

TransIT-X2[®] Dynamic Delivery System for CRISPR/Cas9

Plasmid DNA 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. Before each use , warm to room temperature and vortex gently. |
| Product Guarantee | 1 year from the date of purchase, when properly stored and handled. |

► CRISPR PLASMID DNA 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 × 10⁵ cells/ml.

For suspension cells: Plate cells at a density of 2.5 - 5.0 × 10⁵ cells/ml.

2. Culture cells overnight.

B. Prepare TransIT-X2[®]:DNA complexes (immediately before transfection)

1. Warm TransIT-X2[®] to room temperature and vortex gently before using.
2. Place ___µl of Opti-MEM[®] I Reduced Serum Medium in a sterile tube.
3. Add ___µl of total DNA (combined plasmid DNA encoding Cas9 and/or guide RNA). Mix gently by pipetting.
4. Add ___µl TransIT-X2[®] to the diluted DNA mixture. Mix gently by pipetting.
5. Incubate at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add the TransIT-X2[®]:DNA complexes (prepared in Step B) drop-wise to different areas of the well.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the TransIT-X2[®]:DNA 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 |
| Plasmid DNA (Cas9 and/or guide RNA) | 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



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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