

TransIT-siQUEST® Transfection Reagent

Product	Quantity*	Product No.
TransIT-siQUEST® Transfection Reagent	0.4 ml	MIR 2114
	1 ml	MIR 2110
	5 ml (5 x 1 ml)	MIR 2115
	10 ml (10 x 1 ml)	MIR 2116

*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.

1.0 DESCRIPTION

1.1 General Information

Cellular uptake of long double stranded RNA (dsRNA) has been shown to induce RNA interference in a diverse group of organisms as well as insect cells in culture. RNA interference leads to the inhibition of protein expression through a sequence-specific, dsRNA-mediated destruction of the target messenger RNA (mRNA). Attempts to induce RNA interference using long dsRNA in mammalian cell lines have been met with limited success, due in part to the induction of the interferon response, which results in a general inhibition of protein synthesis. It has been shown that when short RNA duplexes (< 30 base pairs) are introduced into mammalian cells in culture, sequence-specific destruction of target mRNA can be achieved without inducing an interferon response. These short dsRNA, referred to as small interfering RNAs (siRNA), act catalytically at sub-molar ratios 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 RNA interference, induced by the introduction of siRNA, extremely effective at inhibiting target gene expression.^{1,2,3}

Mirus Bio Corporation, in recognition of these significant findings, 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. Transfections are most effective when carried out in complete growth media, with no media change or serum addition required. *TransIT-siQUEST* Reagent efficiently delivers siRNA to a wide range of cell lines, promoting high levels of target gene expression knockdown. These unique features make the *TransIT-siQUEST* Transfection Reagent ideal for siRNA-mediated gene silencing studies. The *TransIT-siQUEST* Reagent is a complement to Mirus Bio's popular *TransIT-TKO*® siRNA Transfection Reagent (see Related Products, Section 5.0). Each unique formulation provides a distinct transfection profile for high efficiency, broad-spectrum siRNA delivery.

1.2 Cell Lines Successfully Tested by Mirus Bio Corporation

A549, CHO-K1, COS-7, HEK 293, HeLa, Hepa1c1c7, HepG2, MCF-7, NIH 3T3, RAW 264.7, and Vero cells.

1.3 Specifications

Concentration: 3.0 mg/ml in 80% ethanol
Storage: Tightly capped at 4°C; DO NOT FREEZE
Stability: 6 months from the date of purchase when stored properly

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each individual cell type. The transfection protocols described in Sections 2.2 and 2.3 should result in efficient transfection of most cell types; however, to ensure optimal results, consider the following variables:

- A. Media conditions** – The *TransIT*-siQUEST Reagent yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection media change.
- B. Cell density (% confluence) at time of transfection**—The recommended confluence for most cell types is 60-80% at the 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 cell densities outside of the recommended range.

Lower cell densities may be necessary for post-transfection incubation times greater than 48 hours. If lower cell densities are plated, ensure that the levels of *TransIT*-siQUEST Reagent and siRNA are titrated accordingly. Alternatively, trypsinize and replat cells 24 hours post-transfection to accommodate longer incubation times. Determine the optimal cell density for each cell type in order to maximize knockdown efficiency. Maintain this density in future experiments for reproducibility.

- C. siRNA concentration**—siRNA used for transfection should be highly pure, sterile, and the correct sequence. As a starting point, use 25 nM siRNA (final concentration in well). Depending on the type of experiment, the optimal final siRNA concentration for transfection is typically within the range of 10 to 50 nM.
- D. *TransIT*-siQUEST Reagent** —As a starting point, test three levels of *TransIT*-siQUEST Reagent, such as 0.5 μ l, 1.5 μ l, and 3 μ l per well of a 24-well plate, using 25 nM siRNA (final concentration in the well). The optimal *TransIT*-siQUEST Reagent volume can be determined by titrating the reagent within the ranges listed in Table 1. The volume of reagent that achieves the highest knockdown efficiency with the lowest cellular toxicity should be used for future transfections.
- E. 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 incubations may be necessary if the target protein has a long cellular half-life and therefore degrades slowly.
- F. Proper Controls**— We recommend using a negative control containing serum-free media, *TransIT*-siQUEST Reagent, and a non-specific siRNA. Other controls to consider are a cells alone control, and a reagent alone control. Run these controls in parallel to serum-free media, *TransIT*-siQUEST Reagent, and specific siRNA, using the same volumes and parameters.

