*Trans*IT-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 *Trans*IT-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 x 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5—5.0 x 10^5 cells/ml.
2. Culture cells overnight.

B. Prepare *Trans*IT-X2®:DNA:gRNA complexes (Immediately before transfection)

1. Warm *Trans*IT-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 *Trans*IT-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 *Trans*IT-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 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, 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
***Trans*IT-X2® Reagent**	1 µl	2 µl	5 µl

▸ Transfection Optimization:

Determine the best *Trans*IT-X2®:DNA ratio for each cell type. Start with 2 µl of *Trans*-IT-X2® per 1 µg of DNA. Vary the concentration of *Trans*IT-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*

Mirus Bio LLC
www.mirusbio.com | techsupport@mirusbio.com | Toll Free (U.S.): 844.MIRUSBIO | Direct: +1.608.441.2852

NOTES

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

Rev.A 051617