# TransIT-TKO® Transfection Reagent

Quick Reference Protocol

Instructions for MIR 2150, 2154, 2155, 2156
Full protocol, SDS and Certificate of Analysis available at mirusbio.com/2150



#### **SPECIFICATIONS**

Storage	Store TransIT-TKO® 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.	

#### ▶ siRNA TRANSFECTION PROTOCOL



Full protocol and additional documentation available at *mirusbio.com/2150* 

### 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 x 10<sup>5</sup> cells/ml.
- 2. Culture overnight. Most cell types should be ≥80% confluent on day of transfection.

### B. Prepare TransIT-TKO® Reagent:siRNA complexes

- 1. Warm TransIT-TKO® to room temperature and vortex gently.
- 2. Place µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
- 3. Add µl TransIT-TKO® Reagent. Mix gently by pipetting.
- Add \_\_\_\_µl of a 10 µM siRNA stock solution (25 nM final concentration). Mix gently by pipetting.
- 5. Incubate at room temperature for 15-30 minutes.

#### C. Distribute complexes to cells

- Add TransIT-TKO® Reagent:siRNA complex mixture drop-wise to different areas of the well.
- 2. Gently rock plate for even distribution of complexes.
- 3. Incubate 24-72 hours.
- 4. Harvest cells and assay for knockdown of gene expression.

Table 1. Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate	
Surface area	1.9 cm <sup>2</sup>	3.8 cm <sup>2</sup>	9.6 cm <sup>2</sup>	
Complete growth medium	0.5 ml	1 ml	2.5 ml	
Serum-free medium	50 μl	100 μΙ	250 μΙ	
TransIT-TKO® Reagent	2.5 μΙ	5 μΙ	10 μΙ	
siRNA (10 μM stock, 25 nM final)	1.4 μΙ	2.8 μΙ	6.8 µl	

## **▶** Transfection Optimization

Determine the best volume of TransIT-TKO® for each cell type. Start with 10  $\mu$ I of TransIT-TKO® per well of a 6-well plate. For further optimization, vary the amount from 8-12  $\mu$ I per well to find the optimal volume.

For more tips and instructions for co-transfection, see <u>full</u> <u>protocol</u>. Cell-type-specific recommendations available at: **Reagent Agent**: mirusbio.com/ra



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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