

Thermo Scientific

DharmaFECT[®] General

Transfection Protocol

The following is a general protocol for use of DharmaFECT transfection reagents to deliver siRNA into mammalian cells and was optimized for adherent cells in a 96-well plate format. Table 1 presents recommended conditions for the most efficient delivery and subsequent silencing of GAPDH and Cyclophilin B siRNA in various cell lines. These conditions include optimized plating densities (Column B), the recommended DharmaFECT transfection reagent (Column A), and recommended DharmaFECT volume per well (Column C). Table 2 provides a range of DharmaFECT volumes for alternate plate formats. We recommend that each experiment includes the following samples in triplicate: 1) Untreated cells, 2) Positive control siRNA (siRNA targeting an endogenous or reporter gene), 3) Negative control siRNA, and 4) your desired test siRNA.

Perform all steps of protocol in a laminar flow cell culture hood using sterile techniques.

Cell Plating

(Optimal cell densities will vary with growth characteristics of specific cells and may need to be determined empirically. See Table 1, Column B for cell line specific recommendations.)

1. Trypsinize and count cells
2. Dilute cells in antibiotic-free complete medium to achieve the appropriate plating density in 100 μ L of solution. (Complete medium is medium that the cells are maintained in, and may contain serum.)
3. Plate 100 μ L of cells into each well of a 96-well plate.
4. Incubate cells at 37°C with 5% CO₂ overnight.

Transfection (for 100 nM of siRNA)

(Performing experiments in triplicate is recommended. All calculations are shown for triplicate samples in 96-well format. To account for loss during pipetting, all volumes are multiplied by 3.5.)

1. Prepare a 2 μ M siRNA solution in 1X siRNA Buffer or another appropriate RNase-free solution.
2. In separate tubes, dilute the appropriate volume of 2 μ M siRNA (Tube 1) and the appropriate DharmaFECT transfection reagent (Tube 2; see Table 1, Column A and C) with serum-free medium. For example, when transfecting A549 cells, prepare the following:
 - a. Tube 1 – Add 17.5 μ L of 2 μ M siRNA to 17.5 μ L serum-free medium. The total volume is 35 μ L.
 - b. Tube 2 – Add 1.4 μ L DharmaFECT 1 to 33.6 μ L of serum-free medium. The total volume is 35 μ L.
3. Mix the contents of each tube gently by pipetting carefully up and down and incubate for 5 minutes at room temperature.
4. Add the content of Tube 1 to Tube 2. In this example, the total volume is 70 μ L. Mix by pipetting carefully up and down and incubate for 20 minutes at room temperature.
5. Add sufficient antibiotic-free complete medium to the mix in step 4 for the desired volume of transfection medium. For this example, add 280 μ L of complete medium for a total volume of 350 μ L transfection medium.
6. Remove culture medium from the wells of the 96-well plate and add 100 μ L of the appropriate transfection medium to each well.
7. Incubate cells at 37°C in 5% CO₂ for 24 – 48 hrs (for mRNA analysis) or 48 – 96 hrs (for protein analysis).
8. If cell toxicity is observed after 24 hours, replace the transfection medium with complete medium and continue incubation. For best results, use samples that are at least 80% viable.

