Dharmacon™
RNAi, Gene Expression & Gene Editing

Duo Transfection Reagent for miRIDIAN™ microRNA Mimics/Inhibitors and Plasmid Co-Transfection

This protocol is optimized for use with 100 ng/well plasmid and 50 nM miRNA mimic or 25 nM miRNA inhibitor in a 96-well plate format. Plasmid concentration may need to be optimized as plasmid size and expression attributes affect optimal conditions. The mimic or inhibitor concentration may be titrated down once optimal delivery conditions are established.

The samples listed in Table 1 are recommended 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 and miRNA Mimic/Inhibitor Co-transfection Experiment:

Samples		Purpose
Plasmid only (with lipid)	[Tube 1a]	Confirm uptake and baseline expression level of plasmid
Plasmid and negative control miRNA mimic/inhibitor (with lipid)	[Tube 1b]	Distinguish miRNA regulation from non-specific effects
Plasmid and test miRNA mimic/inhibitor (with lipid)	[Tube 1c]	Achieve miRNA regulation and expression of plasmid
Mock-transfection (lipid only)	[Tube 1d]	Identify nonspecific effects and cytotoxicity caused by the transfection reagent or procedure
Untreated (no lipid)		Determine baseline phenotype, target miRNA level, and cell viability

Additional Materials Required:

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

Table 2. Reagents for Plasmid and miRNA Mimic/Inhibitor Co-transfection Experiment:

Reagents	Description & 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 not contain antibiotics that may cause cell toxicity during transfection
Serum-free or low-serum medium	For optimal complexing of miRNA mimic/inhibitor, plasmid and DharmaFECT Duo
Plasmid	A plasmid that expresses the desired gene
Test miRNA mimic/inhibitor	miRIDIAN microRNA Mimic/Inhibitor that targets the miRNA to be regulated
Negative control miRNA	A miRNA that does not target any miRNA expressed by the plasmid or any miRNA endogenously expressed by the cells being used, such as miRIDIAN microRNA Mimic Negative Control.

Table 3. Recommended Conditions for miRNA Mimic/Inhibitor and Plasmid Co-transfection using DharmaFECT Duo:

Cell Line	Final Cell Number 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)
ES-D3	2.0×10^4	2.0×10^5	4.2	135.8	0.3
HeLa	2.0×10^4	2.0×10^5	1.4	138.6	0.1
Hep G2	2.0×10^4	2.0×10^5	0.7	139.3	0.05
Jurkat	6.0×10^4	6.0×10^5	8.4	131.6	0.6
MCF7	1.0×10^4	1.0×10^5	2.8	137.2	0.2
MCF10a	1.0×10^4	1.0×10^5	2.8	137.2	0.2
NIH/3T3	1.0×10^4	1.0×10^5	5.6	134.4	0.4

Table 3. 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 hours 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 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. If cells will be assayed after 72 hours, a reduced cell density may be required.
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 4. Recommended miRNA Mimic/Inhibitor Volumes for Specific Final Concentrations:

Final Mimic Concentration (nM)	Volume of 2 µM Mimic Stock Solution (µL)	Final Inhibitor Concentration (nM)	Volume of 2 µM Inhibitor Stock Solution (µL)
50*	8.5*	25*	4.2*
40	6.8	20	3.4
30	5.1	15	2.5
20	3.4	10	1.7
10	1.7	1	0.2

Table 4. This protocol is optimized for use with 100 ng/well plasmid and 50 nM miRNA mimic or 25 nM miRNA inhibitor in a 96-well plate format. It is recommended to start with 50 nM miRNA mimic or 25 nM miRNA inhibitor and once optimal delivery conditions are established to titrate down to the lowest concentration of the miRNA reagent that still produces acceptable miRNA regulation. *Recommended initial volumes

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 miRNA mimic/inhibitor (2 µM) solutions in an RNase-free, pH 7.4-buffered solution.
2. In tubes 1a – 1c, mix plasmid and the appropriate miRNA mimic/inhibitor solutions, if appropriate:
   - 2.1. Tube 1a (plasmid only) - Dilute 17.5 µL plasmid with 17.5 µL serum-free medium. The total volume is 35 µL.
   - 2.2. Tube 1b (plasmid and negative control miRNA mimic/inhibitor) - Mix 17.5 µL plasmid with 17.5 µL negative control miRNA mimic/inhibitor. The total volume is 35 µL.
   - 2.3. Tube 1c (plasmid and test miRNA mimic/inhibitor) - Mix 17.5 µL plasmid with the appropriate amount of test miRNA mimic/inhibitor (See Table 4. It is recommended to start with 8.5 µL mimic and 4.2 µL inhibitor). The total volume is 26 µL for mimics and 21.7 µL for inhibitors.
   - 2.4. Tube 1d (lipid only) – Do not add plasmid or miRNA mimic/inhibitor.
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.
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 61 µL for mimics and 56.7 µL for inhibitors. 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 341 µL for mimics and 336.7 µL for inhibitors. 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 hrs (for mRNA analysis) or 48 – 96 hrs (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.

GE Healthcare

Orders can be placed at:
gelifesciences.com/dharmacon

Customer Support: cs.dharmacon@ge.com
Technical Support: ts.dharmacon@ge.com or
1.800.235.9880; 303.604.9499 if you have any questions.

V1-0914