*Trans*IT-X2® Dynamic Delivery System

Quick Reference Protocol

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

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

SPECIFICATIONS

Storage	Store *Trans*IT-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 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5—5.0 x 10^5 cells/ml.
2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare *Trans*IT-X2®Reagent:DNA complexes

1. Warm *Trans*IT-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 *Trans*IT-X2®. Mix gently by pipetting.
5. Incubate at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add *Trans*IT-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
***Trans*IT-X2® Reagent**	1.5 µl	3 µl	7.5 µl

▸ Transfection Optimization

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 concentration of *Trans*IT-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*

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.MIRUSBIO (844.647.8724) | Direct: +1.608.441.2852

*Trans*IT-X2® Dynamic Delivery System

Quick Reference Protocol

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

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

SPECIFICATIONS

Storage	Store *Trans*IT-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 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5—5.0 x 10^5 cells/ml.
2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare *Trans*IT-X2®Reagent:siRNA complexes

1. Warm *Trans*IT-X2® Reagent to room temperature and vortex gently.
2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___µl *Trans*IT-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 *Trans*IT-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
***Trans*IT-X2® Reagent**	1.5 µl	3 µl	7.5 µl

▸ Transfection Optimization

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 concentration of *Trans*IT-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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*Trans*IT-X2® Dynamic Delivery System

Quick Reference Protocol

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

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

SPECIFICATIONS

Storage	Store *Trans*IT-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 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5—5.0 x 10^5 cells/ml.
2. Culture overnight. Most cell types should be ≥80% confluent at time of transfection.

B. Prepare *Trans*IT-X2®Reagent:DNA:siRNA complexes

1. Warm *Trans*IT-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 *Trans*IT-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
***Trans*IT-X2® Reagent**	1.5 µl	3 µl	7.5 µl

▸ Transfection Optimization

The amount of *Trans*IT-X2® required for co-transfection is dictated by the amount of DNA. Determine the best *Trans*IT-X2® Reagent:DNA ratio for each cell type. Start with 3 µl of *Trans*IT-X2® per 1 µg of DNA. Vary the concentration of *Trans*IT-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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NOTES

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

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