

# 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 $-20^{\circ}\text{C}$ . <b>Before each use</b> , 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 \_\_\_ $\mu\text{l}$  of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add \_\_\_ $\mu\text{l}$  of guide RNA (50  $\mu\text{M}$  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 \_\_\_ $\mu\text{l}$  of mRNA encoding Cas9. Mix gently by pipetting.
5. Add \_\_\_ $\mu\text{l}$  of mRNA Boost Reagent. Mix gently by pipetting.
5. Add \_\_\_ $\mu\text{l}$  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 $\text{cm}^2$	3.8 $\text{cm}^2$	9.6 $\text{cm}^2$
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 $\mu\text{l}$	100 $\mu\text{l}$	250 $\mu\text{l}$
guide RNA (50 $\mu\text{M}$ stock, 50 nM final)	0.5 $\mu\text{l}$	1 $\mu\text{l}$	2.5 $\mu\text{l}$
Cas9 mRNA (1 mg/ml stock)	0.5 $\mu\text{l}$	1 $\mu\text{l}$	2.5 $\mu\text{l}$
mRNA Boost Reagent	0.5 $\mu\text{l}$	1 $\mu\text{l}$	2.5 $\mu\text{l}$
TransIT®-mRNA Transfection Reagent	0.5 $\mu\text{l}$	1 $\mu\text{l}$	2.5 $\mu\text{l}$

#### ► 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](#).

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | Toll Free (U.S.): 844.MIRUSBIO | Direct: +1.608.441.2852



**Reagent Agent<sup>®</sup>**

Reagent Agent<sup>®</sup> 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](https://www.mirusbio.com/ra)

©1996-2017. All rights reserved Mirus Bio LLC. For terms and conditions, visit [www.mirusbio.com](https://www.mirusbio.com).  
Rev.A 051617

---

**Mirus Bio LLC**

[www.mirusbio.com](https://www.mirusbio.com) | [techsupport@mirusbio.com](mailto:techsupport@mirusbio.com) | Toll Free (U.S.): 844.MIRUSBIO | Direct: +1.608.441.2852