Table 1. Recommended titration ranges for *TransIT-siQUEST* Transfection Reagent transfections

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10 cm dish
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
Serum-free Media	9 µl	25 µl	50 µl	100 µl	250 µl	1500 µl
<i>TransIT-siQUEST</i> Transfection Reagent	0.09-0.6 µl	0.26-1.6 µl	0.5-3 µl	1-6 µl	2.5-15 µl	15-90 µl
1 µM stock siRNA (10-50 nM final concentration in well)	0.5-2.6 µl	1.5-7.5 µl	3-15 µl	6-30 µl	15-75 µl	90-450 µl
Complete Growth Media	44 µl	125 µl	250 µl	500 µl	1250 µl	7500 µl

*Surface areas are based on Greiner tissue culture plates and Falcon 10 cm dishes.

NOTE: All volumes in Table 1 are per well of the indicated size.

To dilute siRNA, use 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.

NOTE: The protocols in Sections 2.2 and 2.3 are recommended for performing transfections with the *TransIT-siQUEST* Transfection Reagent in 24-well plates. When performing transfections in different sized wells, the amount of siRNA, serum-free media, *TransIT-siQUEST* Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish (see Table 1). Proper scaling of the total volume of culture medium is important to ensure optimal *TransIT-siQUEST* Reagent and final siRNA concentrations.

2.2 siRNA Transfection in 24-well Plates

A1. Cell Plating for Adherent Cells

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density to obtain ~60-80% confluence the following day (3×10^4 to 1.2×10^5 cells per well of a 24-well plate, depending on cell size and characteristics).^a Plate adherent cells in 500 µl of complete growth media per well.
2. Incubate the cells overnight.^b

A2. Cell Plating for Suspension Cells

1. Immediately prior to transfection, plate cells in 0.25 ml of complete growth media per well, at an appropriate density to obtain ~60-80% confluence at time of transfection ($3-5 \times 10^5$ cells per well). Alternatively, plate cells the day prior to transfection to obtain 60-80% confluence at the time of transfection ($1.5-2.5 \times 10^5$ cells per well).

B. Complex Formation (perform this procedure immediately prior to transfection)

1. In a sterile, plastic tube, add 50 µl (see Table 1) of serum-free medium^c.
2. Add 0.5 to 3 µl (test three different levels, such as 0.5, 1.5 and 3 µl per well of a 24-well plate; see Table 1) of the *TransIT-siQUEST* Transfection Reagent directly into the serum-free media. Mix thoroughly by pipetting or vortexing.
3. Add 10-50 nM siRNA, (the starting recommendation is 25 nM, final concentration in the well) to the diluted *TransIT-siQUEST* Reagent. Mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *TransIT-siQUEST* Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium).

1. For adherent cell types, adjust the volume in the well to 250 µl of complete growth media by removing ~250 µl (half) of the original plating media, to conserve siRNA.
2. Add the *TransIT-siQUEST* Reagent/siRNA complex mixture prepared in step B dropwise to the cells. Gently rock the plate back and forth and from side to side to distribute the complexes evenly. Do not swirl the plate.
3. Incubate for 24-72 hours.^b

NOTE: The above incubation is designed for transfections performed with no media change. If a media change is necessary to remove the transfection complexes, incubate the cells for 24 hours, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.^b

4. Assay for knockdown of target gene expression.

2.3 Sequential Transfection of Plasmid DNA and siRNA

If the transfection of both plasmid DNA and siRNA is required, follow the procedure below.

