PROTOCOL

DharmaFECT™ reverse transfection of siRNA

Three methods for siRNA transfection

Successful gene silencing in siRNA-mediated RNA interference (RNAi) experiments requires efficient uptake of siRNA into the cells of interest. For *in vitro* experiments, transfection is an easy and rapid method of siRNA delivery. Three variations are currently being used: standard/forward transfection, Dharmacon™ Reverse Transfection Format (RTF) plates, and reverse transfection. They differ in the order and timing of the addition of the three necessary components of transfection: siRNA, lipid-based transfection reagent, and cells. In standard transfection, siRNA and lipid are complexed and added to pre-plated cells (Figure 1A). In RTF transfection, cells are added to pre-plated, dried siRNA that is rehydrated and complexed with lipid (Figure 1B). In reverse transfection, all three components are added to the wells at essentially the same time. (Figure 1C). Both RTF and reverse transfections reduce hands-on time for transfection from two days to only one. RTF plates offer the advantage of predispensed siRNAs, while reverse transfection offers the flexibility of testing siRNA reagents at various concentrations or utilizing existing siRNA library resources in a standard transfection format.

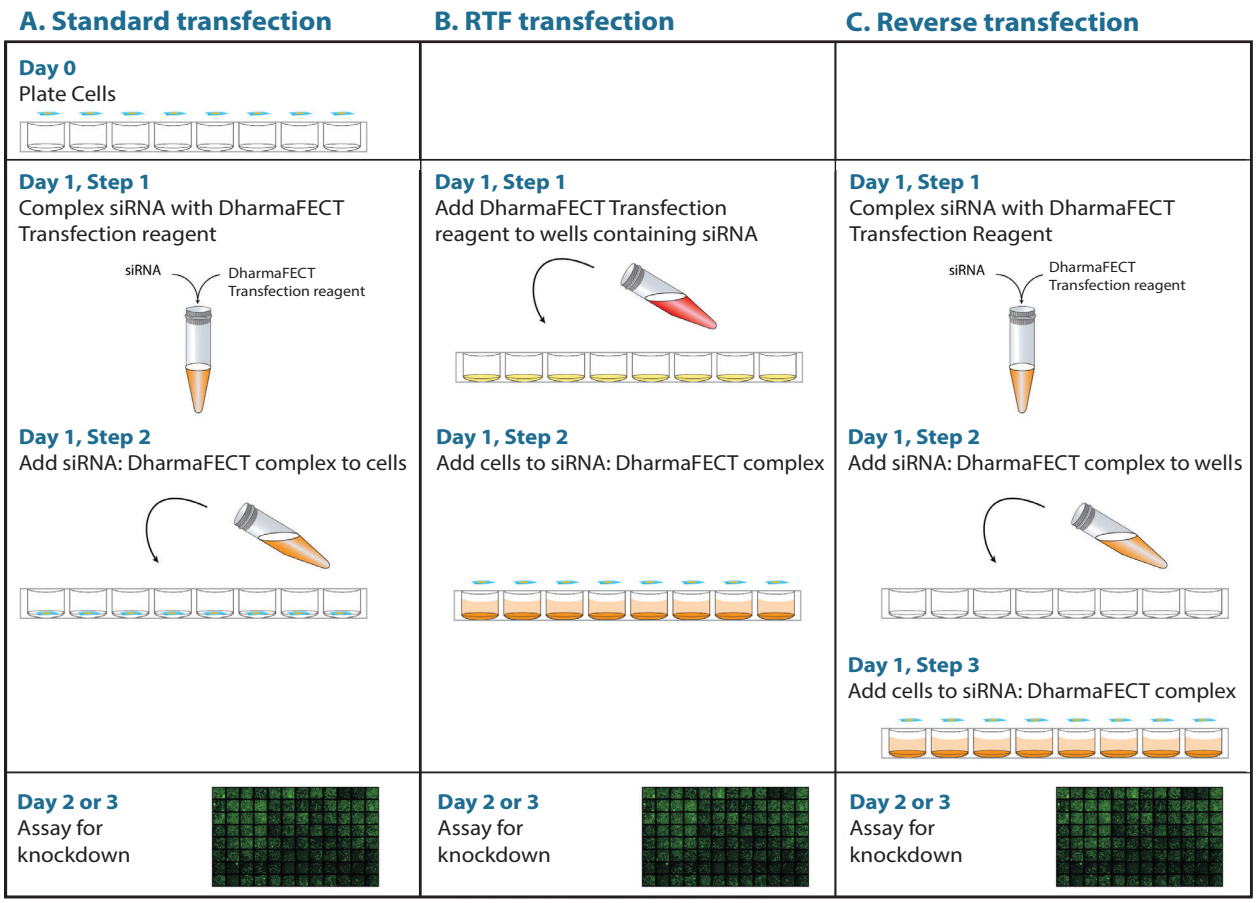

Figure 1. Three transfection methods

Figure 2 shows optimization data for reverse transfection of 1 × 10^4 HeLa cells with 50 nM siRNA in a 96-well plate format. A threshold of 80% cell viability and 75% gene silencing is assigned for successful, non-toxic transfection of siRNA. Other cells and plate formats may require additional optimization to result in good cell viability and gene silencing. Typical parameters that may be varied are cell number, choice of transfection reagent, and the volume of transfection reagent. Table 1 lists recommended conditions for HeLa, HepG2 and MCF7 cell lines.

