



***TransIT*[®]-mRNA Transfection Kit**

INTENDED USE

Mirus Bio Corporation has developed the *TransIT*[®]-mRNA Transfection Kit, which enables highly efficient transfection of single-stranded RNA (including mRNA and viral RNA) with significantly reduced levels of cell damage as compared to other commercially available RNA transfection reagents. Transfections are most effective when carried out in complete growth media, with no media change or serum addition required. These unique features make the *TransIT*[®]-mRNA Transfection Kit ideal for all *in vitro* RNA delivery studies. MIR 2250 (0.5 milliliter) provides sufficient amounts of *TransIT*[®]-mRNA Reagent and mRNA Boost Reagent to perform up to 500 transfections in 12-well plates.

DESCRIPTIONS

mRNA: ssRNA that encodes a protein and has a 5' cap and 3' polyA tail; can be naturally occurring in a mammalian cell or produced *in vitro* to mimic natural mRNA

***in vitro* transcript:** RNA molecule produced from a DNA template using one of the three phage DNA-dependent RNA polymerases (T7, T3, or SP6)

FREQUENTLY ASKED QUESTIONS

General Questions:

Q1. What cell types has Mirus Bio successfully transfected with the *TransIT*[®]-mRNA Transfection Kit?

We have tested A549, CHO-K1, COS-7, HEK 293, HeLa, HepG2, NIH 3T3, and Vero cell lines.

Q2. The *TransIT*[®]-mRNA Transfection Kit contains two components, the *TransIT*[®]-mRNA Reagent and mRNA Boost Reagent. What is the formulation of each component?

The *TransIT*[®]-mRNA Reagent is a proprietary cationic polymer/lipid formulation and is non-liposomal. The mRNA Boost Reagent is a proprietary compound. Both components are supplied in ethanol to ensure sterility and stability.

Q3. How does Mirus Bio produce their RNA for transfection?

We routinely use the mMESSAGE mMACHINE[®] T7 Ultra Kit (Ambion) to generate the *in vitro* transcripts (RNA) that we use for transfection. This kit caps and polyadenylates the transcript so that it will more closely resemble mammalian messenger RNA (mRNA), be translated more efficiently, and be more stable in the cells. Before transfection, we purify the *in vitro* transcribed mRNA using the RNeasy[®] Mini Kit (Qiagen). Other *in vitro* transcription kits should also produce high quality RNA for use in transfections.

Q4. Is it necessary to cap and polyadenylate the *in vitro* transcript before transfection?

Capping and polyadenylation will both increase the level of expression from the transfected RNA and stabilize the RNA within the cell. However, depending on the experiment, it may *not* be necessary to cap and polyadenylate the RNA. 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. In other cases, expression of the transfected RNA may not be the goal, making capping and polyadenylation disadvantageous.

Q5. What size RNA has Mirus Bio delivered using the *TransIT*[®]-mRNA Transfection Kit?

At Mirus Bio, we have transfected four different capped and polyadenylated mRNAs: eGFP mRNA (~1.2 kb), firefly luciferase mRNA (~1.9 kb), β -galactosidase mRNA (~3.2 kb), and hepatitis delta antigen mRNA (~0.94 kb). We have also delivered the hepatitis delta virus genome RNA template (~2.2 kb). Other labs have successfully delivered viral RNA up to 10 kb in length using the *TransIT*[®]-mRNA Transfection Kit.



Q6. Does the size of the transfected RNA affect the transfection efficiency?

No. A common idea is that longer RNAs may require more mass (μg RNA) in the transfection than shorter RNAs, in order to keep relative molar amount of RNA. In fact, we have seen that the mass of RNA used, independent of the length/molarity of the RNA, is a critical factor in optimizing transfection efficiency. The optimal amount of RNA (μg used in the transfection) appears to be dependent on a variety of factors including cell line and the RNA transcript itself. This may involve the secondary structure in the RNA or the assay being used to monitor RNA transfection/expression. For best results, we recommend titrating the mass of each RNA transcript to be used for a particular cell line.

