# *Trans*IT-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 *Trans*IT-siQUEST® Reagent tightly capped at 4°C.<br>***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 0.8-3.0 × $10^5$ cells/ml.
   ***For suspension cells***: Plate cells at a density of 2.5-5.0 × $10^5$ cells/ml.
2. Culture overnight. Most cell types should be ≥ 80% confluent on day of transfection.

**B. Prepare *Trans*IT-siQUEST® Reagent:siRNA complexes**

1. Warm *Trans*IT-siQUEST® to room temperature and vortex gently.
2. Place ___µl of Opti-MEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___µl *Trans*IT-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 *Trans*IT-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 |
| ***Trans*IT-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 *Trans*IT-siQUEST® for each cell type. Start with 7.5 µl of *Trans*IT-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*

**Mirus Bio LLC**

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.MIRUSBIO (844.647.8724) | Direct: +1.608.441.2852

## NOTES

## 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.

Learn more at: mirusbio.com/ra



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