*Trans*IT-siQUEST® Transfection Reagent

Protocol for MIR 2110, 2114, 2115, 2116

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/2110

INTRODUCTION

*Trans*IT-siQUEST® is a broad spectrum siRNA transfection reagent that enables high efficiency siRNA delivery and knockdown of target gene expression in many cell types including primary cells. Transfections with *Trans*IT-siQUEST do not require medium changes and can be carried out in serum-containing medium. In addition to *Trans*IT-siQUEST, Mirus also offers *Trans*IT-X2® and *Trans*IT-TKO® Transfection Reagents for siRNA transfection. Each unique formulation provides high efficiency broad-spectrum siRNA delivery.

SPECIFICATIONS

Storage	Store tightly capped 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-siQUEST to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

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

Product No.	Quantity
MIR 2114	1 × 0.4 ml
MIR 2110	1 × 1.5 ml
MIR 2115	5 × 1.5 ml
MIR 2116	10 × 1.5 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- siRNA
- 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 siRNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency knockdown of target gene expression using *Trans*IT-siQUEST Transfection Reagent. Please refer to **Table 1** on Page 3 for recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** Determine the best 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 appropriate cell density (generally ≥80% confluence) at the time of transfection. If this confluence does not produce optimal results, test cell densities outside of the recommended range.
- **Volume of *Trans*IT-siQUEST Reagent.** Each cell type responds differently to a given transfection reagent. As a starting point, test 1.5 µl of *Trans*IT-siQUEST Reagent per well of a 24-well plate. For further optimization, test three levels of *Trans*IT-siQUEST Reagent, e.g. 0.5 µl, 1.5 µl, and 3 µl per well of a 24-well plate.
- **siRNA dilution**. Dilute siRNA using the manufacturer's recommended buffer. Alternatively, 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.
- **siRNA concentration.** siRNA used for transfection should be highly pure, sterile, and the correct sequence. Depending on the type of experiment, the final siRNA concentration for transfection is typically within the range of 10–50 nM. As a starting point, we recommend 25 nM siRNA (final concentration in well).
- **Proper controls.** Mirus recommends transfecting a non-targeting or nonsense siRNA control sequence to verify that gene expression knockdown or phenotype is attributed to the gene-specific siRNA. Additionally, targeting a gene with multiple siRNA sequences ensures that the resulting phenotype is not due to off-target effects. .
- **Complex formation conditions.** Prepare *Trans*IT-siQUEST Reagent:siRNA 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**.** The *Trans*IT-siQUEST Reagent yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change.
- **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).
- **Transfection incubation time.** The optimal incubation time can be determined empirically by testing a range from 24–72 hours post-transfection, depending on the stability of the target mRNA and its encoded protein. When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post-transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post-transfection incubation may be necessary particularly if the target protein has a long cellular half-life.

Lower cell densities may be necessary when post-transfection incubation times are greater than 48 hours. If lower cell densities are plated, test a range of *Trans*IT-siQUEST reagent to determine the optimal concentration.

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

siRNA TRANSFECTION PROTOCOL

The following procedure describes how to perform siRNA transfection using *Trans*IT-siQUEST Transfection Reagent in 24-well plates. The surface areas of other culture vessels are different and transfection must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-siQUEST Reagent, siRNA and complete culture medium based on the surface area of the cell culture vessel (see **Table 1** below).

Table 1: Recommended starting conditions for siRNA transfections with *Trans*IT-siQUEST 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
*Trans*IT-siQUEST Reagent	0.3 µl	0.8 µl	1.5 µl	3 µl	7.5 µl	46 µl	59 µl
siRNA (10 µM stock) 25 nM final	0.25 µl	0.7 µl	1.4 µl	2.8 µl	6.8 µl	42.5 µl	54 µl

Reverse transfection protocol for high throughput screening available at: http://www.mirusbio.com/hts

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 TransIT-siQUEST need to be pipetted, dilute the Reagent in serum-free medium before each use to avoid pipetting errors. Do not store diluted TransIT-siQUEST Reagent.

