

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 ___ μl of Opti-MEM® I Reduced-Serum Medium in a sterile tube.

3. Add ___ μl TransIT-siQUEST® Reagent. Mix gently by pipetting.

4. 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

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 μl	100 μl	250 μl
TransIT-siQUEST® Reagent	1.5 μl	3 μl	7.5 μl
siRNA (10 μM stock, 25 nM final)	1.4 μl	2.8 μl	6.8 μl

► Transfection Optimization

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