NOTE: Do not transfect plasmid DNA (with a plasmid DNA transfection reagent) and siRNA (with *TransIT*-siQUEST Reagent) at the same time, as the addition of the *TransIT*-siQUEST Reagent may inhibit DNA transfection. Perform the DNA transfection first, followed by the siRNA transfection 4 hours later.

The following recommendations are for the plasmid transfection of cells in one T-75 flask, and the plating and subsequent siRNA transfection of the cells in a 24-well plate. Allot for one T-75 flask per 24 well-plate to be transfected.

A. Cell Plating in a T-75 Flask

1. Approximately 24 hours prior to transfection, plate cells in one T-75 flask (for each 24-well plate needed) at an appropriate cell density to obtain ~50-70% confluence the following day.^a
2. Incubate the cells overnight.^b

NOTE: If transfecting suspension cells, plate cells in a T-75 flask the day of transfection to obtain 50-70% cell confluence.

Complex Formation: *TransIT*[®]-LT1 Reagent and plasmid DNA

NOTE: Mirus Bio offers several reagents for plasmid transfection, including cell line specific reagents. To choose the appropriate reagent, please see Related Products, our website (www.mirusbio.com), or contact Technical Support at techsupport@mirusbio.com or 888-530-0801. The following protocol recommends *TransIT*-LT1 Transfection Reagent, Mirus Bio's broad spectrum transfection reagent, which efficiently transfects DNA into many cell types.

1. In a sterile plastic tube, add 500 μ l of serum-free medium^c.
2. Add 45 μ l of the *TransIT*-LT1 Transfection Reagent (this is a starting recommendation; titration of the reagent may be necessary) directly to the serum-free media. Mix thoroughly by pipetting or vortexing.

3. Incubate at room temperature for 5-20 minutes.

4. Add 15 μ g pDNA (this is a starting recommendation; titration of pDNA may be necessary) to the diluted *TransIT*-LT1 Reagent. Mix by gentle pipetting.

NOTE: If transfecting more than one plasmid, mix the plasmids together in a microcentrifuge tube and incubate for 5-10 minutes at room temperature before adding to the diluted *TransIT*[®]-LT1 Reagent.

5. Incubate at room temperature for 5-20 minutes.

C. Preparation of Cells for pDNA Transfection in a T-75 Flask

NOTE: The *TransIT*-LT1 Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium).

1. Add the *TransIT*-LT1 Reagent/DNA complex mixture from Step B dropwise to the cells in complete growth medium. Gently rock the flask back and forth and from side to side to distribute the complexes evenly. Do not swirl the flask.
2. Incubate for 2-4 hours.^b

D. Transfer of Cells from T-75 flask(s) to a 24-well Plate(s)

1. If transferring adherent cells, trypsinize the T-75 flask according to standard procedures. Transfection reagents can cause suspension cells to adhere; therefore, ensure cells are completely removed from the bottom of the flask.
2. Add 13-14 ml of complete growth media to the T-75 flask. Mix cells thoroughly and plate 500 μ l per well of a 24-well plate (adherent cells) to obtain 60-80% confluence at the time of transfection. Plate 250 μ l of suspension cells to obtain 3-5 $\times 10^5$ cells per well of a 24-well plate.
3. Incubate for 2 hours.^b

E. Complex Formation of *TransIT*-siQUEST Reagent and siRNA

1. In a sterile, plastic tube, add 50 μ l of serum-free medium^c.
2. Add 0.5 to 3 μ l (test three different levels, such as 0.5, 1.5 and 3 μ l per well of a 24-well plate; see Table 1) of the *TransIT*-siQUEST Transfection Reagent directly into the serum-free media. Mix thoroughly by pipetting or vortexing.
3. Add 10-50 nM siRNA (starting recommendation is 25 nM, final concentration in the well) to the diluted *TransIT*-siQUEST Reagent. Mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

F. Preparation of Cells for siRNA Transfection in 24-well Plates

NOTE: The *TransIT*-siQUEST Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium).

