*Trans*IT-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 *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 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 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 overnight.

B. Prepare *Trans*IT-X2®:RNP 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 (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.
6. Immediately add ___µl *Trans*IT-X2® to the RNP:ssODN mixture. Mix gently by pipetting.
7. Incubate at room temperature for 15 minutes.

C. Distribute complexes to cells

1. Add the *Trans*IT-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, 24 nM final in well)	0.12 µl	0.24 µl	0.6 µl
***Trans*IT-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.

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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