



TransIT-TKO[®] siRNA Transfection Reagent

INTENDED USE

Mirus Bio Corporation has developed *TransIT-TKO* Transfection Reagent, which enables highly efficient siRNA transfection with significantly reduced levels of cell damage as compared to cationic-liposome based transfection reagents. *TransIT-TKO* Reagent, when complexed with siRNA, knocks down target gene expression in a variety of cell lines. *TransIT-TKO* Reagent is proven to perform efficiently in conjunction with Mirus' *TransIT[®]* Transfection Reagents for plasmid delivery and is complement to the *TransIT-siQUEST[®]* Reagent (see www.mirusbio.com). Each unique formulation provides a distinct transfection profile for high efficiency, broad-spectrum siRNA delivery. Each milliliter of *TransIT-TKO* Reagent (MIR 2150) is sufficient quantity to perform up to 1000 transfections in 24-well plates, depending on the specific cell type.

FREQUENTLY ASKED QUESTIONS

General Questions and Answers:

Q1. What cell types has Mirus transfected with the *TransIT-TKO* siRNA delivery reagent?

We have tested A549, BNL CL.2, BHK-21, C2C12, C6, CHO-K1, COS-7, Daoy, DB-TRG-05MG, DI-TNC1, DU 145, HEK 293, HeLa, Hepa1c1c7, HepG2, Jurkat, keratinocyte, MCF-7, Neuro-2a, NIH 3T3, PC-3, primary mouse hepatocyte, RAW 264.7, SK-N-MC, THP-1 and Vero cell lines

Q2. What kind of formulation is *TransIT-TKO*? Is it liposomal?

TransIT-TKO Transfection Reagent is a cationic proprietary polymer/lipid formulation, and is non-liposomal. It is supplied in ethanol to ensure sterility.

Q3. How does *TransIT-siQUEST* Reagent differ from *TransIT-TKO* Reagent?

Due to their distinct formulations, each reagent has a unique transfection profile depending on the cell line being transfected. Generally, one particular reagent will be better suited for a particular cell line. *TransIT-siQUEST* Reagent is supplied in 80% ethanol while *TransIT-TKO* Reagent is supplied in 100% ethanol.

Q4. How do the *TransIT-TKO*/siRNA complexes knockdown target gene expression?

When short RNA duplexes are introduced into mammalian cells in culture, sequence-specific inhibition of target mRNA can be achieved without triggering the interferon response. These short dsRNAs, referred to as short interfering RNAs (siRNA), act in concert with cellular proteins to cleave greater than 95% of the target mRNA in the cell. The RNA interference effect can be long lasting and may be detectable after many cell divisions. These properties make siRNA extremely effective at inhibiting target gene expression.^{1,2,3} Using *TransIT-TKO* Transfection Reagent, low levels of siRNA are sufficient to successfully knockdown both transient and stable transgene expression. In transient transfection assays, transgene expression can be silenced whether the siRNA is transfected before, during, or after the transfection of the expression vector.

Q5. Can *TransIT-TKO[®]* be used to transfect siRNA into primary cells?

Yes. We have successfully transfected the following primary cells: human astrocytes, human neurons, and mouse hepatocytes.

Q6. Can *TransIT*-TKO be used to transfect suspension cells?

Yes. Follow the *TransIT*-TKO protocol for adherent cells, and collect cells by centrifugation at the time of harvest/assay as needed. It is important to note that the transfection efficiency can be dependent on cell density. We recommend starting with 300,000-500,000 cells per well of a 24 well plate on the day of transfection (150,000-250,000 cells per well of a 24 well plate if plated the day before transfection).

Q7. How should I store the *TransIT*-TKO Reagent?

The *TransIT*-TKO Reagent should be stored at 4°C and tightly capped to prevent evaporation.

Q8. Where can I find references in which other researchers have used *TransIT*-TKO and siRNA to successfully knockdown gene expression?

Mirus' product references are available in the Technical Resources section of our website. Click on "Product Citations" and choose *TransIT*-TKO Transfection Reagent.

