QIAGEN Supplementary Protocol

Guidelines for co-transfection of adherent cells with siRNA and plasmid DNA using TransMessenger® Transfection Reagent

The following procedure is for co-transfection of adherent cells using siRNA and plasmid DNA in one well of a 24-well plate. This procedure is provided as a starting point for optimization of siRNA and plasmid DNA co-transfection in mammalian cells using TransMessenger Transfection Reagent.

Protocols and handbooks for Transfection Reagents available from QIAGEN can be downloaded from the Transfection Tools website — www.qiagen.com/transfectiontools/.

For detailed information about TransMessenger Transfection Reagent, and for general guidelines about RNA transfection, please read the *TransMessenger Transfection Reagent Handbook* before beginning this procedure.

IMPORTANT: Please consult the Safety Information section in the *TransMessenger Transfection Reagent Handbook* before beginning this procedure.

Procedure

1. **The day before transfection, seed 0.5–1 x 10^5 cells (depending on the cell type) per well of a 24-well plate in 0.5 ml appropriate growth medium containing serum and antibiotics.**

   Make sure that cells are in good condition and are seeded 24 h before transfection. Cells should be 50–80% confluent on the day of transfection.

2. **Incubate cells under their normal growth conditions (typically 37°C and 5% CO_2).**

3. **On the day of transfection, condense the siRNA and plasmid DNA by combining the reagents in the following order. First dilute 2.4 µl Enhancer R in the appropriate volume of Buffer EC. Add 0.1 µg siRNA and 0.2 µg plasmid DNA, and mix by vortexing. The final volume should be 100 µl.**

   Note: Since a quantity of less than 0.1 µg siRNA may be sufficient for silencing RNA transcribed from the co-transfected plasmid DNA, the optimal amount of siRNA to use in this procedure should be determined by performing a titration experiment.

   IMPORTANT: Always mix Enhancer R with Buffer EC before adding siRNA and plasmid DNA. The ratio of total nucleic acids to Enhancer R should be 1:8.

4. **Incubate at room temperature (15–25°C) for 2–5 min, and then centrifuge the mixture for a few seconds to collect drops from the top of the tube.**

5. **Add 2.5 µl TransMessenger Transfection Reagent to the siRNA-plasmid DNA-Enhancer R mixture. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   IMPORTANT: The amount of TransMessenger Reagent required for optimal performance may depend on the cell line and the amounts of plasmid DNA and siRNA used. For specific cell types and targets, optimal conditions could be different from those described here.

6. **Incubate the samples for 10 min at room temperature to allow transfection-complex formation.**

7. **While complex formation is taking place, gently aspirate the growth medium from the plate, and carefully wash cells once with sterile PBS using 1.5–2 times the volume of media used for cell seeding.**

   IMPORTANT: Do not allow the cells to become dry. Minimize the amount of time they are without medium.

8. **Add 100 µl cell growth medium (without serum or antibiotics) to the tube containing the nucleic acid–TransMessenger Reagent complexes. Mix by pipetting up and down twice, then immediately add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**

   Note: The use of media without serum or antibiotics avoids the potential introduction of RNases. It is possible that transfection in the presence of serum may give improved results. Double-stranded siRNA is more resistant to degradation by RNases than single-stranded RNA. Therefore, if RNase contamination is not a concern or if your serum is validated to have no detectable RNase activity, medium containing serum can be used.

9. **Incubate cells with the transfection complexes for 3 h under their normal growth conditions.**

   Note: If no adverse effects are observed, the incubation time can be increased to 4h.

10. **Remove complexes from the cells, wash cells once with PBS, and then add 500 µl fresh growth medium containing serum and antibiotics to the cells.**

11. **Incubate cells under their normal growth conditions and monitor gene silencing after an appropriate incubation time (e.g., 24 h after transfection). Change the medium as required.**

    Note: The optimal time point for gene silencing analysis is dependent on cell type, the gene targeted, and method of analysis. A time course experiment should be performed to determine the appropriate incubation time.

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