*Trans*IT-X2® Dynamic Delivery System

Protocol for MIR 6000, 6003, 6004, 6005, 6006

Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/literature

INTRODUCTION

*Trans*IT-X2® Dynamic Delivery System is an advanced, non-liposomal polymeric system that enables high efficiency transfection of many cell types, including primary cells. *Trans*IT-X2 can be used for DNA delivery, siRNA delivery or simultaneous delivery of DNA and siRNA. Transfections with *Trans*IT-X2 do not require medium changes and can be carried out in serum-containing medium.

SPECIFICATIONS

Storage	Store *Trans*IT-X2 Dynamic Delivery System at –20°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-X2 to room temperature and vortex gently before each use.

*Trans*IT-X2 Dynamic Delivery System is supplied in the following formats.

Product No.	Quantity
MIR 6003	1 × 0.3 ml
MIR 6004	1 × 0.75 ml
MIR 6000	1 × 1.5 ml
MIR 6005	5 × 1.5 ml
MIR 6006	10 × 1.5 ml

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Protocols for delivery of CRISPR components using *Trans*IT-X2, including RNP, can be found at mirusbio.com/literature.

For Research Use Only

BEFORE YOU START: DNA TRANSFECTION

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency plasmid DNA transfection using the *Trans*IT-X2 Dynamic Delivery System. **Table 1** presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are desirable. DNA prepared using miniprep kits is not recommended as it might contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove endotoxin from your DNA preparation.

Do not use DNA prepared using miniprep kits for transfection.

- **Ratio of *Trans*IT-X2 to DNA.** Determine the best *Trans*IT-X2:DNA ratio for each cell type. Start with 3 µl of *Trans*IT-X2 per 1 µg of DNA. Vary the amount of *Trans*IT-X2 from 2–6 µl per 1 µg DNA to find the optimal ratio. **Table 1** provides recommended starting conditions based on cell culture vessel size.

NOTE: Optimal starting conditions for many cell types can be found using our online transfection database, Reagent Agent® (www.mirusbio.com/ra).

- **Complex formation conditions.** Prepare *Trans*IT-X2:DNA 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.

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

- **Presence of antibiotics.** Antibiotics may inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added directly to cells grown in complete culture medium containing serum and low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24–72 hours but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time.

Table 1. Recommended starting conditions for DNA transfections with *Trans*IT-X2 Dynamic Delivery System

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
DNA (1 µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-X2	0.3 µl	0.78 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

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 *Trans*IT-X2 need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-X2.

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections using the *Trans*IT-X2 Dynamic Delivery System in 6-well plates. The surface areas of other culture vessels are different, and transfections must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-X2, DNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 1** on Page 2).

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

Transient Plasmid DNA Transfection Protocol per Well of a 6-Well Plate

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells in 2.5 ml complete growth medium per well in a 6-well plate. For most cell types, cultures should be ≥ 80% confluent at the time of transfection.

   For adherent cells: Plate cells at a density of 0.8–3.0 × 10^5 cells/ml.

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

Divide cultured cells 18–24 hours before transfection to ensure active cell division at the time of transfection.

B. Prepare *Trans*IT-X2:DNA complexes (Immediately before transfection)

1. Warm *Trans*IT-X2 to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 2.5 µg (2.5 µl of a 1 µg/µl stock) plasmid DNA.
4. Pipet gently to mix completely.
5. Add 7.5 µl *Trans*IT-X2 to the diluted DNA mixture. For further optimization of your cell type, test additional amounts of *Trans*IT-X2 (please refer to "Before You Start" on Page 2).
6. Pipet gently to mix completely.
7. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

Warm *Trans*IT-X2 to room temperature and vortex gently before each use.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-X2:DNA 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-X2:DNA complexes.
3. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay as required.

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

Transfection complexes, visualized as small particles, are sometimes observed following transfection. The complexes are not toxic to cells and do not impact transfection efficiency or transgene expression.

Note: For generating stable cell transfectants, passage cells 48–72 hours post-transfection in complete growth medium containing appropriate selection antibiotics such as G418 or Hygromycin B. Maintain selection for 1-2 weeks to allow for selection of cells that have undergone stable integration of DNA.

BEFORE YOU START: siRNA TRANSFECTION

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 the *Trans*IT-X2 Dynamic Delivery System. Please refer to **Table 2** on Page 5 for recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.
- **Volume of *Trans*IT-X2.** Each cell type responds differently to a given transfection reagent. As a starting point, test 7.5 µl of *Trans*IT-X2 per well of a 6-well plate. For further optimization, test three amounts of *Trans*IT-X2, e.g. 5 µl, 7.5 µl, and 10 µl per well of a 6-well plate.

