Optimizing Plasmid DNA Delivery with *Trans*IT® Transfection Reagents

Quick Reference Protocol for DNA Transfection Optimization in a 12-well Plate Format

OPTIMIZATION PROTOCOL FOR PLASMID DNA DELIVERY

The following protocol describes a Reagent-to-DNA titration designed to determine the optimal DNA transfection conditions for any cell type using Mirus Bio Broad Spectrum and Cell Type Specific Reagents. NOTE: Additional optimization is required for Mirus Transfection Kits containing multiple reagents.

A. Plate cells

1. Plate cells in 1 ml complete growth medium per well in a 12-well plate.
   For adherent cells: Plate cells at a density of 0.8 - 3.0 x 10^5 cells/ml.
   For suspension cells: Plate cells at a density of 2.5 - 5.0 x 10^5 cells/ml.
2. Culture overnight. Most adherent cell types should be 75-90% confluent on day of transfection.

B. Prepare *Trans*IT® Reagent:DNA complexes

1. Warm *Trans*IT® Reagent to room temperature and vortex gently.
2. Prepare transfection complex Master Mixes in sterile tubes by combining OptiMEM® I Reduced-Serum Medium, plasmid DNA and *Trans*IT® Reagent according to Table 1. After each component addition, mix thoroughly by pipetting or brief vortexing.

Table 1. Recommended volumes for Reagent:DNA titration

Master Mix	Test Wells	Reagent:DNA Ratio	Serum-free Medium	*Trans*IT® Reagent	DNA (1 µg/µl stock)
1	A1, A2	2:1	220 µl	4.4 µl	2.2 µl
2	A3, A4	3:1	217.8 µl	6.6 µl	2.2 µl
3	B1, B2	4:1	215.6 µl	8.8 µl	2.2 µl
4	B3, B4	5:1	213.4 µl	11 µl	2.2 µl

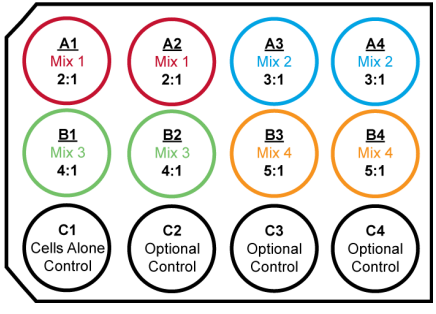
NOTE: Master mixes contain a 10% surplus volume for each component to reduce pipetting error and ensure that equivalent volumes can be transferred to replicate wells.

3. Incubate complexes at room temperature for 15-30 minutes.

C. Distribute complexes to cells

1. Add 103 µl *Trans*IT® Reagent:DNA complex mixtures drop-wise to different areas of the wells according to Figure 1 (at right).
2. Gently rock plate for even distribution of complexes.
3. Incubate 24-72 hours at 37°C, 5% CO_2.
4. Harvest cells and assay as required.

Figure 1. Recommended Reagent:DNA titration conditions



NOTE: It is important to include an untransfected Cells Alone Control (C1) to assess cell health following transfection. Additional controls to consider include: Reagent Alone, DNA Alone and Medium Only.

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ADDITIONAL FACTORS THAT INFLUENCE TRANSFECTION EFFICIENCY

Cell Passage Number: Cellular behavior and response varies with passage number. Maintain a similar cell passage number between experiments to ensure reproducibility. A low passage number can make cells more sensitive to transfection whereas a high passage number can render cells refractory to transfection.

Cell Confluency: Use healthy, actively dividing cells to maximize transfection efficiency. We recommend plating cells the night before transfection at a density that will promote cell division and obtain 75-90% confluency for transfection the following day.

DNA Quality: Use only high quality, endotoxin-free DNA for transfections. Contaminants such as protein, carbohydrate and lipids may affect transfection efficiency and gene expression levels. Ensure that the plasmid preparation exhibits an A260/A280 ratio of > 1.8.

DNA Quantity: For suspension cells such as THP-1, higher expression is observed when using higher amounts of DNA per well (e.g. 2 µg of plasmid DNA per well of a 12-well plate would yield higher expression compared to 1 µg of plasmid DNA per well). When using higher amounts of DNA per well, increase the volume of transfection reagent proportionately to maintain the optimal Reagent:DNA ratio. For example, add 6 µl of *Trans*IT® Reagent when using 2 µg DNA per well of a 12-well tissue culture plate.

Complex Formation Time: After mixing the DNA and transfection reagent, incubate to form complexes for 15-30 minutes at room temperature before adding the mix to your cells. Transfection efficiency will decrease if the complex formation time exceeds an hour. In some cell types, shorter complex formation times (e.g. 10 minutes) are advantageous for transfection.

Post-transfection Incubation Time: Depending on the transgene being expressed and the experimental design, post-transfection incubation time can have a dramatic effect on experimental outcome. Protein expression is typically detectable as early as 4 hours post-transfection and can persist for many days. In general, maximal protein expression occurs 48 hours post-transfection. The time point for optimal gene expression can be determined by varying post-transfection incubation times from 4 to 72 hours.

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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