

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



SPECIFICATIONS

Storage	Store TransIT-siQUEST® 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/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 ___ μ l of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___ μ l TransIT-siQUEST® Reagent. Mix gently by pipetting.
4. Add ___ μ l of a 10 μ 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 ²	3.8 cm ²	9.6 cm ²
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 μ l	100 μ l	250 μ l
TransIT-siQUEST® Reagent	1.5 μ l	3 μ l	7.5 μ l
siRNA (10 μ M stock, 25 nM final)	1.4 μ l	2.8 μ l	6.8 μ l

▶ Transfection Optimization

Determine the best volume of TransIT-siQUEST® for each cell type. Start with 7.5 μ l of TransIT-siQUEST per well of a 6-well plate. For further optimization, vary the amount from 5-10 μ 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

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Reagent Agent[®]

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.

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