*Trans*IT®-mRNA Transfection Kit for CRISPR/Cas9 mRNA and gRNA Delivery

Instructions for use with MIR 2225, 2250, 2251, 2255, 2256

SPECIFICATIONS

Storage	Store both *Trans*IT®-mRNA Reagent and mRNA Boost Reagent 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 mRNA + 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 (per well). Most cell types should be ~80% confluent on day 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®-mRNA Reagent:mRNA Boost:RNA complexes

1. Warm *Trans*IT®-mRNA and Boost Reagent to room temperature and vortex gently.
2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___µl of guide RNA (50 uM 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 mRNA encoding Cas9. Mix gently by pipetting.
5. Add ___µl of mRNA Boost Reagent. Mix gently by pipetting.
5. Add ___µl *Trans*IT®-mRNA Transfection Reagent. Mix gently by pipetting.
6. Incubate at room temperature for 2-5 minutes to allow complexes to form.

C. Distribute transfection complexes to cells

1. Add the complexes (prepared in Step B) drop-wise to different areas of the wells.
2. Gently rock the culture vessel to evenly distribute the complexes.
3. Incubate 24-72 hours. NOTE: A post-transfection media exchange is not necessary.
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 mRNA (1 mg/ml stock)	0.5 µl	1 µl	2.5 µl
mRNA Boost Reagent	0.5 µl	1 µl	2.5 µl
***Trans*IT®-mRNA Transfection Reagent**	0.5 µl	1 µl	2.5 µl

▸ Transfection Optimization:

Cell type, cell confluency, reagent volume, and post-transfection incubation time are a few of the key parameters that affect the outcome of transfection experiments. For more on transfection optimization, see the Trans-IT®-mRNA full protocol (PDF) or Tips from the Bench.

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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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