Transient siRNA transfection protocol for cells in 24-well plates

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells using the following guidelines. For most cell types, cultures should be ≥80% confluent at the time of transfection.

   For adherent cells: Plate cells at density of 0.8-3.0 x 10^5 cells/ml.

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

B. Prepare *Trans*IT-siQUEST Reagent:siRNA complexes (Immediately before transfection)

1. Warm *Trans*IT-siQUEST Reagent to room temperature and vortex gently before using.
2. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 1.5 µl of *Trans*IT-siQUEST Reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the *Trans*IT-siQUEST Reagent (please refer to "Before You Start" on Page 2).
4. Add 1.4 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

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

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-siQUEST Reagent:siRNA complexes (prepared in Step B) 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-siQUEST Reagent:siRNA complexes.
3. Incubate for 24–72 hours or as required. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay for knockdown of target gene expression.

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

Note: When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary, particularly if the target protein has a long cellular half-life.

PLASMID DNA & siRNA SEQUENTIAL TRANSFECTION PROTOCOL

For plasmid DNA transfection, we recommend either *Trans*IT®-LT1 (MIR 2300) or *Trans*IT®-2020 (MIR 5400) Transfection Reagents. These broad spectrum plasmid DNA transfection reagents can be used to efficiently deliver DNA into a wide variety of cell types. For more information, visit *www.mirusbio.com.*

The following procedure describes how to perform sequential transfection of plasmid DNA and siRNA transfection using *Trans*IT-LT1 and *Trans*IT siQUEST. The initial plasmid DNA transfection is recommended in a T75 flask for convenience and reduced well-to-well variability for subsequent siRNA transfection.

The following procedure describes how to perform siRNA transfections in 24-well plates. For other tissue culture formats, please refer to **Table 1** on Page 3.

Transient plasmid DNA and siRNA sequential transfection protocol

A. Plate cells in a T75 flask

1. ***For adherent cells:*** Approximately 18–24 hours prior to transfection, plate cells in one T75 flask (for each 24-well plate needed) at an appropriate cell density to obtain ≥80% confluence the following day. Incubate the cell culture overnight.

   For suspension cells: Plate cells in a T75 flask the day of transfection to obtain 50–70% cell confluence

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-LT1 Reagent:DNA complexes (Immediately before transfection)

1. Warm *Trans*IT-LT1 to room temperature and vortex gently before using.
2. Place 500 µL of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 45 µL *Trans*IT-LT1 Reagent. Pipet gently to mix completely.
4. Add 15 µg (15 µl of a 1 µg/µl stock) plasmid DNA. Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

For optimization of plasmid DNA transfection, follow the guidelines as per *Trans*IT-LT1 (MIR2300) user protocol.

C. Distribute the TransIT-LT1: DNA complexes to cells in complete growth medium

1. Add the *Trans*IT-LT1 Reagent:DNA complexes (prepared in Step B) drop-wise to cells in complete growth medium. 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-LT1 Reagent:DNA complexes.
3. Incubate for 2–4 hours.

D. Transfer cells from T75 flask to 24-well plates

1. Trypsinize the T75 flask according to standard procedures.

   For adherent cells: Add 13–14 ml of complete growth medium to the T75 flask. Mix cells thoroughly and plate 500 µl per well of a 24-well plate (adherent cells) to obtain ≥80% confluence at the time of transfection.

   For suspension cells: Transfer 250 µl of suspension cells to obtain a cell density of 3–5 x 10^5 cells per well of a 24-well plate. Transfection reagents can cause suspension cells to adhere; therefore, ensure cells are completely removed from the bottom of the flask.
2. Incubate for 2 hours.

E. Prepare TransIT-siQUEST:siRNA complexes (Immediately before transfection)

1. Warm *Trans*IT-siQUEST Reagent to room temperature and vortex gently before using.
2. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 1.5 µl of *Trans*IT-siQUEST Reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the TransIT-siQUEST Reagent (please refer to "Before You Start" on Page 2).
4. Add 1.4 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

F. Distribute the TransIT-siQUEST:siRNA complexes to cells in complete growth medium

1. Add the transfection complexes (prepared in Step E) drop-wise to different areas of the wells containing plated cells.
3. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the transfection complexes.
4. Incubate for 24–72 hours or as required. It is not necessary to replace the complete growth medium with fresh medium.
5. Harvest cells and assay for expression and/or knockdown of target gene expression.

