



TransIT-siQUEST[®] Transfection Reagent

INTENDED USE

Mirus Bio Corporation has developed *TransIT-siQUEST* Transfection Reagent, which enables highly efficient siRNA transfection with significantly reduced levels of cell damage compared to cationic-liposome based transfection reagents. When complexed with siRNA, the *TransIT-siQUEST* Reagent knocks down target gene expression in a variety of cell lines. *TransIT-siQUEST* is a complement to the *TransIT-TKO[®]* Reagent (see www.mirusbio.com). Each unique formulation provides a distinct transfection profile for high efficiency, broad-spectrum siRNA delivery. Each milliliter of *TransIT-siQUEST* Reagent (MIR 2110) 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-siQUEST* Reagent?

We have tested A549, CHO-K1, COS-7, HEK 293, HeLa, Hepa1c1c7, HepG2, MCF-7, NIH 3T3, primary mouse hepatocyte, RAW 264.7, and Vero cell lines.

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

TransIT-siQUEST Reagent is a proprietary cationic polymer/lipid formulation, and it 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 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-siQUEST* Reagent/siRNA complexes knockdown target gene expression?

The introduction of short RNA duplexes into cultured mammalian cells can cause sequence-specific destruction of a target mRNA without triggering an interferon response. These double stranded 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-siQUEST* 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 when the siRNA is transfected 4 hours after the transfection of the expression vector (please see the *TransIT-siQUEST* Reagent protocol).

Q5. Can *TransIT-siQUEST* Reagent be used to transfect siRNA into primary cells?

Yes. We have successfully transfected primary mouse hepatocytes.

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

Yes. Follow the *TransIT-siQUEST* Reagent protocol for transfection of adherent cells as written in the protocol, 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-siQUEST* Reagent?

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

Q8. Where can I find references in which other researchers have used *TransIT-siQUEST* Reagent 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-siQUEST* Transfection Reagent.

Q10. How should I dilute my siRNA?

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

TransIT-siQUEST Reagent Protocol Questions and Answers:



Q11. Should I add the *TransIT*-siQUEST Reagent /siRNA complexes to cells in serum-containing media or serum-free media?

For all of the cell lines that we have tested to date, the highest knockdown 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.

Q12. Do I have to change the media or add media after transfection with *TransIT*-siQUEST Reagent and siRNA?

We recommend leaving the transfection complexes on the cells for 24 hours after transfection before performing a complete media change. A media change performed earlier may affect the efficiency of knockdown. If toxicity is observed, more media can be added to the cells 4-24 hours post transfection.

Q13. Will antibiotics interfere with my transfection efficiency?

We use a low level (0.1x to 1x final concentration) of antibiotics in our cell culture and routinely see no adverse effects on knockdown efficiencies. Higher levels of antibiotics may interfere with transfection.

Q14. Can I transfect my DNA and siRNA at the same time?

No. Due to the cationic nature of the *TransIT*-siQUEST Reagent, it is recommended that plasmid DNA is delivered first using a DNA transfection reagent, followed by siRNA transfection using *TransIT*-siQUEST Reagent 4 hours later. Refer to the *TransIT*-siQUEST Reagent protocol for recommendations on performing this type of sequential transfection.

Q15. Can different siRNA duplexes directed against the same target gene produce different levels of knockdown?

Yes. We have found that different siRNA sequences may result in different levels of target gene knockdown. Design several siRNA sequences for a particular target gene to improve the probability of efficient gene knockdown.

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. Mirus offers *Label IT*[®] 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*-siQUEST Reagent, and I do not see gene knockdown. What can be the cause?

Ensure that you understand and are adhering to the recommended protocol. Even slight variations in the procedure can affect siRNA delivery therefore knockdown efficiency.

- Suboptimal volume of *TransIT*-siQUEST Reagent
Determine the optimal *TransIT*-siQUEST Reagent volume for each cell type by testing 0.5 μ l, 1 μ l and 3 μ l of reagent per well of a 24-well plate. See Table 1 of the *TransIT*-siQUEST protocol for recommended starting volumes for several plate sizes.
- Suboptimal siRNA concentration
Determine the optimal siRNA concentration by titrating from 10 nM to 50 nM final concentration in well. However, in some cases, a higher concentration of siRNA may be necessary to see adequate target gene knockdown.
- 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 may denature the siRNA duplex.
- Poor quality of transfecting siRNA
Avoid siRNA degradation by using RNase-free handling procedures and plastic ware. Ensure that the sequence of siRNA is correct for your gene of interest.
- Serum present during *TransIT*-siQUEST 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*-siQUEST 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 cell culture 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 not included
To ensure effective quantification of knockdown, include the following controls: cells only (for visual comparisons), *TransIT*-siQUEST Reagent alone, and *TransIT*-siQUEST Reagent plus a non-specific siRNA. In order to verify that the transfections are working and producing an effective knockdown, use *TransIT*-siQUEST 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*-siQUEST Reagent and siRNA, I see cellular toxicity. What can I do?

On common cell lines, using the *TransIT*-siQUEST protocol, we observe little to no toxicity. If your cell line is sensitive to the *TransIT*-siQUEST/siRNA complexes, decrease the amount of 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*-siQUEST 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*-siQUEST Reagent/siRNA complex mixture was used in transfection
Reduce the amount of *TransIT*-siQUEST Reagent/siRNA complex mixture in the transfection. See Table 1 of the *TransIT*-siQUEST Reagent protocol for recommended starting concentrations.
- Cell density was too low at time of transfection
Repeat the transfection at a higher cell density, such as 60-80% confluence.
- 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 an additional volume of complete media at 4-24 hours post-transfection.
- Complexes were added to cells in serum-free media
TransIT-siQUEST Reagent/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 the transfection reagent. We recommend maintaining a similar passage number between experiments to maintain reproducibility.
- Include Proper controls
Include the following controls to aid in assessing cellular toxicity: cells alone (for visual comparisons), *TransIT*-siQUEST Reagent alone, and *TransIT*-siQUEST Reagent plus a non-specific siRNA.
- *TransIT*-siQUEST 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.

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.