

## INTRODUCTION

TransIT-TKO<sup>®</sup> 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. TransIT-TKO was introduced in 2001 and was the first commercially available siRNA transfection reagent. Co-transfection of siRNA and DNA is also feasible with TransIT-TKO and has been tested across a variety of cell types. Transfections with TransIT-TKO do not require medium changes and can be carried out in serum-containing medium. In addition to TransIT-TKO, Mirus also offers TransIT-siQUEST<sup>®</sup> Transfection Reagent for siRNA transfection. Each unique formulation provides high efficiency broad-spectrum siRNA delivery.

## SPECIFICATIONS

<b>Storage</b>	Store tightly capped at 4°C. <b>Before each use</b> , warm to room temperature and vortex gently.
<b>Product guarantee</b>	1 year from the date of purchase, when properly stored and handled.



Warm TransIT-TKO to room temperature and vortex gently before each use.

## MATERIALS

### Materials Supplied

TransIT-TKO Transfection Reagent is supplied in **one** of the following formats.

Product No.	Quantity
MIR 2154	1 × 0.4 ml
MIR 2150	1 × 1.0 ml
MIR 2155	5 × 1.0 ml
MIR 2156	10 × 1.0 ml

### Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- siRNA
- Serum-free medium (e.g. Opti-MEM<sup>®</sup> 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 *TransIT-TKO* Transfection Reagent. Please refer to **Table 1** on Page 4 for recommended starting conditions dependent 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 50–80% confluence) at the time of transfection. If this confluence does not produce optimal results, test cell densities outside of the recommended range.
- **Volume of *TransIT-TKO* Reagent.** Each cell type responds differently to a given transfection reagent. As a starting point, test 2.5 µl of *TransIT-TKO* Reagent per well of a 24-well plate. For further optimization, test three levels of *TransIT-TKO* Reagent, e.g. 1 µl, 2.5 µl, and 4 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 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, targeting a gene with multiple siRNA sequences ensures that the resulting phenotype is not due to off-target effects.
- **Complex formation conditions.** Prepare *TransIT-TKO* 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 *TransIT-TKO* 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 *TransIT-TKO* reagent to determine optimal concentration.



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

## Additional Tips for DNA and siRNA Co-Transfection

Observe the following recommendations in addition to the tips for siRNA transfection when performing a co- transfection of DNA and siRNA. This procedure is further outlined in the DNA and siRNA Co-transfection Protocol on page 5. The suggestions below yield high efficiency knockdown of target gene expression using *TransIT-TKO* Transfection Reagent for DNA and siRNA delivery. Please refer to **Table 2** on page 5 for recommended starting conditions dependent on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is 50–70% confluence. Determine the optimal cell density for each cell type in order 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<sup>®</sup> Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.
- **Ratio of *TransIT-TKO* Reagent to DNA.** Determine the best *TransIT-TKO* Reagent:DNA ratio for each cell type. Start with 1  $\mu$ l of *TransIT-TKO* Reagent per 1  $\mu$ g of DNA. Vary the concentration of *TransIT-TKO* Reagent from 2–8  $\mu$ l per 1  $\mu$ g DNA to find the optimal ratio. **Table 2** provides recommended starting conditions based on cell culture vessel size.
- **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 *TransIT-TKO* Reagent:DNA complexes in serum-free growth medium. Mirus recommends Opti-MEM I Reduced-Serum Medium.
- **Cell culture conditions:** Culture cells in the appropriate medium. The *TransIT-TKO* Reagent 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 *TransIT-TKO* reagent to determine 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 TransIT-TKO 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, TransIT-TKO Reagent, siRNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 1**).

**Table 1:** Recommended starting conditions for siRNA transfection with TransIT-TKO 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 <sup>2</sup>	1.0 cm <sup>2</sup>	1.9 cm <sup>2</sup>	3.8 cm <sup>2</sup>	9.6 cm <sup>2</sup>	59 cm <sup>2</sup>	75 cm <sup>2</sup>
Complete growth medium (half volume protocol)	44 µl	125 µl	0.25 ml	0.5 ml	1.25 ml	7.5 ml	9.8 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
TransIT-TKO Reagent	0.5 µl	1.3 µl	2.5 µl	5 µl	10 µl	77 µl	98 µl
siRNA (1 µM stock) 25 nM final	1.33 µl	3.78 µl	7.5 µl	15 µl	37.5 µl	225 µl	293 µl



This protocol recommends using half the volume of complete growth medium than used in traditional transfection protocols. This ensures maximum siRNA transfection efficiency. If full culture volume is desired, double the volume of complete growth medium and siRNA. Do not double the volume of TransIT-TKO.



