

TransIT®-mRNA Transfection Kit for CRISPR/Cas9 mRNA and gRNA Delivery



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

SPECIFICATIONS

Storage	Store both TransIT®-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 $\sim 80\%$ confluent on day of transfection.

For adherent cells: Plate cells at a density of $0.8\text{--}3.0 \times 10^5$ cells/ml.

For suspension cells: Plate cells at a density of $2.5\text{--}5.0 \times 10^5$ cells/ml.

2. Culture cells overnight.

B. Prepare TransIT®-mRNA Reagent:mRNA Boost:RNA complexes

1. Warm TransIT®-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 μM 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 TransIT®-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
TransIT®-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 TransIT®-mRNA [full protocol \(PDF\)](#) or [Tips from the Bench](#).

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