*Trans*IT®-mRNA Transfection Kit

Quick Reference Protocol

Instructions for MIR 2225, 2250, 2255, 2256

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/2250

SPECIFICATIONS

Storage	Store both *Trans*IT®-mRNA Reagent and mRNA Boost Reagent tightly capped at 4°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.

RNA TRANSFECTION PROTOCOL

Full protocol and additional documentation available at ***mirusbio.com/2250***

Fill in volumes below based on culture vessel used for transfection (Table 1).

A. Plate cells

1. Plate cells in ___ml complete growth medium (per well).
   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. Most cell types should be ≥80% confluent on day of transfection.

B. Prepare *Trans*IT®-mRNA Reagent:mRNA Boost:RNA complexes

1. Warm *Trans*IT®-mRNA and mRNA Boost Reagents to room temperature and vortex gently.
2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___µl RNA. Mix gently by pipetting.
4. Add ___µl of mRNA Boost Reagent. Mix gently by pipetting.
5. Add ___µl of *Trans*IT®-mRNA Reagent. Mix gently by pipetting.
6. Incubate at room temperature for **2-5 minutes**.

C. Distribute complexes to cells

1. Add *Trans*IT®-mRNA Reagent:mRNA Boost:RNA complex mixture drop-wise to different areas of the well.
2. Gently rock plate for even distribution of complexes.
3. Incubate 4-48 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
RNA (1 µg/µl stock)	0.5 µl	1 µl	2.5 µl
***Trans*IT®-mRNA Reagent**	1 µl	2 µl	5 µl
mRNA Boost Reagent	1 µl	2 µl	5 µl

Transfection Optimization

Determine the best *Trans*IT®-mRNA:RNA and mRNA Boost:RNA ratio for each cell type. Start with 2 µl of *Trans*IT®-mRNA Reagent per 1 µg of RNA. Vary the amount of *Trans*IT®-mRNA Reagent from 1–3 µl per 1 µg RNA to find the optimal ratio. Vary the amount of mRNA Boost Reagent from 1–3 µl per 1 µg of RNA.

For additional optimization tips, see full protocol.

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.C 0917