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-TKO need to be pipetted, dilute the reagent in 100% ethanol before each use to avoid pipetting errors. **Do not** store diluted TransIT-TKO Reagent.

### Transient siRNA transfection protocol per well of a 24-well plate

#### A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells using the following guidelines. Ideally cells should be 50–80% confluent prior to transfection.  
**For adherent cells:** Plate cells in 0.5 ml complete growth medium/well at an optimal concentration (generally  $2-6 \times 10^5$  cells/well).  
**For suspension cells:** Plate cells in 0.25 ml of complete growth medium per well, at a density of  $8-10 \times 10^5$  cells per well.

2. Incubate the cell cultures overnight.

#### B. Prepare TransIT-TKO Reagent:siRNA complexes (Immediately before transfection)

1. Warm TransIT-TKO Reagent to room temperature and vortex gently before using.
2. Place 50 µl of Opti-MEMI Reduced-Serum Medium in a sterile tube.
3. Add 2.5 µl of TransIT-TKO Reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the TransIT-TKO Transfection Reagent (please refer to “Before You Start” on Page 2).
4. Add 7 µl of a 1 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 TransIT-TKO to room temperature and vortex gently before each use.

**C. Distribute the complexes to cells in complete growth medium**

1. **For adherent cells:** Adjust the volume in the well to 0.25 ml of complete growth medium by removing 0.25 ml (half) of the original plating medium. Reducing the volume of medium in each well substantially decreases the amount of siRNA required per well.  
**For suspension cells:** It is not necessary to replace the complete growth medium with fresh medium.
2. Add the *TransIT-TKO* Reagent:siRNA complexes (prepared in Step B) drop-wise to different areas of the wells.
3. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *TransIT-TKO* Reagent:siRNA 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 knockdown of target gene expression.



There is no need to change fresh 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.

**DNA & siRNA CO-TRANSFECTION PROTOCOL**

Co-transfection of DNA and siRNA is possible with *TransIT-TKO* Transfection Reagent across a variety of cell types. Delivery of DNA with *TransIT-TKO* into your cell type should first be determined prior to attempting the co-transfection.

The following procedure describes how to perform the co-transfection of DNA and siRNA using *TransIT-TKO* to cells 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, *TransIT-TKO* Reagent, siRNA, DNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 2**). It is highly recommended to first optimize DNA transfection efficiency with *TransIT-TKO* in your particular cell type before attempting the co-transfection.



Please optimize delivery of DNA with *TransIT-TKO* prior to attempting co-transfection of siRNA and DNA.

**Table 2.** Recommended starting conditions for DNA and siRNA transfections with *TransIT-TKO* 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 <sup>2</sup>	1.0 cm <sup>2</sup>	1.9 cm <sup>2</sup>	3.8 cm <sup>2</sup>	9.6 cm <sup>2</sup>	59 cm <sup>2</sup>	75 cm <sup>2</sup>
Complete growth medium (half of volume protocol)	44 µl	125 µl	0.25 ml	0.5 ml	1.25 ml	7.5 ml	9.8 ml
<b>DNA complex</b>							
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
<i>TransIT-TKO</i> Reagent	0.1 µl	0.26 µl	0.5 µl	1.0 µl	2.5 µl	15 µl	19 µl
<b>siRNA complex</b>							
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
siRNA (1 µM stock) 25 nM final	1.55 µl	4.43 µl	8.75 µl	17.5 µl	43.8 µl	263 µl	340 µl
<i>TransIT-TKO</i> Reagent	0.5 µl	1.3 µl	2.5 µl	5 µl	10 µl	77 µl	98 µl



If small volumes of *TransIT-TKO* need to be pipetted, dilute the reagent in 100% ethanol before each use to avoid pipetting errors. **Do not** store diluted *TransIT-TKO* Reagent.

## Transient DNA and siRNA co-transfection protocol per well of a 24-well plate

### A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells using the following guidelines. Ideally cells should be 50–80% confluent prior to transfection.

**For adherent cells:** Plate cells in 0.5 ml complete growth medium/well at an optimal concentration (generally  $2-6 \times 10^5$  cells/well).

