

TransIT-X2® Dynamic Delivery System for CRISPR/Cas9 Ribonucleoprotein (RNP) + DNA Oligo (ssODN) 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 RIBONUCLEOPROTEIN (RNP) + ssODN 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 adherent 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 $2.5-5.0 \times 10^5$ cells/ml.

2. Culture overnight.

B. Prepare TransIT-X2®:RNP 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 (12 nM final concentration per well). Mix gently by pipetting. NOTE: If using 2-part crRNA + tracrRNA, 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 a 30 µM Cas9 protein stock solution (6 nM final concentration per well). Mix gently by pipetting.
5. Incubate at room temperature for 10 minutes.
6. Add ___µl ssODN (12 nM final concentration per well) to tube containing RNP mixture.
7. Immediately add ___µl TransIT-X2® to the RNP:ssODN mixture. Mix gently by pipetting.
8. Incubate at room temperature for 15 minutes.

C. Distribute complexes to cells

1. Add the TransIT-X2®:RNP:ssODN complexes (prepared in Step B) drop-wise to different areas of the well. Gently rock plate for even distribution of complexes.
2. Incubate 24-72 hours.
3. 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
guide RNA (50 µM stock, 12 nM final in well)	0.12 µl	0.24 µl	0.6 µl
Cas9 Protein (30 µM stock, 6 nM final in well)	0.1 µl	0.2 µl	0.5 µl
ssODN (50 µM stock, 12 nM final in well)	0.12 µl	0.24 µl	0.6 µl
TransIT-X2® Reagent	1 µl	2 µl	5 µl

► Transfection Optimization:

The 2:1:2 ratio of guide RNA to Cas9 protein to ssODN (12 nM gRNA:6 nM Cas9:12 nM ssODN; final concentration per well) used in this protocol is a starting point for RNP + DNA oligo transfection. Further ratio optimization may be required for some cell types.

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

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