

TransIT-X2® Dynamic Delivery System for CRISPR/Cas9
Plasmid and gRNA Delivery



Instructions for use with MIR 6000, 6003, 6004, 6005, 6006, 6010

SPECIFICATIONS

Storage	Store TransIT-X2® Dynamic Delivery System tightly capped at –20°C. <i>Before each use</i> , warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

► CAS9 DNA + gRNA TRANSFECTION PROTOCOL

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

- A. Plate cells**
- 1. Approximately 18-24 hours before transfection, plate cells in ___ml complete growth medium. Most cell types should be ~80% confluent 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 cells overnight.
- B. Prepare TransIT-X2®:DNA:gRNA complexes (Immediately before transfection)**
- 1. Warm TransIT-X2® to room temperature and vortex gently before using.
 - 2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
 - 3. Add ___µl of a 50 µM guide RNA stock solution (50 nM final concentration per well). Mix gently by pipetting. NOTE: If using 2-part crRNA + tracrRNA, first combine at a 1:1 molar ratio and incubate for 10 minutes at room temperature to anneal. Then add to tube containing OptiMEM®.
 - 4. Add ___µl of plasmid DNA encoding Cas9. Mix gently by pipetting.
 - 5. Add ___µl TransIT-X2® to the diluted DNA/gRNA mixture. Mix gently by pipetting.
 - 6. Incubate at room temperature for 15-30 minutes.
- C. Distribute complexes to cells**
- 1. Add the TransIT-X2®:DNA:gRNA complexes (prepared in Step B) 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 cm²	3.8 cm²	9.6 cm²
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 µl	100 µl	250 µl
guide RNA (50 µM stock, 50 nM final)	0.5 µl	1 µl	2.5 µl
Cas9 Plasmid DNA (1 mg/ml stock)	0.5 µl	1 µl	2.5 µl
TransIT-X2® Reagent	1 µl	2 µl	5 µl

► Transfection Optimization:

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

For more on transfection optimization, see the TransIT-X2® [full protocol \(PDF\)](#) or [Tips from the Bench](#). Cell-type-specific recommendations are 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.

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