1. For adherent cell types, adjust the volume in the well to 250 μ l of complete growth media by removing ~250 μ l (half) of the original plating media, to conserve siRNA.
2. Add the *TransIT*-siQUEST Reagent/siRNA complex mixture prepared in step E dropwise to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly. Do not swirl the plate.
3. Incubate for 24-72 hours.^b

NOTE: The above incubation is designed for transfections performed with no media change. If a media change is necessary to remove the transfection complexes, incubate the cells for 24 hours, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.^b

5. Assay for inhibition of target gene expression.

^a Since the optimal cell density (confluence) for efficient transfection can vary between cell types, this should be determined for each cell type. Maintain the optimal seeding protocol for each cell type between experiments.

^b Standard incubation conditions for mammalian cells are 37°C in 5% CO₂. Other cell types, such as insect cells, require different temperatures and CO₂ concentrations. Use conditions appropriate for the cell type of interest.

^c The *TransIT*-siQUEST Reagent/siRNA complex may not form properly if the complex formation medium contains serum, resulting in poor transfection efficiencies. Any serum-free media can be used for complex formation, provided it does not contain polyanions such as dextran sulfate and heparin.

3.0 TROUBLESHOOTING**Low Knockdown Efficiency****• Suboptimal *TransIT*-siQUEST Reagent**

Determine the optimal *TransIT*-siQUEST Reagent volume by titrating the reagent from 0.5 μ l to 3 μ l per well of a 24-well plate. See Section 2.1D for recommended starting conditions. It may be necessary to titrate outside of this range depending on the cell type. Use the volume of reagent that gives the highest knockdown efficiency with the lowest cellular toxicity for future transfections.

• Suboptimal siRNA concentration

Determine the optimal siRNA concentration by titrating from 10 to 50 nM (final concentration in the well). We recommend starting with 25 nM of siRNA (final concentration in the well). In some instances higher concentrations of siRNA such as 100 or 200 nM may be necessary to achieve sufficient knockdown of the gene of interest.

• Low Transfection Efficiency

Follow transfection protocol steps carefully. To assess transfection efficiency of siRNA, use Mirus' Label IT[®] siRNA Tracker Kits (see Related Products, Section 5.0).

• 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.

• Incorrect siRNA Sequence

Ensure that the sequence of siRNA is correct for the gene of interest. More than one sequence may need to be tested for optimal knockdown efficiency.

- **Poor quality of transfecting siRNA**
Avoid siRNA degradation by using RNase-free handling procedures and plastic ware. For high quality siRNA design and manufacture, Mirus recommends Dharmacon Research, Inc. (dharmacon.com). Degradation of siRNA can be detected on acrylamide gels.
- **Fetal calf serum present during *TransIT*-siQUEST Reagent/siRNA complex formation**
Use serum-free medium during complex formation steps.
- **Cell density (% confluence) not optimal at time of transfection**
The recommended cell density for most cell types at the time of transfection is 60-80% confluence. Determine the optimal cell density, which may be outside the recommended range, for each cell type in order to maximize transfection efficiency. Maintain the optimal 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. If polyanions are necessary for the health of the cells, the transfection media can be replaced with the optimal cell growth media 24 hours post-transfection.
- **Proper controls were not included**
To ensure assay validation, include the following controls: cells only (for visual comparisons), *TransIT*-siQUEST Reagent alone, and *TransIT*-siQUEST Reagent with a non-specific siRNA. To verify efficient transfection and knockdown, use *TransIT*-siQUEST Reagent to deliver an siRNA targeted against a ubiquitous gene, such as GAPDH or Lamin A/C, followed by western blotting or target mRNA quantification.