	Species	Cell Line	Recommended Transfection Conditions (96-well format)			Relative GAPDH Knockdown (%)
			(A) DharmaFECT Reagent	(B) Cell Density (per well)	(C) DharmaFECT Volume per well (µL)	
Human	A549	Lung carcinoma	1	2.5 x 10 ⁴	0.4	92
	BxPC3	Pancreas adenocarcinoma	1	5.0 x 10 ³	0.8	98
	Capan-1	Pancreas adenocarcinoma	4	5.0 x 10 ³	0.8	77
	DU 145	Prostate carcinoma	1	1.0 x 10 ⁴	0.2	94
	HEK293	Kidney transformed embryonic cells	1	2.5 x 10 ⁴	0.4	92
	HeLa	Cervical epithelial adenocarcinoma	1	5.0 x 10 ³	0.4	95
	HeLa S3	Cervical epithelial adenocarcinoma	1	5.0 x 10 ³	0.4	97
	Hep G2	Hepatocellular carcinoma	4	1.0 x 10 ⁴	0.4	91
	H1299	Lung carcinoma	2	1.0 x 10 ⁴	0.2	93
	HT-1080	Fibrosarcoma	4	5.0 x 10 ³	0.2	96
	HT-29	Colorectal carcinoma	4	5.0 x 10 ³	0.4	99
	HUVEC	Umbilical vein endothelial cells	1	2.5 x 10 ⁴	0.05	70
	LNCaP	Prostate carcinoma	3	2.5 x 10 ⁴	1.6	98
	MDA-MB453	Breast adenocarcinoma	2	5.0 x 10 ³	0.2	91
	MCF7	Breast adenocarcinoma	1	1.0 x 10 ⁴	0.2	90
	PC-3	Prostate carcinoma	2	1.0 x 10 ⁴	0.2	88
	SKBR3	Breast adenocarcinoma	1	2.5 x 10 ⁴	0.4	93
THP-1*	Acute monocytic leukemia	2	5.0 x 10 ³	0.1	73	
Rodent	A7R5	Rat aortic smooth muscle	2	5.0 x 10 ³	0.1	95
	C2C12	Mouse myoblasts	3	5.0 x 10 ³	0.2	87
	CHO K1	Chinese hamster ovary	4	1.0 x 10 ⁴	0.8	92
	H9C2	Rat heart myoblasts	1	1.0 x 10 ⁴	0.2	96
	mIMCD3	Mouse renal inner medullary	4	5.0 x 10 ³	0.4	58
	NRK-49F	Rat kidney fibroblast	2	1.0 x 10 ⁴	0.2	92
	RAT2	Rat fibroblast	2	5.0 x 10 ³	0.2	85
3T3 L1	Mouse embryonic fibroblast	3	2.5 x 10 ⁴	1.6	97	
Monkey	COS 7	African green monkey kidney	2	5.0 x 10 ³	0.4	94

*Cells adapted for growth in suspension.

Table 1. Transfection optimization survey: GAPDH and Cyclophilin B silencing as a measure of efficient transfection. Cells were transfected with 100 nM siRNA targeting either the housekeeping gene GAPDH or Cyclophilin B. To determine the conditions for the most efficient delivery of siRNA, each cell line was tested at three plating densities (5 x 10³, 1 x 10⁴, 2.5 x 10⁴ cells per well) with a range of DharmaFECT volumes (0.05 - 1.6 µL/well) for all four DharmaFECT transfection reagents. The best combination of the three parameters for each cell line is reported above. These results should serve as starting points in optimization of transfection conditions for a particular cell line. The GAPDH siRNA has been validated for reducing mRNA level by 75% or more under optimal transfection conditions, therefore the GAPDH knockdown observed represents a relative measure of transfection efficiency. In cases where knockdown is less than 75%, the transfection efficiency is not considered optimal and may be subject to poor uptake and/or release into the cytoplasmic compartment.

Plate Format		Tube 1 Volumes per well		Tube 2 Volumes per well		Plating Volume (µL/well)
Plating Format (wells/plate)	Surface Area (cm ² /well)	2 µM siRNA (µL)	Serum-free Medium (µL)	DharmaFECT (µL)	Serum-free Medium (µL)	Transfection Medium
96	0.3	5	5	0.05 - 0.5	9.95 - 9.5	100
24	2	25	25	0.5 - 2.0	49.5 - 48.0	500
12	4	50	50	1.0 - 3.0	99.0 - 97.0	1000
6	10	100	100	2.0 - 6.0	198.0 - 194.0	2000

Table 2. 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. *Note: 100 nM = 100 nmol/L = 100 pmol/mL = 100 fmol/µL

Contact Information

For technical questions regarding the use of siRNA reagents, please contact Dharmacon Products Technical Support at:

In America/Asia

800-235-9880

303-604-9499

dharmacon.lab@thermofisher.com

In Europe/Israel

00800 73724648

32-53-85-71-84

perbio.eurotech@thermofisher.com

In Other Countries

Please contact your appropriate

distributor as listed on

www.thermo.com/dharmacondistributors

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