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

Note: When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary, particularly if the target protein has a long cellular half-life.

TROUBLESHOOTING GUIDE

Problem	Solution
POOR siRNA KNOCKDOWN EFFICIENCY	
*Trans*IT-siQUEST Reagent was not mixed properly.	Warm *Trans*IT-siQUEST to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-siQUEST Reagent:siRNA ratio	For optimization, test three levels of *Trans*IT-siQUEST Reagent, e.g. 0.5, 1.5, and 3 µl per well of a 24-well plate, using 25 nM siRNA (final concentration in the well). Additional levels may need to be titrated depending on the cell type.
Suboptimal siRNA concentration	Determine the optimal siRNA concentration by titrating from 10–50 nM (final concentration in the well). We recommend starting with 25 nM siRNA (final concentration in the well). In some instances, higher concentrations of siRNA up to 100 nM may be necessary to achieve sufficient knockdown of the gene of interest. Please refer to "Before You Start" on page 4.
Proper controls were not included	1. Serum-free medium alone 2. Serum-free medium + *Trans*IT-siQUEST Reagent + a non-targeting siRNA To verify efficient transfection and knockdown, use *Trans*IT-siQUEST Reagent to deliver a siRNA targeted against a ubiquitous gene, e.g. GAPDH or Lamin A/C, followed by target western blotting or mRNA quantification. To assess delivery efficiency of siRNA, use Mirus' *Label* IT® siRNA Tracker™ Intracellular Localization Kits or a prelabeled *Label* IT RNAi Delivery Control (please refer to Related Products on Page 8)
Denatured siRNA	To dilute siRNA, use the manufacturer's recommended buffer or 100 mM NaCl, 50 mM Tris, pH 7.5 in RNase-free water. **Do not** use water as this can denature the siRNA at low concentration during long-term storage.
Incorrect siRNA Sequence	Ensure that the sequence of the siRNA is correct for the gene of interest. More than one sequence is recommended for optimal knockdown efficiency and to ensure on-target effects.
Poor quality of siRNA	Avoid siRNA degradation by using RNase-free handling procedures and plastic ware. Degradation of siRNA can be detected on acrylamide gels.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-siQUEST 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 e.g. 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 optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g. 24–72 hours). When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary if the target protein has a long cellular half-life.
Cell-type dependence	Some cell types might exhibit better knockdown efficiencies with an alternative siRNA delivery reagent from Mirus: TransIT-X2® Dynamic Delivery System or *Trans*IT-TKO® Transfection Reagent.

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add transfection complexes drop-wise to the 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	*Trans*IT-siQUEST efficiently transfects cells cultured in serum-free medium; however, toxicity may be higher if serum is not present. If toxicity is a problem, consider adding serum to the culture medium.
Medium change or addition may be necessary	If incubating for 48–72 hours, it may be necessary to change the complete medium 24 hours post-transfection. Alternatively, add additional complete medium 4–24 hours post-transfection.
Knockdown of an essential gene	If the siRNA is directed against a gene that is essential to the cell, cytotoxicity may be observed due to knockdown of the target gene. Include a transfection control with non-targeting siRNA to compare the cytotoxic effects of the gene being knocked down.
Cell density not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this density 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

- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT-TKO®Transfection Reagent
- *Trans*IT-2020 Transfection Reagent
- *Trans*IT-PRO® Transfection Kit
- *Trans*IT Cell Line Specific Transfection Reagents and Kits
- *Trans*IT-LT1 Transfection Reagent
- Ingenio™ Electroporation Solution and Kits
- *Label* IT Plasmid Delivery Controls
- *Label* IT Tracker Intracellular Nucleic Acid Localization Kits
- *Label* IT RNAi Delivery Controls
- *Label* IT siRNA Tracker Intracellular Localization Kits

For details on the above mentioned products, visit www.mirusbio.com

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.mirusbio.com/ra

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