1.0 ml complete growth medium per well in a 12-well plate. Ideally cells should be ≥80% confluent prior to transfection.

   For adherent cells: Plate cells at a density of 0.8–3.0 × 10^5 cells/ml.

   For suspension cells: Plate cells at a density of 2.5–5.0 × 10^5 cells/ml.
2. Incubate the cell cultures overnight.

Divide cultured cells 18–24 hours before transfection such that the cells reach optimal cell density at the time of transfection.

B. Prepare *Trans*IT-mRNA Reagent:mRNA Boost:RNA complexes (Immediately before transfection)

1. Warm *Trans*IT-mRNA and mRNA Boost reagents to room temperature and vortex gently before using.
2. Place 100 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 1 µg (1 µl of a 1 µg/µl stock) RNA. Pipet gently to mix completely.
4. Add 2 µl mRNA Boost Reagent to the diluted RNA mixture. Pipet gently to mix completely.
5. Add 2 µl *Trans*IT-mRNA Reagent to the diluted RNA mixture. Pipet gently to mix completely.
6. Incubate at room temperature for 2–5 minutes to allow sufficient time for complexes to form. *Do not incubate the complexes for more than 5 minutes.*

C. Distribute the complexes to cells in complete growth medium

1. Add the complexes (prepared in Step B) drop-wise to different areas of the wells. It is not necessary to replace the complete growth medium with fresh medium.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-mRNA Reagent:mRNA Boost:RNA complexes.
3. Incubate for 4–48 hours depending on the nature of the transfected RNA and the goal of the experiment.
4. Harvest cells and assay as required.

Warm *Trans*IT-mRNA and mRNA Boost reagents to room temperature and vortex gently before each use.

Do not let the complexes incubate longer than 5 minutes as this may decrease transfection efficiency.

*Trans*IT-mRNA is a low-toxicity reagent. There is no need to change culture medium after transfection, unless required by your cell type or culture conditions. If required, perform a medium change at least 4 hours post-transfection.

For protein translation assays, the recommended starting incubation range is from 12–18 hours.

For non-capped and/or non polyadenylated transcripts, incubation times may be shorter.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW RNA TRANSFECTION EFFICIENCY	
*Trans*IT-mRNA or mRNA Boost Reagent was not mixed properly.	Warm *Trans*IT-mRNA and mRNA Boost Reagents to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-mRNA Reagent:mRNA Boost Reagent ratio	Determine the optimal reagent levels to use for RNA transfection by testing a range of *Trans*IT-mRNA Reagent levels (1, 2, and 3 µl) with a range of mRNA Boost Reagent amounts (1, 2, and 3 µl). Following this guideline, nine different transfection complexes will be formed and transfected in order to identify the best transfection conditions for the particular RNA and cell type.
Suboptimal complex formation time	We recommend incubating the transfection complexes for 2–5 minutes. Longer incubation times may result in lower transfection efficiency.
Suboptimal amount of *Trans*IT-mRNA Reagent	Determine optimal amount of *Trans*IT-mRNA Reagent for each cell type. Titrate the *Trans*IT-mRNA Reagent from 1–3 µl per well of a 12-well plate, e.g. test 1, 2 and 3 µl of *Trans*IT-mRNA Reagent per well of a 12-well plate.
Suboptimal amount of mRNA Boost Reagent	Determine optimal amount of mRNA Boost Reagent for each cell type. Titrate the mRNA Boost Reagent from 1–3 µl per well of a 12-well plate, e.g. test 1, 2, and 3 µl of *Trans*IT-mRNA Reagent per well of a 12-well plate.
Suboptimal amount of RNA	Determine the RNA concentration accurately. Use RNA preps that have an $A_{260/280}$ absorbance ratio of 1.8–2.0. The optimal RNA concentration generally ranges between 1–3 µg/well of a 12-well plate. Start with 1.0 µg/well of a 12-well plate. Consider testing more or less RNA while scaling the amount of reagent accordingly.
Low-quality RNA	Use highly purified, sterile and contaminant-free RNA for transfection. We recommend using purifying the RNA using a column procedure such as Qiagen's RNeasy spin columns. Avoid RNase contamination as degradation of the RNA substrate will significantly diminish expression of the transfected RNA.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT mRNA Reagent:mRNA Boost:RNA 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.
Transfection incubation time	Determine the best transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g. 12–72 hours). The optimal incubation time is generally 24–48 hours.
Proper controls were not included	To verify efficient transfection, use *Trans*IT-mRNA Reagent to deliver a reporter transcript such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding RNA that is capped and polyadenylated. To assess delivery efficiency of RNA, use Mirus' *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits to label the RNA substrate for intracellular tracking (please refer to Related Products on Page 7).

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Cell density not optimal at time of transfection	Determine the best cell density for each cell type to maximize transfection efficiency. Use this density to ensure reproducibility. For most cell types, ≥80% confluence at the time of transfection is recommended.
	Divide the culture at least 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at time of transfection.
Transfection complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-mRNA:mRNA Boost Reagent:RNA transfection complexes drop-wise to different areas of the wells with plated 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 were added to cells cultured in serum-free medium	Allow *Trans*IT-mRNA Reagent:mRNA Boost:RNA 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.
Low-quality RNA	Use highly purified, sterile and contaminant-free RNA for transfection.
	We recommend using purifying the RNA using a column procedure such as Qiagen's RNeasy spin columns.
	Avoid RNase contamination as degradation of the RNA substrate will significantly diminish expression of the transfected RNA.
Translated RNA is toxic to cells	If the target RNA is translated, include a control with non-coding RNA and *Trans*IT-mRNA Transfection Kit to compare the cytotoxic effects of the RNA being translated.
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
- *Trans*IT Cell Line Specific Transfection Reagents and Kits
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT-2020 Transfection Reagent
- *Trans*IT-LT1 Transfection Reagent
- *Trans*IT-PRO™ Transfection Kit

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