

TransIT®-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 <i>TransIT</i> ®-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 10⁵ cells/ml.
For suspension cells: Plate cells at a density of 2.5—5.0 x 10⁵ cells/ml.
2. Culture overnight. Most cell types should be ≥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 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 *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 |
| <i>TransIT</i> ®-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](#).

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