High Cellular Toxicity

- **Media change or addition may be necessary**
If incubating for 48-72 hours, it may be necessary to change the complete media 24 hours post-transfection. Alternatively, add additional complete media 4-24 hours post-transfection.
- **Excessive amount of *TransIT*-siQUEST Reagent**
Reduce the amount of *TransIT*-siQUEST Reagent in the transfection. See Table 1 for recommended starting conditions. Use the volume of reagent that gives the highest knockdown efficiency with the lowest cellular toxicity for future transfections.
- **Cell density (% confluence) was too low at time of transfection**
Grow cells to a higher cell density and repeat the transfection.
- ***TransIT*-siQUEST Reagent/siRNA complex mixture and cells were not mixed thoroughly**
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.
- **Complexes were added to cells in serum-free media**
TransIT-siQUEST/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, cytotoxicity may be observed. If you must add the complexes to 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. Maintain a similar passage number between experiments to ensure reproducibility.
- **Knockdown of an essential gene**
If the siRNA is directed against a gene that is essential to the cell, cytotoxicity may be observed.

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

For a list of citations using Mirus Bio products, please visit the Technical Resources section of our website (www.mirusbio.com).

4.0 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.

5.0 RELATED PRODUCTS

RNA Interference Products:*

- TransIT*-TKO[®] siRNA Transfection Reagent (Product # MIR 2150)
- siXpress*[®] PCR Vector Systems (Product # MIR 7300, 7301, 7302)
- Label IT*[®] siRNA Tracker Intracellular Localization Kit with *TransIT*-TKO[®] Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
- Label IT*[®] siRNA Tracker Intracellular Localization Kit with *TransIT*[®]-*siQUEST*[™] Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
- Label IT*[®] siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

For endotoxin removal from DNA:*

- MiraCLEAN*[®] Endotoxin Removal Kit (Product #5900)

For DNA tracking studies:

- Label IT*[®] Tracker[™] Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)
- Label IT*[®] Plasmid Delivery Control, Cy[™]3 or Fluorescein, (Product # MIR 7904, 7905, 7906, 7907)

For determination of gene expression efficiency:

- Beta-Gal Staining Kit (Product # MIR 2600)

Plasmid transfection reagents:*

- TransIT*[®]-LT1 Transfection Reagent (Product # MIR 2300)
- TransIT*[®]-LT2 Transfection Reagent (Product # MIR 2400)
- TransIT*[®]-Express Transfection Reagent (Product # MIR 2000)
- TransIT*[®]-HeLaMONSTER[®] Transfection Kit (Product # MIR 2900)
- TransIT*[®]-Keratinocyte Transfection Reagent (Product # MIR 2800)
- TransIT*[®]-CHO Transfection Kit (Product # MIR 2170)
- TransIT*[®]-3T3 Transfection Kit (Product # MIR 2180)
- TransIT*[®]-293 Transfection Kit (Product # MIR 2700)
- TransIT*[®]-COS Transfection Kit (Product # MIR 2190)
- TransIT*[®]-Insecta Transfection Reagent (Product # MIR 2200)
- TransIT*[®]-Jurkat Transfection Reagent (Product # MIR 2120)
- TransIT*[®]-Prostate Transfection Kit (Product # MIR 2130)
- TransIT*-Neural[®] Transfection Reagent (Product # MIR 2140)
- TransIT*[®]-mRNA Transfection Kit (Product # MIR 2250)
- TransIT*-TKO[®] siRNA Transfection Reagent (Product # MIR 2150)
- TransIT*[®]-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo Gene Delivery Kits:*

- TransIT*[®]-*In Vivo* Gene Delivery System (Product # MIR 5100)
- TransIT*[®]-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
- TransIT*[®]-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)
- TransIT*[®]-QR Hydrodynamic Delivery Solution (Product # MIR 5240)
- TransIT*[®]-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

*These products are available in additional sizes.

Mirus Bio Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811; 6,593,465 and patents pending.

The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

This product is sold to the Buyer with a limited license to use this product for research only. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio Corporation.

TransIT, *TransIT*-TKO, *TransIT*-Neural, *MiraCLEAN*, *HeLaMONSTER*, *siXpress*, and *Label IT* are registered trademarks of Mirus Bio Corporation.

Tracker and *siQUEST* are trademarks of Mirus Bio Corporation.

©2004, Mirus Bio Corporation. All rights reserved.