# **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 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.

## ▶ 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  $0.8-3.0 \times 10^5$  cells/ml.

For suspension cells: Plate cells at a density of 2.5—5.0 x 10<sup>5</sup> 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  $\mu$ M 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

- 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 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 μΙ	100 μΙ	250 μΙ
guide RNA (50 μM stock, 50 nM final)	0.5 μΙ	1 μΙ	2.5 μΙ
Cas9 Plasmid DNA (1 mg/ml stock)	0.5 μΙ	1 μΙ	2.5 μΙ
TransIT-X2® Reagent	1 μΙ	2 μΙ	5 μΙ

#### ▶ Transfection Optimization:

Determine the best TransIT-X2\*:DNA ratio for each cell type. Start with 2  $\mu$ l of TransIT-X2\* per 1  $\mu$ g of DNA. Vary the concentration of TransIT-X2\* from 2-6  $\mu$ l per 1  $\mu$ g total DNA to find the optimal ratio.

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



 $Reagent\ Agent^*\ is\ an\ online\ tool\ designed\ to\ help\ determine\ the\ best\ solution\ for\ nucleic\ acid\ delivery\ based\ on\ in-house\ data,\ customer\ feedback\ and\ citations.$ 

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