Table 1. Final conditions for reverse transfection of siRNA in 96-well plate

Condition	HeLa	HepG2	MCF7
Cell number/well	1 × 10^4	1 × 10^4	1 × 10^4
DharmaFECT™ formulation	1	4	4
DharmaFECT volume/well	0.12 µL	0.12 µL	0.06 µL

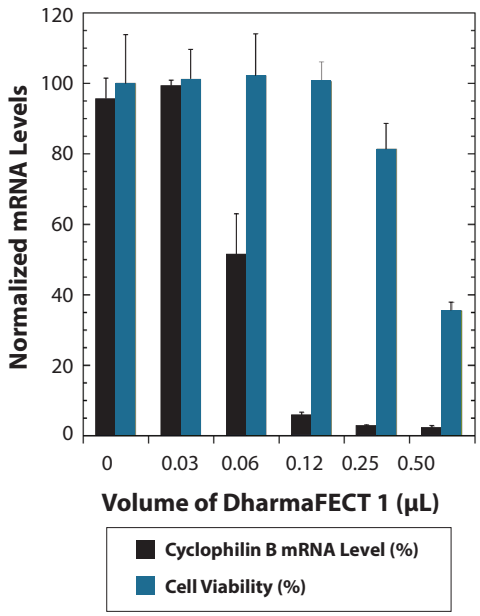

Figure 2. Reverse transfection optimization. HeLa cells (1 × 10^4 cells/well) were transfected with 50 nM siGENOME™ Cyclophilin B control siRNA (Cat #D-001136-01) and varying DharmaFECT 1 (Cat #T-2001) volumes (0.03-0.50 µL/well) in 96-well plates using the protocol described on the next page. Cyclophilin B mRNA level was measured using bDNA at 48 hours and expressed as percent of sample treated with siGENOME Non-targeting siRNA (Cat #D-001210-01). Cell viability was measured by Thermo Scientific™ alamarBlue™ assay at 48 hours and expressed as percent of untreated sample. All values are expressed as mean ± SD, n=3.

Reverse transfection protocol

It is recommended to include the control samples listed in Table 2 in every transfection experiment. All steps of the protocol should be performed in a laminar flow cell culture hood using sterile technique. Performing transfection experiments in triplicate is recommended to allow statistical analysis of the results. To account for normal loss during pipetting, all reagents should be prepared in excess.

Table 2. Recommended samples for siRNA transfection experiments

Samples	Purpose
Untreated	Determine baseline phenotype, target gene level, and cell viability
Negative Control siRNA	Distinguish sequence-specific silencing from non-specific affects
Positive Control siRNA	Measure efficiency of siRNA uptake into cells
Test siRNA	Knockdown of target gene

1. Prepare the following solutions: 1.1. Stock siRNA solutions (100 µM) in an RNase-free, pH 7.4-buffered solution. 1.2. Dilute DharmaFECT 1 by adding 1.8 µL DharmaFECT 1 in 373.2 µL serum-free and antibiotic-free cell culture medium. Total volume is 375 µL.
2. In three separate tubes, mix 100 µL diluted DharmaFECT 1 and 0.25 µL of the appropriate siRNA solutions (negative control, positive control and test siRNAs).
3. Aliquot 25 µL of the siRNA:DharmaFECT complex into triplicate wells in a 96-well plate.
4. Incubate plates for 30 minutes at room temperature.
5. Trypsinize HeLa cells and prepare a 1 × 10^5 cells/mL solution in antibiotic-free complete medium.
6. Add 0.1 mL cell solution to each well containing siRNA: Dharmacon DharmaFECT complex. The final volume is 125 µL/well.
7. Incubate cells at 37 °C in 5% CO_2 for 24-48 hours (for mRNA analysis) or 48-96 hours (for protein analysis).
8. If cell toxicity is observed after 24 hours, replace the transfection medium with complete medium and continue incubation. Cell viability may be determined with alamarBlue™, MTT, or other assays for metabolic activity. For best results, use samples that are at least 80% viable.

Materials

Transfection experiments require standard cell culture reagents and instruments appropriate for maintenance of the cells being used. Reagents for assaying cell viability and gene silencing are also needed. Table 3 list specific reagents required for the reverse transfection protocol described here. Assays for mRNA level, protein level, or other phenotypic change may be performed to assess silencing. Because RNAi is an mRNA-specific event, we highly recommend assaying for reduction at the mRNA level using reverse transcription quantitative real-time PCR (RT-qPCR), northern blot analysis, branched DNA, or other similar methods. Typical time points for detecting target knockdown with lipid-mediated siRNA delivery are 24–48 hours for mRNA and 48–96 hours for protein. Accell siRNA delivery is typically assessed at 72 hours. Time course studies are recommended to identify optimal time points for assessing knockdown of a specific gene of interest.

Table 3. Reagents for siRNA reverse transfection protocol

Reagents	Description and use
Cells	For best transfection efficiency, use cells in log-phase growth at passage number 20 or lower
Antibiotic-free complete medium	Medium in which the cells are normally maintained and may contain up to 20% serum, but does not contain antibiotics which may cause cell toxicity during transfection
Serum-free and antibiotic-free cell culture medium*	For dilution and optimal complexing of siRNA and DharmaFECT™
Transfection Reagent	DharmaFECT™ 1 is recommended for most cell types, but additional formulations are offered for optimization studies. See the recommended formulation for your cell type at dharmacon.horizondiscovery.com
Test siRNA	An siRNA that targets the gene of interest. Search for your gene at dharmacon.horizondiscovery.com
Negative Control siRNA	An siRNA that does not target any gene in the cells being used, to distinguish sequence-specific silencing from non-specific effects
Positive Control siRNA	A validated siRNA that targets an abundantly expressed housekeeping or reference gene, to measure efficiency of siRNA uptake into cells.

*Serum-free and antibiotic-free cell culture medium is recommended for preparation of transfection mix (for example, MEM-RS, HyClone Cat # SH30564).

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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