*Trans*IT-X2® Dynamic Delivery System for CRISPR/Cas9 Plasmid DNA 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.

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 × 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5 - 5.0 × 10^5 cells/ml.
2. Culture cells overnight.

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

1. Warm *Trans*IT-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 *Trans*IT-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 *Trans*IT-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 *Trans*IT-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
***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*

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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

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