Q9. How should I dilute the siRNA?

Use 100 mM NaCl in 50 mM Tris , pH 7.5, made with RNase-free water. Do not use water alone to dilute siRNA, as this may result in denaturation of the siRNA duplex, especially at low concentrations. siRNA can also be diluted in annealing buffer that is supplied with your siRNA.

TransIT*-TKO Protocol Questions and Answers:*Q10. Should I add the *TransIT*-TKO/siRNA complexes to cells in serum-containing media or serum-free media?**

On all of the cell lines that we have tested to date, highest transfection efficiencies are achieved when the complexes are added to cells in their complete media (serum-containing). However, be sure to form the complexes in serum-free media before transfection, as serum can interfere with complex formation.

Q11. Do I have to change the media or add media after transfection with *TransIT*-TKO/siRNA?

We recommend leaving the transfection complexes on for 24 hours after transfection before changing the media or adding more media. A media change can be performed earlier, but this may affect the efficiency of siRNA transfection.

Q12. Will antibiotics interfere with my transfection efficiency?

We use a low level (0.1x to 1x final concentration) of antibiotics in the cell culture media which is used for our *TransIT*-TKO and siRNA transfections and routinely see no adverse effects on transfection efficiencies. Higher levels of antibiotics may interfere with transfection. If toxicity is observed, more media can be added to the cells 4-24 hours post transfection.

Q13. Can I transfect my DNA and siRNA at the same time?

Yes. We do not observe a difference in transfection efficiency between transfecting DNA and siRNA simultaneously and transfecting the siRNA four hours after the DNA transfection. Be sure to closely follow Section 2.3 of the *TransIT*-TKO protocol, as order of addition of the *TransIT*-LT1, DNA, *TransIT*-TKO and siRNA is important.

Q14. Can I transfect two different siRNA duplexes at the same time with *TransIT*-TKO Reagent?

Yes. The *TransIT*-TKO Reagent can be used to transfect two different siRNA duplexes at the same time. No adjustment in the amount of *TransIT*-TKO Reagent per well is needed.

Q15. Can different sequence siRNA duplexes achieve different levels of knockdown of the same target gene?

Yes. We have found that different siRNA sequences may result in different levels of target gene knockdown. Try a few different sequences for a particular target gene if you are not achieving the knockdown efficiency that you expect.

Q16. How can I assess siRNA transfection efficiency for my cell type?

To assess delivery, the siRNA can be fluorescently labeled then visualized under a microscope. We have developed the *LabelIT*[®] siRNA Tracker Intracellular Localization Kits that provide the necessary reagents to directly label and transfect siRNA, in an efficient and non-destructive manner. Both subcellular localization and functionality can be monitored in the same experiment following the delivery of the labeled sample into mammalian cells. It is important to note that a higher concentration of siRNA may need to be used to visualize the fluorescent signal.

Troubleshooting questions:**Q17. I used the *TransIT*-TKO Reagent, and I do not see gene knockdown. What can I do next?**

