Quick-Start Protocol March 2016

HiPerFect® Transfection Reagent

The HiPerFect Transfection Reagent (cat. nos. 301702, 301704, 301705, 301707 and 301709) should be stored at 2–8°C upon arrival.

Further information

- *HiPerFect Transfection Reagent Handbook*: **www.qiagen.com/HB-0471**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- These protocols are for transfection of eukaryotic cells (adherent or suspension) with siRNA or miRNA in 24-well plates.
- Cells should be in optimal physiological condition at the time of transfection. The optimal number of seed cells depends on the cell type and time of analysis.
- Nucleic acid amounts in the protocol refer to siRNA. For appropriate amounts of miRNA mimic or miRNA inhibitor to transfect, refer to the manufacturer's instructions. Where possible, dilute miRNA mimic or miRNA inhibitor in the same volume as recommended for siRNA in the protocol.
- The amount of HiPerFect Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. Tables 1 and 2 provide suggestions for optimizing the ratio of siRNA to HiPerFect Transfection Reagent for adherent and suspension cells, respectively.
- For an up-to-date list of cell lines successfully transfected using HiPerFect Transfection Reagent, visit the Transfection Cell Database at **www.qiagen.com/TransfectionTools**.

Fast-forward transfection of adherent cells with siRNA or miRNA in 24-well plates

1. Shortly before transfection, seed 0.4–1.6 x 10^5 cells per well of a 24-well plate with 0.5 ml appropriate culture medium containing serum and antibiotics. Alternatively, seed cells after step 3 of this protocol.
2. For the short time until transfection, incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).
3. Dilute 37.5 ng siRNA in 100 µl culture medium without serum (this will give a final siRNA concentration of 5 nM after adding complexes to cells in step 5). Add 3 µl HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing.
4. Incubate for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.
5. Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.
6. Incubate the cells with the transfection complexes under their normal growth conditions, and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.

Table 1. Starting points for optimizing transfection of adherent cell lines in 24-well plates

Amount of siRNA	75 ng (10 nM)	75 ng (10 nM)	75 ng (10 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl
Amount of siRNA	37.5 ng (5 nM)	37.5 ng (5 nM)	37.5 ng (5 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl
Amount of siRNA	7.5 ng (1 nM)	7.5 ng (1 nM)	7.5 ng (1 nM)
Volume of HiPerFect Reagent	1.5 µl	3 µl	4.5 µl

Transfection of suspension cells with siRNA or miRNA in 24-well plates

Preparation of suspension cell lines, including Jurkat and K562

1. The day before transfection, dilute cells to a density of 3 x 10^5 per ml in appropriate culture medium containing serum and antibiotics in a spinner flask. Incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).
2. On the day of transfection, plate 2 x 10^5 cells per well of a 24-well plate with 100 µl culture medium containing serum and antibiotics.
3. Dilute 750 ng siRNA in 100 µl culture medium without serum (this will give a final siRNA concentration of 100 nM after adding medium in step 4, page 4). Continue with "Transfection of suspension cells", next page.

Preparation of macrophage cell lines, including J774.A1 and RAW 264.7

1. Shortly before transfection, seed 0.4–2 x 10^5 cells per well of a 24-well plate with 100 µl appropriate culture medium containing serum and antibiotics. Alternatively, seed cells after step 3 of this protocol.
2. For the short time until transfection, incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).
3. Dilute 375 ng siRNA in 100 µl culture medium without serum (this will give a final siRNA concentration of 50 nM after adding medium in step 4, page 4). Continue with "Transfection of suspension cells", next page.

Preparation of differentiated macrophage cell lines, including THP-1

1. Approximately 24 h before transfection, seed 2 x 10^4 cells per well of a 24-well plate with 0.5 ml appropriate culture medium containing serum and antibiotics. Add the appropriate amount of differentiation-inducing agent to the cells and incubate overnight under normal growth conditions (typically 37°C and 5% CO_2).

   Note: For differentiation of THP-1 cells, use 100 ng/ml PMA.

2. Shortly before transfection, remove the culture medium from the cells and add 100 µl fresh culture medium containing serum and antibiotics. For the short time until transfection, incubate the cells under normal growth conditions (typically 37°C and 5% CO_2).
3. Dilute 375 ng siRNA in 100 µl culture medium without serum (this will give a final siRNA concentration of 50 nM after adding medium in step 4, page 4). Continue with "Transfection of suspension cells".

Table 2. Starting points for optimizing transfection of suspension cells in 24-well plates

Amount of siRNA	750 ng (100 nM)	750 ng (100 nM)	750 ng (100 nM)
Volume of HiPerFect Reagent	3 µl	6 µl	9 µl
Amount of siRNA	375 ng (50 nM)	375 ng (50 nM)	375 ng (50 nM)
Volume of HiPerFect Reagent	3 µl	6 µl	9 µl
Amount of siRNA	187.5 ng (25 nM)	187.5 ng (25 nM)	187.5 ng (25 nM)
Volume of HiPerFect Reagent	3 µl	6 µl	9 µl

Transfection of suspension cells

1. Add 6 µl HiPerFect Transfection Reagent to the diluted siRNA and mix by vortexing. Incubate the samples for 5–10 min at room temperature (15–25°C) to allow the formation of transfection complexes.
2. Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.
3. Incubate the cells with the transfection complexes under their normal growth conditions for 6 h.
4. Add 400 µl culture medium containing serum and antibiotics to the cells and incubate until analysis of gene silencing (e.g., 6–72 h after transfection, depending on the experimental setup). Change the medium as required.

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