QIAGEN Supplementary Protocol

Fast-Forward cotransfection of adherent cells with siRNA and DNA in 24-well plates using Attractene Transfection Reagent

The Fast-Forward Protocol is provided as a starting point for optimization of siRNA–DNA cotransfection of adherent cells in 24-well plates without preplating of cells 24 hours prior to transfection. The amounts given are for one well of a 24-well plate. For some sensitive cell lines, it may be necessary to use the Traditional Protocol where cells are plated the day before transfection (see page 4). The Traditional Protocol can be used for sensitive cell lines.

IMPORTANT: Please read the handbook supplied with Attractene Transfection Reagent, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning these procedures. Attractene Transfection Reagent is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Culture medium
- DNA and siRNA of interest (predesigned siRNA for every human, mouse, and rat gene is available at www.qiagen.com/GeneGlobe)
- Cells of interest

Important points before starting

- To achieve optimal transfection for a given cell line and DNA–siRNA combination, the amount of DNA and Attractene Transfection Reagent should first be optimized. Table 1 shows a pipetting scheme for optimizing DNA transfection of cells in the presence of siRNA in 24-well plates. As a starting point, we recommend using 0.4 µg DNA, 6 pmol nonfunctional, negative control siRNA, and 1.5 µl reagent (in bold in Table 1).
- After optimization using the scheme shown in Table 1, transfect 6 pmol of a functional siRNA, instead of the nonfunctional, negative control siRNA, in combination with the DNA and Attractene Reagent amounts selected and test for knockdown efficiency after an appropriate incubation time.

Table 1. Pipetting scheme for optimizing DNA–siRNA cotransfection in one well of a 24-well plate

Amount of DNA	0.2 µg	0.2 µg	0.2 µg
Amount of negative control siRNA	6 pmol	6 pmol	6 pmol
Volume of Attractene Reagent	0.5 µl	0.75 µl	1.5 µl
Amount of DNA	0.4 µg	**0.4 µg**	0.4 µg
Amount of negative control siRNA	6 pmol	**6 pmol**	6 pmol
Volume of Attractene Reagent	1 µl	**1.5 µl**	3 µl
Amount of DNA	0.6 µg	0.6 µg	0.6 µg
Amount of negative control siRNA	6 pmol	6 pmol	6 pmol
Volume of Attractene Reagent	1.5 µl	2.25 µl	4.5 µl

The recommended starting point for transfection is shown in bold.

- It may be necessary to increase the amount of functional siRNA (e.g., to 12 pmol) to achieve significant knockdown (≥70% knockdown). In this case, the amount of negative control siRNA in the negative control experiment should also be increased by the same amount.
- Using an increased amount of siRNA may require an increase in the volume of Attractene Reagent to achieve optimal transfection. To determine the optimal volume, titrate using the pipetting scheme shown in Table 1, but using the higher negative control siRNA amount (e.g., 12 pmol negative control siRNA instead of 6 pmol). Finally, proceed with the experiment using the optimized amounts of functional siRNA and reagent.

Procedure

1. **Dilute 0.4 µg DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: 0.1 µg/µl) with medium without serum, proteins, or antibiotics, to a total volume of 60 µl.**

   For example, if the DNA concentration is 1 µg/µl, dilute 0.4 µl DNA in 59.6 µl medium.

2. **Add 6 pmol siRNA to the diluted DNA. This will give a final siRNA concentration of 10 nM after adding complexes to cells in step 7.**

   For example, if the siRNA solution is 20 µM, add 0.28 µl to the diluted DNA.

3. **Add 1.5 µl Attractene Transfection Reagent. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**

4. **Incubate the samples for 10–15 min at room temperature (15–25°C) to allow transfection complex formation. Continue with steps 5 and 6 during the incubation time.**

Note: Transfection complex formation takes a minimum of 10–15 min. The transfection complexes will remain stable during the time it takes to prepare the cells for transfection. However, the incubation time should not be extended for longer than is necessary for cell preparation.

5. **Harvest the cells by trypsinization and suspend in culture medium containing serum and antibiotics.**

   Note: The cells should be healthy and in logarithmic growth phase.

   It is important that serum and antibiotics are present in the culture medium at this point because transfections are performed without changing the medium. The cultivation of cells over this time without serum would deprive the cells of essential growth factors. This does not apply to cells that are routinely cultivated without serum.

6. **Count the cells in the harvested cell suspension and adjust the cell density to 0.4–1.6 x 10^5 cells in 500 µl (depending on the cell line). The optimal cell density should be determined for each cell line.**

   Note: For example, cell density should be adjusted to 0.8–3.2 x 10^5 cells per ml for a final cell density at transfection of 0.4–1.6 x 10^5 cells in 500 µl.

   As plating and transfection of cells are carried out on the same day, higher cell densities are required than would be necessary if the cells had a longer incubation time prior to transfection. As a guideline, the required cell number is usually 2–3-fold higher than that used for the Traditional Protocol (where cells are plated on the day before transfection).

7. **Add 500 µl of the cell suspension to a well of a 24-well plate. Next, add the transfection complexes to the well. Mix by pipetting up and down twice.**

8. **Incubate the cells with the transfection complexes under their normal growth conditions (typically 37°C and 5% CO_2).**

   In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.

9. **Assay the knockdown of the transfected gene after an appropriate incubation time.** The length of the incubation time depends on the assay and the transfected gene.

Cotransfection of adherent cells with siRNA and DNA in 24-well plates using Attractene Transfection Reagent (Traditional Protocol)

This protocol is provided as a starting point for optimization of siRNA–DNA cotransfection of adherent cells in 24-well plates. In this protocol, cells are plated 24 hours prior to transfection. The amounts given are for one well of a 24-well plate. This protocol can be used for sensitive cell lines.

Procedure

1. **The day before transfection, seed 2–8 x 10^4 cells (depending on the cell type) in 500 µl of an appropriate culture medium containing serum and antibiotics.**
2. **Incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).**

   Cells should be 40–80% confluent on the day of transfection.
3. **On the day of transfection, dilute 0.4 µg DNA dissolved in TE buffer, pH 7–8 (minimum DNA concentration: 0.1 µg/µl) with medium without serum, proteins, or antibiotics, to a total volume of 60 µl.**
4. **Add 6 pmol siRNA to the diluted DNA. This will give a final siRNA concentration of 10 nM after adding complexes to cells in step 8.**

   For example, if the siRNA solution is 20 µM, add 0.28 µl to the diluted DNA.
5. **Add 1.5 µl Attractene Transfection Reagent to the DNA solution. Mix by pipetting up and down or vortexing. Centrifuge for a few seconds to remove any liquid from the top of the tube if necessary.**
6. **Incubate the samples for 10–15 min at room temperature (15–25°C) to allow complex formation.**
7. **While complex formation takes place, gently aspirate the medium from the cells and add 500 µl fresh medium (containing serum and antibiotics) to the cells.**
8. **Add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
9. **Incubate the cells with the transfection complexes under their normal growth conditions (typically 37°C and 5% CO_2).**

   In most cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the complexes after 6–18 hours and add fresh culture medium.
10. **Assay the knockdown of the transfected gene after an appropriate incubation time.** The length of the incubation time depends on the assay and the transfected gene.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature . Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx .

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