PROTOCOL

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DharmaFECT™ Duo transfection reagent—for siRNA and plasmid co-transfection

This protocol is optimized for use with 100 ng/well plasmid and 100 nM siRNA in a 96-well plate format. This protocol may also serve as guidelines for microRNA and plasmid co-transfection. Table 4 provides a range of DharmaFECT volumes for alternate plate formats. Plasmid concentration may need to be optimized as plasmid size and expression attributes affect optimal conditions. To reduce off-target effects, siRNA concentration may be titrated down once optimal delivery conditions are established.

It is recommended to include the samples listed in Table 1 in every transfection experiment. All steps of the protocol should be performed in a laminar flow cell culture hood using sterile technique. Performing each sample in triplicate wells is recommended to allow statistical analysis of the results.

Table 1. Recommended samples for plasmid/siRNA co-transfection experiment:

Samples		Purpose
Plasmid only (with DharmaFECT Duo)	[Tube 1a]	Confirm uptake and baseline expression level of plasmid
Plasmid and negative control siRNA (with DharmaFECT Duo)	[Tube 1b]	Distinguish sequence-specific silencing from non-specific effects
Plasmid and test siRNA (with DharmaFECT Duo)	[Tube 1c]	Achieve silencing of target gene and expression of plasmid
Mock-transfection (DharmaFECT Duo only)	[Tube 1d]	Identify nonspecific effects and cytotoxicity caused by the transfection reagent or procedure
Untreated (no DharmaFECT Duo)	[Tube 1d]	Determine baseline phenotype, target gene level, and cell viabilitye

Additional materials required

Transfection experiments require standard cell culture reagents and instruments appropriate for maintenance of cells. Reagents for assaying cell viability and gene silencing are also needed. Table 2 lists specific reagents required, in addition to DharmaFECT Duo

Table 2. Reagents for plasmid/siRNA co-transfection experiment

Reagents	Description and use
Cells	For best transfection efficiency, use cells in log-phase growth at a low passage number
Antibiotic-free complete medium	Medium in which the cells are cultured, which may contain up to 20% serum, but does notcontain antibiotics that may cause cell toxicity during transfection
Serum-free or low-serum medium	For optimal complexing of siRNA, plasmid and DharmaFECT Duo
Plasmid	A plasmid that expresses the desired gene
Test siRNA	siRNA that targets the gene to be silenced
Plasmid	A plasmid that expresses the desired gene
Negative control siRNA	An siRNA that does not target any gene expressed by the plasmid or any gene endogenously expressed by the cells being used, such as Dharmacon™ ON-TARGET*plus*™ Non-targeting siRNA

Cell plating

Optimal cell number for plating will vary with growth characteristics of specific cells and may need to be determined empirically.

1. Trypsinize and count cells.
2. Dilute cells in antibiotic-free complete medium to the appropriate stock density as described in Table 3.
3. Plate 0.1 mL cell suspension into each well of a 96-well plate.
4. Incubate cells at 37 °C with 5% CO_2 overnight.

Table 3. Recommended samples for plasmid/siRNA co-transfection experiment. The indicated number of cells was plated in 96-well plate format and incubated overnight as described in the protocol. The psiCHECK™-2 Vector (Promega, 100 ng/well) and Renilla luciferase-targeting siRNA (100 nM) were complexed with a range of DharmaFECT Duo volumes (0.05 - 0.6 µL/well). Firefly luciferase expression was assessed 48 hour post-transfection using the Dual-Glo™ Luciferase Assay System (Promega). Renilla™ Luciferase mRNA knockdown was assessed using branched DNA analysis (Panomics, Fremont, CA). Cell viability was assessed using the alamarBlue™ Assay (Thermo Fisher Scientific). Cell viability and silencing values are normalized to untreated cells.

Cell line	Final cell # per well	Stock cell density (cells/mL)	Volume of DharmaFECT Duo (µL)	Volume of serum-free medium (µL)	Final volume of DharmaFECT Duo (µL/well)	Cell viability (%)	Silencing (%)
ES-D3	2.0×10^4	2.0×10^5	4.2	135.8	0.3	87	97
HeLa	2.0×10^4	2.0×10^5	1.4	138.6	0.1	79	81
Hep G2	2.0×10^4	2.0×10^5	0.7	139.3	0.05	72	91
Jurkat	6.0×10^4	6.0×10^5	8.4	131.6	0.6	89	96
MCF7	1.0×10^4	1.0×10^5	2.8	137.2	0.2	80	99
MCF10a	1.0×10^4	1.0×10^5	2.8	137.2	0.2	78	98
NIH/3T3	1.0×10^4	1.0×10^5	5.6	134.4	0.4	83	97

Transfection

All volumes are multiplied by 3.5 to account for the triplicate samples and loss during pipetting.

1. Prepare stock plasmid (20 µg/mL) and siRNA (2 µM) solutions in an RNase-free, pH 7.4-buffered solution.
2. In tubes 1a–1c, mix plasmid and the appropriate siRNA solutions, if appropriate:
   a. Tube 1a (plasmid only)—Dilute 17.5 µL plasmid with 17.5 µL serum-free medium. The total volume is 35 µL.
   b. Tube 1b (plasmid and negative control siRNA) - Mix 17.5 µL plasmid with 17.5 µL negative control siRNA. The total volume is 35 µL.
   c. Tube 1c (plasmid and test siRNA)—Mix 17.5 µL plasmid with 17.5 µL test siRNA. The total volume is 35 µL.
   d. Tube 1d (lipid only)—Do not add plasmid or siRNA.
3. In tube 2, dilute sufficient DharmaFECT Duo in serum-free medium to give a total volume of 140 µL, as indicated in Table 3. This volume needs to be increased when more than one test siRNA is being used.
4. Mix the contents of all tubes gently by pipetting carefully up and down.
5. Incubate tubes for 5 minutes at room temperature.
6. Add 35 µL of Tube 2 content to Tube 1a–1d, bringing the total volume to 70 µL. Mix by pipetting carefully up and down.
7. Incubate for 20 minutes at room temperature.
8. Add 280 µL antibiotic-free complete medium to each mix in step 7. The total volume is 350 µL. These are the transfection media.
9. Remove medium from the wells of the 96-well plate containing cells and replace with 100 µL of the appropriate transfection medium to each well.
10. Incubate cells at 37° C in 5% CO_2 for 24 - 48 hours (for mRNA analysis) or 48–96 hours (for protein analysis).
11. 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 with at least 70% cell viability.

Table 4. Recommended volumes for transfecting 100 nM* siRNA in various plating formats. DharmaFECT volumes per well represent guidelines and may need to be optimized. Increasing volumes by 10% may help account for variances when pipetting liquids.

Plate format		Tube 1a volumes per well		Tube 1b and 1c volumes per well		Tube 1d volume per well	Tube 2 volumes per well		Total plating volume (µL/well)
Plating format (wells/plate)	Surface area (cm^2/well)	20 µg/mL plasmid (µL)	Serum-free medium (µL)	20 µg/mL plasmid (µL)	2 µM siRNA (µL)	Serum-free medium (µL)	DharmaFECT (µL)	Serum-free medium (µL)	Transfection medium
96	0.3	5	5	5	5	10	0.05–0.5	9.95–9.5	100
24	2	25	25	25	25	50	0.5–2.0	49.5–48.0	500
12	4	50	50	50	50	100	1.0–3.0	99.0–97.0	1000
6	10	100	100	100	100	200	2.0–6.0	198.0–194.0	2000

*Note: 100 nM = 100 nmol/L = 100 pmol/mL = 100 fmol/µL.

If you have any questions

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