

TransIT®-mRNA Transfection Kit

Quick Reference Protocol

Instructions for MIR 2225, 2250, 2251, 2255, 2256
Full protocol, SDS and Certificate of Analysis available at mirusbio.com/2250



SPECIFICATIONS

Storage	Store both TransIT®-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 $0.8 - 3.0 \times 10^5$ cells/ml.
For suspension cells: Plate cells at a density of $2.5 - 5.0 \times 10^5$ cells/ml.
 - 2. Culture overnight. Most cell types should be $\geq 80\%$ confluent on day of transfection.
- B. Prepare TransIT®-mRNA Reagent:mRNA Boost:RNA complexes**
- 1. Warm TransIT®-mRNA and mRNA Boost Reagents to room temperature and vortex gently.
 - 2. Place ___µl of Opti-MEM® 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 TransIT®-mRNA Reagent. Mix gently by pipetting.
 - 6. Incubate at room temperature for **2-5 minutes**.
- C. Distribute complexes to cells**
- 1. Add TransIT®-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
TransIT®-mRNA Reagent	1 µl	2 µl	5 µl
mRNA Boost Reagent	1 µl	2 µl	5 µl

► Transfection Optimization

Determine the best TransIT®-mRNA:RNA and mRNA Boost:RNA ratio for each cell type. Start with 2 µl of TransIT®-mRNA Reagent per 1 µg of RNA. Vary the amount of TransIT®-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](#).



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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