**For suspension cells:** Plate cells in 0.25 ml of complete growth medium per well, at a density of  $8-10 \times 10^5$  cells per well

2. Incubate the cells overnight.

### B. Prepare Complexes (Immediately before transfection)

**Note:** Complex formation of *TransIT-TKO* Reagent:DNA and *TransIT-TKO* Reagent:siRNA (Steps 1 & 2) should be synchronized to allow for simultaneous addition to cells.

#### 1. Prepare *TransIT-TKO* Reagent:DNA complexes

- a. Warm *TransIT-TKO* Reagent to room temperature and vortex gently before using.
- b. Place 50  $\mu$ l of Opti-MEM I Reduced-Serum Medium in a sterile tube
- c. Add 0.5  $\mu$ l of *TransIT-TKO* Reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the *TransIT-TKO* Transfection Reagent (please refer to “Before You Start” on page 3).
- d. Add 0.5  $\mu$ g (0.5  $\mu$ l of 1  $\mu$ g/ $\mu$ l stock) plasmid DNA.

**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-TKO* to avoid preferential complex formation of either plasmid.

- e. Pipet gently to mix completely
- f. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

#### 2. Prepare *TransIT-TKO* Reagent:siRNA complexes

- a. Warm *TransIT-TKO* Reagent to room temperature and vortex gently before using.
- b. Place 50  $\mu$ l of Opti-MEM I Reduced-Serum Medium in a sterile tube.
- c. Add 2.5  $\mu$ l of *TransIT-TKO* Reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the *TransIT-TKO* Transfection Reagent (please refer to “Before You Start” on Page 2).
- d. Add 7  $\mu$ l of a 1  $\mu$ M siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
- e. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

#### 3. Combine complexes

- a. After *TransIT-TKO* Reagent:DNA and *TransIT-TKO* Reagent:siRNA complex formation, add the complexes together.
- b. Pipet gently to mix completely.
- c. Incubate at room temperature for 5 minutes.



For simultaneous addition of DNA and siRNA complex, coordinate complex formation of each one separately prior to combining and adding to cells.



Warm *TransIT-TKO* to room temperature and vortex gently before each use.

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

1. **For adherent cells:** Adjust the volume in the well to 0.25 ml of complete growth medium by removing 0.25 ml (half) of the original plating medium. Reducing the volume of medium in each well substantially decreases the amount of siRNA required per well.  
**For suspension cells:** It is not necessary to replace the complete growth medium with fresh medium.
2. Add the co-transfection complexes (prepared in Step C) drop-wise to different areas of the wells.
3. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the co-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 knockdown of target gene expression.

**Note:** If preferred, DNA transfection can also be performed using *TransIT<sup>®</sup>-LT1* Transfection Reagent (MIR2300) or *TransIT<sup>®</sup>-2020* (MIR 5400) while delivering siRNA with *TransIT-TKO*. To deliver simultaneously, *TransIT-TKO* can be replaced with either *TransIT-LT1* or *TransIT-2020* in Step 1. Follow Step 2 and 3 as directed. Please refer to the detailed protocol for *TransIT-LT1* or *TransIT-2020* to optimize DNA transfection with either of these reagents.



*TransIT-TKO* can be replaced with *TransIT-LT1* or *TransIT-2020* for delivery of DNA.

Please refer to complete protocols for *TransIT-LT1* (MIR2300) and *TransIT-2020* (MIR5400) for DNA transfection procedures and optimization.

## TROUBLESHOOTING GUIDE

Problem	Solution
<b>POOR siRNA KNOCKDOWN EFFICIENCY</b>	
TransIT-TKO Reagent was not mixed properly.	Warm <i>TransIT-TKO</i> to room temperature and vortex gently before each use.
Suboptimal <i>TransIT-TKO</i> Reagent:siRNA ratio	For optimization, test three levels of <i>TransIT-TKO</i> Reagent, e.g. 1, 2.5, and 4 µl per well of a 24-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.
Proper controls were not included	<ol style="list-style-type: none"> <li>1. Serum-free medium alone</li> <li>2. Serum-free medium + <i>TransIT-TKO</i> Reagent + a non-targeting siRNA</li> </ol> To verify efficient transfection and knockdown, use <i>TransIT-TKO</i> 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’ <i>Label IT<sup>®</sup></i> siRNA Tracker™ Intracellular Localization Kits or a pre-labeled <i>Label IT<sup>®</sup></i> 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. <b>Do not</b> 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 may need to be tested 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 all <i>TransIT-TKO</i> Reagent complexes in serum-free growth medium. We recommend Opti-MEMI 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 e.g. 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.
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: <i>TransIT-siQUEST</i> Transfection Reagent. Mirus Bio offers the <i>TransIT-siPAK</i> Trial Kit which contains samples of both the <i>TransIT-siQUEST</i> and <i>TransIT-TKO</i> Transfection Reagents.