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

- **Suboptimal volume of *TransIT*-TKO Reagent**
Determine the optimal *TransIT*-TKO Reagent concentration by titrating the reagent from 1 μ l to 4 μ l per well of a 24-well plate. See Table 1 of the *TransIT*-TKO protocol for recommended starting concentrations.
- **Suboptimal siRNA concentration**
Determine the optimal siRNA concentration by titrating 10 nM to 50 nM final concentration in the well. See Table 1 of the *TransIT*-TKO protocol for recommended starting concentrations.
- **Denatured siRNA**
Use recommended buffer (100 mM NaCl, 50 mM Tris, pH 7.5 in RNase-free water) or annealing buffer to dilute siRNA. Do not use water as this can denature the siRNA duplex.
- **Poor quality of transfecting siRNA**
Avoid siRNA degradation by using RNase-free handling procedures and plasticware. Degradation can be detected on acrylamide gels. Ensure that the sequence of siRNA is correct for your gene of interest.
- **Serum present during *TransIT*-TKO Reagent/siRNA 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 is 60-80% confluence at time of transfection (3×10^4 to 1.2×10^5 cells per well of a 24-well plate, depending on cell size and characteristics). If this confluency does not produce optimal results, test various cell densities outside the recommended range. Lower cell densities may be necessary for transfection incubation times over 48 hours. If lower cell densities are plated, ensure that the levels of *TransIT*-TKO Reagent and siRNA are titrated accordingly. Determine the optimal cell density for each cell type in order to maximize gene knockdown. 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.
- **Cell morphology has changed**
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.
- **Poor detection of gene knockdown**
The target mRNA is usually degraded within the first 24 hours post-transfection and can be measured using assays such as qRT-PCR and Northern blots. If target gene knockdown is assayed by detection of the protein, the half-life of the protein encoded by the target mRNA can have a dramatic effect on the (post-transfection) incubation time necessary to see significant knockdown. When using protein-based assays (Western blots, Elisa's, etc), the stability of the target protein should therefore be taken into consideration when determining the optimal time to assay the cells after transfection.

- Proper controls are included
To ensure effective quantification of knockdown, include the following controls: cells only (for visual comparisons), *TransIT*-TKO Reagent alone, and *TransIT*-TKO Reagent plus a non-specific siRNA. In order to verify that the transfections are working and producing an effective knockdown, use *TransIT*-TKO Reagent to deliver a siRNA targeted against a ubiquitous gene, such as GAPDH or Lamin A/C, followed by Western blotting or target mRNA quantification.

Q18. After I transfect with *TransIT*-TKO and siRNA, I see cellular toxicity. Is this normal? What can I do?

On common cell lines, using the *TransIT*-TKO Reagent protocol, we observe little to no toxicity. If your cell line is sensitive to the *TransIT*-TKO/siRNA complexes, decrease the amount of *TransIT*-TKO Reagent per well, or change the media after 24 hours. Ensure you are adding your complexes to the cells in complete growth media (serum-containing). If serum-free media is used, higher toxicity may be observed. A *TransIT*-TKO Reagent alone control can be performed to ensure that the siRNA duplex is not targeting a gene that is crucial for cell viability.

- Excessive amount of *TransIT*-TKO Reagent/siRNA complex mixture was used in transfection
Reduce the amount of *TransIT*-TKO Reagent/siRNA complex mixture in the transfection. See Table 1 of the *TransIT*-TKO protocol for recommended starting concentrations.
- Cell density was too low at time of transfection
Grow cells to a higher cell density and repeat the transfection.
- Media change or addition may be necessary in some cell lines
If incubating for 48-72 hours, it may be necessary to change the complete media 24 hours post-transfection, or simply add another volume of complete media at 16-24 hours post-transfection.
- Complexes were added to cells in serum-free media
TransIT-TKO/siRNA complexes should be added to cells in complete media (serum-containing media) 5-20 minutes after complex formation. If these complexes are added to cells in serum-free media, cells may exhibit more cytotoxicity. If you must add the complexes to cells in serum-free media, add complete media after 4 hours to minimize toxic effects.
- Cell morphology has changed
If the passage number of the cells is too high or too low they may be more sensitive to cellular toxicity. We recommend maintaining a similar passage number between experiments to maintain reproducibility.
- *TransIT*-TKO Reagent/siRNA complex mixture and cells were not mixed thoroughly following addition to the cells
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.
- Include proper controls
Include the following controls to aid in assessing cellular toxicity: cells alone (for visual comparisons), *TransIT*-TKO Reagent alone, and *TransIT*-TKO Reagent plus a non-specific siRNA.

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

siRNA Technology References

1. Elbashir, S.M. et al. (2001) *Nature* **411**: 494-498.
2. Caplen, N.J. et al (2001) *Prot. Natl. Acad. Sci.* **98**: 9742-9747.
3. Sharp, P.A. (2001) *Genes and Development* **15**: 485-490.