PROTOCOL

horizon™
INSPIRED CELL SOLUTIONS

Co-transfection of miRIDIAN™ microRNA reagents and plasmid using DharmaFECT™ Duo transfection reagent

This protocol is optimized for use with 100 ng/well plasmid and 50 nM miRIDIAN 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 transfection reagent (Cat# T-2010-xx).

Table 2. Reagents for plasmid and miRNA mimic/inhibitor 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 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 transfection reagent
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 Contro (Dharmacon Cat # CN-001000-01)

Table 3. Recommended conditions for miRNA mimic/inhibitor and plasmid co-transfection using DharmaFECT Duo. 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 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)
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

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 4. Recommended miRNA mimic/inhibitor volumes for specific final concentrations. 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

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

*Recommended initial volumes

Transfection

All volumes are multiplied by 3.5 to miRNA mimic/inhibitor account for the triplicate samples and loss during pipetting. This will result in 12 transfections in 96 well plate format: 4 combinations (tube 1a to d) in triplicate.

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–1d, mix plasmid and the appropriate miRNA mimic/inhibitor solutions, if appropriate:
   a. Tube 1a (plasmid only)—Dilute 17.5 µL of stock plasmid (20 µg/mL) with 17.5 µL serum-free medium. The total volume is 35 µL.
   b. Tube 1b of stock plasmid (20 µg/mL) and negative control miRNA mimic/inhibitor)—Mix 17.5 µL of stock plasmid (20 µg/mL) with 17.5 µL negative control miRNA mimic/inhibitor at 2µM. The total volume is 35 µL.
   c. Tube 1c (plasmid and test miRNA mimic/inhibitor)—Mix 17.5 µL of stock plasmid (20 µg/mL) with the appropriate amount of test miRNA mimic/inhibitor at 2µM (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. Adjust to 35 µL with water.
   d. Tube 1d (lipid only)—Do not add plasmid or miRNA mimic/inhibitor. Adjust to 35 µL with water.
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 4 tubes are considered.
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 6. 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.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

psiCHECK-2 Vector and Dual-Glo are trademarks of Promega. Renilla is a trademark of Panomics. alamarBlue Assay is a trademark of Thermo Fisher Scientific All trademarks are the property of Horizon Discovery Company unless otherwise specified. ©2018 Horizon Discovery Group Company—All rights reserved. First published June 2014. UK Registered Head Office: Building 8100, Cambridge Research Park, Cambridge, CB25 9TL, United Kingdom.

horizon™
INSPIRED CELL SOLUTIONS

V20818