

# TransIT-siQUEST® Transfection Reagent

## Quick Reference Protocol

Instructions for MIR 2110, 2111, 2114, 2115, 2116

Full protocol, SDS and Certificate of Analysis available at [mirusbio.com/2110](http://mirusbio.com/2110)



### SPECIFICATIONS

Storage	Store TransIT-siQUEST® Reagent tightly capped at 4°C. <i>Before each use</i> , 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/2110](http://mirusbio.com/2110)

### 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  $0.8\text{-}3.0 \times 10^5$  cells/ml.

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

2. Culture overnight. Most cell types should be  $\geq 80\%$  confluent on day of transfection.

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

1. Warm TransIT-siQUEST® to room temperature and vortex gently.

2. Place \_\_\_  $\mu\text{l}$  of Opti-MEM® I Reduced-Serum Medium in a sterile tube.

3. Add \_\_\_  $\mu\text{l}$  TransIT-siQUEST® Reagent. Mix gently by pipetting.

4. Add \_\_\_  $\mu\text{l}$  of a 10  $\mu\text{M}$  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

1. Add TransIT-siQUEST® 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 $\mu\text{l}$	100 $\mu\text{l}$	250 $\mu\text{l}$
TransIT-siQUEST® Reagent	1.5 $\mu\text{l}$	3 $\mu\text{l}$	7.5 $\mu\text{l}$
siRNA (10 $\mu\text{M}$ stock, 25 nM final)	1.4 $\mu\text{l}$	2.8 $\mu\text{l}$	6.8 $\mu\text{l}$

### ► Transfection Optimization

Determine the best volume of TransIT-siQUEST® for each cell type. Start with 7.5  $\mu\text{l}$  of TransIT-siQUEST per well of a 6-well plate. For further optimization, vary the amount from 5-10  $\mu\text{l}$  per well to find the optimal volume.

For additional optimization tips, see [full protocol](#). Cell-type-specific recommendations available at [Reagent Agent: mirusbio.com/ra](http://Reagent Agent: mirusbio.com/ra)

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## 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)

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