

TransIT-X2® Dynamic Delivery System

Quick Reference Protocol

DNA Delivery Instructions for MIR 6000, 6003, 6004, 6005, 6006, 6010

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6000



SPECIFICATIONS

Storage	Store TransIT-X2® Dynamic Delivery System tightly capped 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.

▶ TRANSFECTION PROTOCOL | PLASMID DNA



Full protocol and additional documentation available at mirusbio.com/6000

Fill in volumes below based on culture vessel used for transfection (Table 1).

A. Plate cells

1. Plate cells in ___ ml complete growth medium (per well or flask) 18-24 hours prior to transfection to ensure cells are actively dividing at the time of transfection.

For adherent cells: Plate cells at a density of 0.8—3.0 x 10⁵ cells/ml.

For suspension cells: Plate cells at a density of 2.5—5.0 x 10⁵ cells/ml.

2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare TransIT-X2® Reagent:DNA complexes

1. Warm TransIT-X2® Reagent to room temperature and vortex gently.
2. Place ___ μl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___ μl plasmid DNA. Mix gently by pipetting.
4. Add ___ μl of TransIT-X2®. Mix gently by pipetting.
5. Incubate at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add TransIT-X2®:DNA complex mixture drop-wise to different areas of the well.
2. Gently rock plate for even distribution of complexes.
3. Incubate 24-72 hours.
4. Harvest cells and assay as required.

Table 1. Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate
Surface area	1.9 cm ²	3.8 cm ²	9.6 cm ²
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 μl	100 μl	250 μl
DNA (1 μg/μl stock)	0.5 μl	1 μl	2.5 μl
TransIT-X2® Reagent	1.5 μl	3 μl	7.5 μl

▶ Transfection Optimization

Determine the best TransIT-X2®:DNA ratio for each cell type. Start with 3 μl of TransIT-X2® per 1 μg of DNA. Vary the concentration of TransIT-X2® from 2–6 μl per 1 μg DNA to find the optimal ratio.

For additional optimization tips, see [full protocol](#). Cell-type-specific recommendations available at [Reagent Agent: mirusbio.com/ra](http://mirusbio.com/ra)

TransIT-X2[®] Dynamic Delivery System

Quick Reference Protocol

siRNA Delivery Instructions for MIR 6000, 6003, 6004, 6005, 6006, 6010

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6000



SPECIFICATIONS

Storage	Store TransIT-X2 [®] Dynamic Delivery System tightly capped 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.

▶ TRANSFECTION PROTOCOL | siRNA



Full protocol and additional documentation available at mirusbio.com/6000

Fill in volumes below based on culture vessel used for transfection (Table 2).

A. Plate cells

1. Plate cells in ___ml complete growth medium (per well or flask) 18-24 hours prior to transfection to ensure cells are actively dividing at the time of transfection.

For adherent cells: Plate cells at a density of 0.8—3.0 x 10⁵ cells/ml.

For suspension cells: Plate cells at a density of 2.5—5.0 x 10⁵ cells/ml.

2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare TransIT-X2[®] Reagent:siRNA complexes

1. Warm TransIT-X2[®] Reagent to room temperature and vortex gently.
2. Place ___μl of OptiMEM[®] I Reduced-Serum Medium in a sterile tube.
3. Add ___μl TransIT-X2[®]. Mix gently by pipetting.
4. Add ___μl of a 10 μM siRNA stock solution (25 nM final concentration in well). Mix gently by pipetting.
5. Incubate at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add TransIT-X2[®]:siRNA complex mixture drop-wise to different areas of the well.
2. Gently rock plate for even distribution of complexes.
3. Incubate 24-72 hours.
4. Harvest cells and assay for knockdown of gene expression.

Table 2. Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate
Surface area	1.9 cm ²	3.8 cm ²	9.6 cm ²
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 μl	100 μl	250 μl
siRNA (10 μM stock, 25 nM final)	1.4 μl	2.8 μl	6.8 μl
TransIT-X2 [®] Reagent	1.5 μl	3 μl	7.5 μl

▶ Transfection Optimization

Determine the best TransIT-X2[®]:DNA ratio for each cell type. Start with 3 μl of TransIT-X2[®] per 1 μg of DNA. Vary the concentration of TransIT-X2[®] from 2–6 μl per 1 μg DNA to find the optimal ratio.

*For additional optimization tips, see [full protocol](#). Cell-type-specific recommendations available at **Reagent Agent:** mirusbio.com/ra*

TransIT-X2® Dynamic Delivery System



Quick Reference Protocol

DNA and siRNA Delivery Instructions for MIR 6000, 6003, 6004, 6005, 6006, 6010

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6000

SPECIFICATIONS

Storage	Store TransIT-X2® Dynamic Delivery System tightly capped 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.

▶ TRANSFECTION PROTOCOL | DNA & siRNA



Full protocol and additional documentation available at mirusbio.com/6000

Fill in volumes below based on culture vessel used for transfection (Table 3).

A. Plate cells

1. Plate cells in ___ ml complete growth medium (per well or flask) 18-24 hours prior to transfection to ensure cells are actively dividing at the time of transfection.

For adherent cells: Plate cells at a density of 0.8—3.0 x 10⁵ cells/ml.

For suspension cells: Plate cells at a density of 2.5—5.0 x 10⁵ cells/ml.

2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare TransIT-X2® Reagent:DNA:siRNA complexes

1. Warm TransIT-X2® Reagent to room temperature and vortex gently.
2. Place ___ μl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___ μl plasmid DNA. Mix gently by pipetting.
4. Add ___ μl of a 10 μM siRNA stock solution (25 nM final concentration in well). Mix gently by pipetting.
5. Add ___ μl TransIT-X2® Reagent. Mix gently by pipetting.
6. Incubate at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add co-transfection complex mixture drop-wise to different areas of the well.
2. Gently rock plate for even distribution of complexes.
3. Incubate 24-72 hours.
4. Harvest cells and assay as required.

Table 3. Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate
Surface area	1.9 cm ²	3.8 cm ²	9.6 cm ²
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 μl	100 μl	250 μl
DNA (1 μg/μl stock)	0.5 μl	1 μl	2.5 μl
siRNA (10 μM stock, 25 nM final)	1.4 μl	2.8 μl	6.8 μl
TransIT-X2® Reagent	1.5 μl	3 μl	7.5 μl

▶ Transfection Optimization

The amount of TransIT-X2® required for co-transfection is dictated by the amount of DNA. Determine the best TransIT-X2® Reagent:DNA ratio for each cell type. Start with 3 μl of TransIT-X2® per 1 μg of DNA. Vary the concentration of TransIT-X2® from 2–6 μl per 1 μg DNA to find the optimal ratio.

For additional optimization tips, see [full protocol](#). Cell-type-specific recommendations available at [Reagent Agent: mirusbio.com/ra](#)

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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: [mirusbio.com/ra](https://www.mirusbio.com/ra)

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