  NOTE: Optimal starting conditions for many cell types can be found using our online transfection database, Reagent Agent® www.mirusbio.com/ra.
- **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 at low concentrations.
- **siRNA concentration.** siRNA used for transfection should be highly pure, sterile, and the correct sequence. Depending on the type of experiment, the optimal 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 the gene expression knockdown or phenotype is attributed to the gene-specific siRNA. Additionally, independent transfection of multiple siRNA sequences targeting a given gene minimizes the possibility that the observed phenotype is due to off-target effects.
- **Complex formation conditions.** Prepare *Trans*IT-X2: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**.** *Trans*IT-X2 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. At lower cell densities, less *Trans*IT-X2 may be optimal.

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

siRNA TRANSFECTION PROTOCOL

The following procedure describes how to perform siRNA transfections using the *Trans*IT-X2 Dynamic Delivery System in 6-well plates. The surface areas of other culture vessels are different, and transfections must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-X2, siRNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 2**).

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

Table 2: Recommended starting conditions for siRNA transfection with *Trans*IT-X2 Dynamic Delivery System

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
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
*Trans*IT-X2	0.3 µl	0.78 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

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 *Trans*IT-X2 need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-X2.

Transient siRNA Transfection Protocol per Well of a 6-Well Plate

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 a density of 0.8–3.0 × 10^5 cells/ml.

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

B. Prepare *Trans*IT-X2:siRNA complexes (Immediately before transfection)

Warm *Trans*IT-X2 to room temperature and vortex gently before each use.

1. Warm *Trans*IT-X2 to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 6.8 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
4. Add 7.5 µl of *Trans*IT-X2. Pipet gently to mix completely. For further optimization of your cell type, test additional amounts of *Trans*IT-X2 (please refer to "Before You Start" on Page 4).
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-X2: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-X2: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.

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.

BEFORE YOU START: CO-TRANSFECTION

Important Tips for DNA & siRNA Co-Transfection

Observe the following recommendations when performing co-transfection of DNA and siRNA. The suggestions below yield high efficiency gene delivery and knockdown of target gene expression using *Trans*IT-X2 Dynamic Delivery System. Please refer to **Table 3** on page 8 for recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preps that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are desirable. DNA prepared using miniprep kits is not recommended as it might contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.
- **Volume of *Trans*IT-X2.** The amount of *Trans*IT-X2 required in co-transfection experiments is dictated by the µg of DNA. Determine the best *Trans*IT-X2:DNA ratio for each cell type. Start with 3 µl of *Trans*IT-X2 per 1 µg of DNA. Vary the amount of *Trans*IT-X2 from 2–6 µl per 1 µg DNA to find the optimal ratio. **Table 3** on page 8 provides recommended starting conditions based on cell culture vessel size.

  NOTE: Optimal starting conditions for many cell types can be found using our online transfection database, Reagent Agent® (www.mirusbio.com/ra).
- **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 optimal 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 plasmid-only control to verify gene expression and provide a reference for determining gene expression knockdown.
- **Complex formation conditions.** Prepare *Trans*IT-X2:DNA complexes in serum-free growth medium. Mirus recommends Opti-MEM I Reduced-Serum Medium.
- **Cell culture conditions.** Culture cells in the appropriate medium. *Trans*IT-X2 yields improved efficiencies when transfections are performed in complete growth medium without a post-transfection medium change. There is no need to perform a medium change to remove the transfection complexes.
- **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 serum and low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24–72 hours but will vary depending on the goal of the experiment, nature of the plasmid used, and the half-life of the expressed protein.

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-X2 volumes to determine the optimal concentration.

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

DNA & siRNA CO-TRANSFECTION PROTOCOL

The following procedure describes how to perform co-transfection of DNA and siRNA using *Trans*IT-X2 in 6-well plates. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-X2, DNA, siRNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 3**).

Table 3. Recommended starting conditions for DNA and siRNA co-transfections with *Trans*IT-X2 Dynamic Delivery System

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
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1.0 µl	2.5 µl	15 µl	19 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
*Trans*IT-X2	0.3 µl	0.78 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

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 *Trans*IT-X2 need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-X2.