Q7. Can the *TransIT*[®]-mRNA Transfection Kit be used to transfect suspension cells?

Yes. Follow the *TransIT*[®]-mRNA Transfection Kit protocol for transfection of suspension cells, and collect cells by centrifugation at the time of harvest and assay as needed.

Q8. How should I store the *TransIT*[®]-mRNA Transfection Kit?

Both the *TransIT*[®]-mRNA Reagent and mRNA Boost Reagent should be stored tightly capped at 4°C to prevent evaporation. Prior to use, warm both reagents to room temperature and gently vortex to dissolve any precipitate that may have formed.

Q9. Where can I find references in which other researchers have used the *TransIT*[®]-mRNA Transfection Kit?

As citations become available, they can be listed in the technical resources section of our website.

Q10. Can I use the *TransIT*[®]-mRNA Transfection Kit for siRNA delivery?

No. Mirus Bio recommends using either *TransIT*-TKO[®] or *TransIT*[®]-siQUEST[™] for siRNA delivery. Each reagent contains a unique formulation designed to achieve optimal transfection efficiency in a variety of cell types.

***TransIT*[®]-mRNA Reagent Protocol Questions:**

Q11. Should I add the RNA/mRNA Boost Reagent/*TransIT*[®]-mRNA Reagent complexes to cells in serum-free media or in media containing serum?

The highest transfection efficiencies are achieved when the complexes are added to cells in complete growth media (serum-containing). Prior to transfection, be sure to form the complexes in serum-free media before transfection, as serum can interfere with complex formation and may lead to degradation of the RNA.

Q12. Do I have to change the media or add media after transfection with the *TransIT*[®]-mRNA Transfection Kit?

When using the *TransIT*[®]-mRNA Transfection Kit, a media change or addition after transfection is not necessary.

Q13. Will antibiotics interfere with my transfection efficiency?

At Mirus Bio, we use a low level (100X stock of penicillin/streptomycin/amphotericin diluted to 0.1-1X final concentration) of antibiotics in our complete growth media when performing RNA transfections using the *TransIT*[®]-mRNA Transfection Kit, with no adverse effects on transfection efficiencies. Higher levels of antibiotics may interfere with transfection. Although transfection complexes can be added to cells in their complete growth media containing antibiotics, the complex formation procedure should be performed in serum-free media that does not contain antibiotics.

Q14. After transfection using the *TransIT*[®]-mRNA Transfection Kit, how long should I let my samples incubate?

The optimal incubation time can be determined by testing a range from 4 to 48 hours. We routinely use capped, polyadenylated mRNA expressing firefly luciferase and see an optimal incubation range from 12 to 18 hours post-transfection. However, the incubation time may be shorter if the transfected RNA is not capped or polyadenylated. Alternately, viral rescue by RNA transfection may require more time to generate infectious virus.

Q15. Can I transfect two different RNA substrates at the same time using *TransIT*[®]-mRNA Transfection Kit ?

Yes. We have used a viral model system to demonstrate that both a viral genomic RNA and a viral mRNA can be transfected simultaneously. Initiation of viral replication indicated that transfection of both RNAs into the same cells was successful. Transfection of either RNA by itself did not promote viral replication.

Troubleshooting Questions:

Q16. I used the *TransIT*[®]-mRNA Transfection Kit, and I see low transfection efficiency. What can I do next?

Ensure that you understand and are adhering to the recommended protocol. Even slight variations in the procedure can adversely affect transfection efficiency.