## TROUBLESHOOTING GUIDE continued

Problem	Solution
<b>POOR DNA TRANSFECTION EFFICIENCY (FOR CO-TRANSFECTION)</b>	
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
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.
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.
Precipitate formation during transfection complex formation	<p>During complex formation, scale all reagents according to Table 2 on page 5 including serum-free media, <i>TransIT-TKO</i>, plasmid DNA, and siRNA.</p> <p>Precipitation maybe 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.</p>
Proper experimental controls were not included	<p>To assess delivery efficiency of plasmid DNA, use Mirus' <i>LabelIT<sup>®</sup> Tracker<sup>™</sup></i> Intracellular Nucleic Acid Localization Kit to label the target plasmid or Mirus' prelabeled <i>LabelIT</i> Plasmid Delivery Controls (please refer to Related Products on Page 12).</p> <p>Likewise, to assess delivery efficiency of siRNA, use Mirus' <i>LabelIT<sup>®</sup> siRNA Tracker<sup>™</sup></i> Intracellular Localization Kits or prelabeled <i>LabelIT<sup>®</sup> RNAi</i> Delivery Control (please refer to Related Products on page 12).</p> <p>To verify efficient transfection, use <i>TransIT-TKO</i> Reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.</p>
<i>TransIT-TKO</i> Reagent was not mixed properly	Warm <i>TransIT-TKO</i> to room temperature and vortex gently before each use.
Suboptimal <i>TransIT-TKO</i> Reagent:DNA ratio	Determine the best <i>TransIT-TKO</i> Reagent: DNA ratio for each cell type. Titrate the <i>TransIT-TKO</i> Reagent from 2–8 µl per 1 µg DNA. Refer to “Before You Start” on Page 3.
Suboptimal DNA concentration	<p>Determine the DNA concentration accurately. Use plasmid DNA preps that have an <math>A_{260/280}</math> absorbance ratio of 1.8–2.0.</p> <p>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 <i>TransIT-TKO</i> Transfection Reagent accordingly.</p>
Low-quality plasmid DNA	<p>Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection.</p> <p>We recommend using Mirus Bio's MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation.</p> <p>Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells.</p> <p><b>Do not</b> use DNA prepared using miniprep kits as it might contain high levels of endotoxin.</p>

## TROUBLESHOOTING GUIDE continued

Problem	Solution
<b>HIGH CELLULAR TOXICITY</b>	
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	<i>TransIT-TKO</i> 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, 50–80% 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

- 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
- *TransIT*-2020 Transfection Reagent
- *TransIT*-PRO™ Transfection Kit
- *TransIT* Cell Line Specific Transfection Reagents and Kits
- *TransIT-InVivo* Gene Delivery Kits
- *TransIT*-LT1 Transfection Reagent
- *TransIT*-QR and *TransIT*-EE Delivery Solutions and Kits
- *TransIT*-siPAK Kit
- *TransIT*-siPAK Plus Kit
- *TransIT*-siQUEST Transfection Reagent

For details on the above mentioned products, visit [www.mirusbio.com](http://www.mirusbio.com) or [www.TheTransfectionExperts.com](http://www.TheTransfectionExperts.com).

Contact Mirus Bio for additional information.



The Transfection Experts

Mirus Bio LLC  
545 Science Drive  
Madison, WI 53711  
Toll-free: 888.530.0801  
Direct: 608.441.2852  
Fax: 608.441.2849

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Use of Mirus Bio *TransIT*® polyamine transfection reagents are covered by U.S. Patent No. 5,744,335, No. 6,180,784, No. 7,101,995, No. 7,601,367 and patents pending. Mirus Bio *Label IT*® nucleic acid labeling and modifying reagents are covered by U.S. Patent No. 6,262,252, No. 6,593,465, No. 7,049,142, No. 7,326,780 and No. 7,491,538. Cy™3 and Cy™5 products or portions thereof are manufactured under license from Carnegie Mellon University and are covered by U.S. Patent No. 5,268,486.

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For publications citing the use of *TransIT*-TKO Transfection Reagent, visit [www.mirusbio.com](http://www.mirusbio.com).