Transient DNA & siRNA Co-Transfection Protocol per Well of a 6-Well Plate

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 a density of 0.8–3.0 × 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5–5.0 × 10^5 cells/ml.
2. Incubate the cells overnight.

B. Prepare co-transfection complexes (Immediately before transfection)

1. Warm *Trans*IT-X2 to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 2.5 µg (2.5 µl of 1 µg/µl stock) plasmid DNA. Pipet gently to mix completely.
4. Add 6.8 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
5. Add 7.5 µl of *Trans*IT-X2. Pipet gently to mix completely. For further optimization of your cell type, test additional amounts of *Trans*IT-X2 (please refer to “Before You Start” on page 7).
6. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

C. Distribute the complex mixture to cells in complete growth medium

1. Add the co-transfection 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 co-transfection 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.

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TROUBLESHOOTING GUIDE

POOR DNA TRANSFECTION EFFICIENCY	
Problem	**Solution**
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Suboptimal *Trans*IT-X2:DNA ratio	Determine the best *Trans*IT-X2: DNA ratio for each cell type. Titrate the *Trans*IT-X2 from 2–6 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Determine the DNA concentration accurately. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8–2.0. The optimal DNA concentration generally ranges between 1–3 µg/well of a 6-well plate. Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of *Trans*IT-X2 accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin- and contaminant-free DNA for transfection. We recommend using Mirus MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells. **Do not** use DNA prepared using miniprep kits as it might contain high levels of endotoxin.
Cells not actively dividing at the time of transfection	Divide the culture at least 18–24 hours before transfection to ensure that the cells are actively dividing and reach optimal cell density at time of transfection.
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g.12–72 hours). The best incubation time is generally 24–48 hours.
*Trans*IT-X2 was not mixed properly	Warm *Trans*IT-X2 to room temperature and vortex gently before each use.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents according to **Table 1** on Page 2, or **Table 3** on Page 8 including serum-free media, *Trans*IT-X2, and plasmid DNA (and siRNA for co-transfection). Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold.
Proper experimental controls were not included	To assess delivery efficiency of plasmid DNA, use Mirus *Label* IT® Nucleic Acid Labeling Kits to label the target plasmid or Mirus prelabeled *Label* IT® Plasmid Delivery Controls. To verify efficient transfection, use *Trans*IT-X2 to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.

TROUBLESHOOTING GUIDE continued

HIGH CELLULAR TOXICITY	
Problem	**Solution**
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.
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.
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-X2 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.
siRNA 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.

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TROUBLESHOOTING GUIDE CONTINUED

POOR siRNA KNOCKDOWN	
Problem	**Solution**
Suboptimal *Trans*IT-X2:siRNA ratio	For optimization, test three levels of *Trans*IT-X2, e.g. 5, 7.5, and 10 µl per well of a 6-well plate, using 25 nM siRNA (final concentration in the well). It may be necessary to titrate outside of this range 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 200 nM may be necessary to achieve sufficient knockdown of the gene of interest. Refer to "Before You Start" on Page 4.
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, such as *Trans*IT-siQUEST® and *Trans*IT-TKO® Transfection Reagents.
Incorrect siRNA sequence	Ensure that the sequence of the siRNA is correct for the gene of interest. More than one sequence may need to be tested for optimal knockdown efficiency and to ensure on-target effects.
Proper controls were not included	We recommend including the following controls: 1. Serum-free medium alone 2. Serum-free medium + *Trans*IT-X2 + non-targeting siRNA To verify efficient transfection and knockdown, use *Trans*IT-X2 to deliver a siRNA targeted against a ubiquitous gene, e.g. GAPDH or Lamin A/C, followed by target Western blotting or mRNA quantification using qPCR. To assess delivery efficiency of plasmid DNA, use Mirus *Label* IT® Nucleic Acid Labeling Kits to label the target plasmid or Mirus prelabeled *Label* IT® Plasmid Delivery Controls.
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.
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 all *Trans*IT-X2 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. The presence of polyanions such as dextran sulfate or heparin can inhibit transfection. Use transfection medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hours post transfection.
*Trans*IT-X2 was not mixed properly.	Warm *Trans*IT-X2 to room temperature and vortex gently before each use.

RELATED PRODUCTS

- Ingenio® Electroporation Solution and Kits
- *Label* IT® Nucleic Acid Labeling Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® RNAi Delivery Controls
- MiraCLEAN® Endotoxin Removal Kits
- Cell Culture Antibiotic Solutions
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT-siQUEST® Transfection Reagent
- *Trans*IT-TKO® Transfection Reagent

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.

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