- **Optimize the amount of *TransIT*[®]-mRNA Reagent**
Determine the optimal amount of *TransIT*[®]-mRNA Reagent by titrating the reagent from 1 µl to 3 µl per well of a 12-well plate. See Table 1 of the *TransIT*[®]-mRNA Transfection Kit protocol for recommended starting conditions.
- **Optimize the amount of mRNA Boost Reagent**
Determine the optimal amount of mRNA Boost Reagent by titrating the reagent from 0.5 µl to 2 µl per well of a 12-well plate. See Table 1 of the *TransIT*[®]-mRNA Transfection Kit protocol for recommended starting conditions.
- **Optimize the amount of RNA**
Determine the optimal RNA amount by titrating from 0.5 to 1.5 µg of RNA per well of a 12-well plate. For certain applications, the optimal amount may be outside of the recommended range. As a starting point, use 1 µg of RNA per well of a 12-well plate.
- **Optimize the ratio of the *TransIT*[®]-mRNA Reagent to mRNA Boost Reagent**
In order to thoroughly determine the optimal reagent levels to use for a RNA transfection, test a range of *TransIT*[®]-mRNA Reagent levels (1, 2, and 3 µl) each with a range of mRNA Boost Reagent amounts (0.5, 1, and 2 µl). In this example, nine different transfection complexes will be formed and transfected in order to identify the best transfection conditions for the particular RNA and cell type.
- **Optimize the incubation time for complex formation prior to transfection**
We recommended incubating the complexes for 2-5 minutes. Longer incubation times may result in lower transfection efficiency.
- **Use high quality, purified RNA**
RNA used for transfection should be highly purified and sterile. We recommend purifying the RNA using a column procedure such as RNeasy[®] spin columns (Qiagen). Avoid RNase contamination as degradation of the RNA substrate will significantly diminish expression of the transfected RNA.
- **Use serum-free media during RNA/mRNA Boost Reagent/*TransIT*[®]-mRNA Reagent complex formation**
Be sure to use serum-free medium when forming the complexes.
- **Optimize cell density (% confluence) at time of transfection**
The recommended cell density for most cell types at the time of transfection is 60-90% confluence. Determine the optimal cell density, which may be outside the recommended range, for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Eliminate potential inhibitors of transfection**
The presence of polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use serum-free medium and complete growth medium that do not contain these or other polyanions.
- **Use cells of similar passage number**
If the passage number of the cells is too high or too low, transfection efficiency can be adversely affected. We recommend maintaining a similar passage number between experiments to ensure reproducibility.

Q17. Should I expect toxicity when using the *TransIT*[®]-mRNA Transfection Kit? How can I minimize cellular toxicity?

On cell lines tested, we observe little to no cellular toxicity using the *TransIT*[®]-mRNA Reagent. Ensure that you understand and are adhering to the recommended protocol to obtain optimal results. Even slight variations in the procedure can adversely affect transfection efficiency.



Frequently Asked Questions

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- Rock culture vessel following addition of RNA/mRNA Boost Reagent/*TransIT*[®]-mRNA transfection complexes
Mix thoroughly to evenly distribute the complexes to all of the 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.
- Optimize the amount of *TransIT*[®]-mRNA Reagent, mRNA Boost Reagent, or RNA was used in the transfection
Reduce or increase the amount of component(s) used in the transfection. See Table 1 of the *TransIT*[®]-mRNA Transfection Kit protocol for recommended starting conditions.
- Optimize cell density (% confluence) at the time of transfection
Grow cells to a higher cell density and repeat the transfection. We recommend performing the transfection when cells are 60-90% confluent.
- Transfect cells in complete growth media (containing serum)
Form complexes in serum-free media, and add complexes to cells growing in complete growth media (containing serum). Cytotoxicity is decreased when the complexes are added to cells in complete growth media with no media change post-transfection. NOTE: Prior to transfection, form RNA / mRNA Boost Reagent complexes in serum-free media.
- Use cells of similar passage number
If the passage number of the cells is too high or too low they may be more sensitive to transfection reagents. Maintain a similar passage number between experiments to ensure reproducibility.

For specific questions or concerns, please contact our Technical Support Team at 888.530.0801 or techsupport@mirusbio.com.