

# TransIT-X2<sup>®</sup> 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 <sup>®</sup> Dynamic Delivery System tightly capped at -20°C. <b>Before each use</b> , 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 × 10<sup>5</sup> cells/ml.

**For suspension cells:** Plate cells at a density of 2.5 - 5.0 × 10<sup>5</sup> cells/ml.

2. Culture cells overnight.

#### B. Prepare TransIT-X2<sup>®</sup>:DNA complexes (immediately before transfection)

1. Warm TransIT-X2<sup>®</sup> to room temperature and vortex gently before using.
2. Place \_\_\_µl of Opti-MEM<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup>: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<sup>®</sup>: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 <sup>2</sup>	3.8 cm <sup>2</sup>	9.6 cm <sup>2</sup>
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 <sup>®</sup> Reagent	1 µl	2 µl	5 µl

#### ► Transfection Optimization:

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

For more on transfection optimization, see the TransIT-X2<sup>®</sup> [full protocol \(PDF\)](#) or [Tips from the Bench](#). Cell-type-specific recommendations are available at **Reagent Agent:** [mirusbio.com/ra](http://mirusbio.com/ra)



**Reagent Agent<sup>®</sup>**

Reagent Agent<sup>®</sup> 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://mirusbio.com/ra)

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