

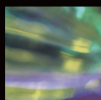
Thermo Scientific DharmaFECT Transfection Reagents



Maintain cell viability with reagents specially formulated to deliver siRNA



Achieve robust siRNA uptake for dependable gene silencing



Expand your RNAi applications with an optimized co-transfection reagent



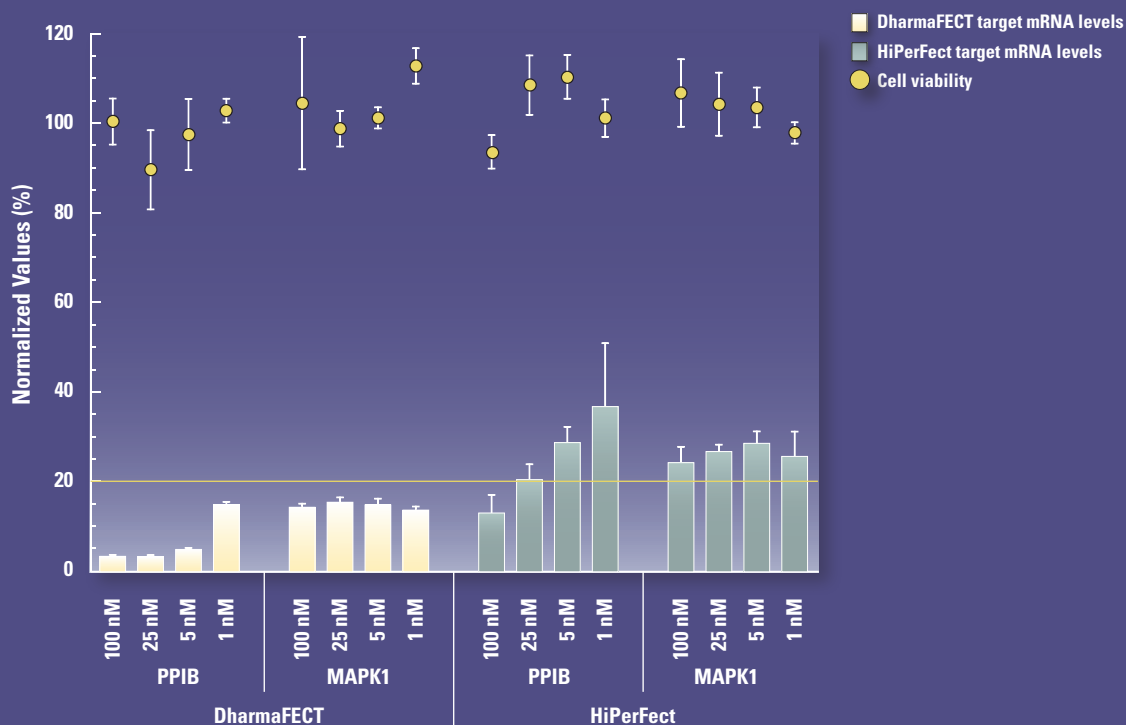
Thermo Scientific DharmaFECT transfection reagents deliver

Efficient siRNA delivery is critical for successful gene silencing. Even highly potent siRNA may appear non-functional without effective delivery. DharmaFECT® transfection reagents provide efficient and reliable siRNA transfection at low siRNA concentrations with low cellular toxicity.

- Specialized formulations for successful delivery of siRNA to more cell lines
- Effective delivery for target silencing at low siRNA concentrations
- Low toxicity even at a broad range of experimental conditions

efficient delivery

DharmaFECT provides highly efficient delivery at low siRNA concentrations



DharmaFECT achieved >80% silencing at all siRNA concentrations, while HiPerFect (Qiagen) only achieved this level with PPIB at 100 nM.

HeLa cells were transfected with SMARTpool™ reagents targeting Cyclophilin B (PPIB) or MAPK1 at concentrations of 1, 5, 25, and 100 nM.

mRNA expression (bars) was determined by branched DNA assay (Panomics Quantigene Reagent System) and cell viability (data points) was determined by alamarBlue (Biosource International).



Multiple formulations ensure siRNA transfection success

No single transfection reagent effectively delivers siRNA to the hundreds of different cell lines being used in RNAi experiments. Why compromise delivery efficiency and cell viability with a "one-size-fits-all" transfection reagent?

DharmaFECT is the only transfection reagent offered in four distinct siRNA-specific formulations to ensure optimal siRNA delivery in a wide range of cell lines.

- No single lipid reagent outperformed the panel of DharmaFECT formulations in a benchmark study of twenty common cell lines
- Independent third-party optimization of siRNA transfection demonstrated DharmaFECT formulations outperforming four competitor lipid reagents in 9 of 10 cell lines¹

multiple formulations

DharmaFECT formulations deliver siRNA effectively to more cell lines

Optimal lipid for siRNA transfection in a 384-well format¹

Adapted from Borawski et al.

Cell Line	Lipid Reagent
HeLa	DharmaFECT 4
SKOV3	DharmaFECT 4
A549	DharmaFECT 2
HCT116	DharmaFECT 1
Huh7	DharmaFECT 3
A673	Lipofectamine 2000
XMD5	DharmaFECT 3
HEK293	DharmaFECT 1
UMR	DharmaFECT 1
MDA-MB-231	DharmaFECT 4

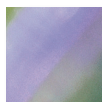
Lipids tested were DharmaFECT 1-4, HiPerFect (Qiagen), TransIT-TKO (Mirus) Lipofectamine 2000 and Oligofectamine (Invitrogen).

¹ Borawski, J., et al. "Optimization Procedure for small interfering RNA Transfection in a 384-well format." J. Biomolecular Screening 12 (2007); 546-559.

Cell Line	DharmaFECT formulation	Successful Transfection			
		Other Manufacturers			
		Lipid A	Lipid B	Lipid C	Lipid D
A549	✓				✓
BxPC3	✓			✓	✓
DU 145	✓	✓	✓	✓	✓
HEK293	✓				✓
HeLa	✓	✓	✓	✓	✓
HeLa S3	✓			✓	✓
Hep G2	✓				✓
HT - 29	✓		✓	✓	✓
LNCaP	✓		✓		
MCF7	✓				
PC - 3	✓				
H1299	✓	✓		✓	
H1080	✓	✓		✓	
CHO K1	✓		✓		✓
A7R5	✓				
3T3 L1	✓				✓
NKR-49F	✓	✓		✓	✓
RAT - 2	✓				
H9C2	✓	✓			
AR42J	✓				
COS 7	✓				

DharmaFECT formulations deliver siRNA effectively to more cell lines than lipids from other manufacturers.

Optimization studies with DharmaFECT 1, 2, 3, 4 and four other lipid transfection reagents were assessed for effective siRNA delivery (>80% knockdown) and low toxicity (>80% viability). Checkmarks indicate successful transfection.



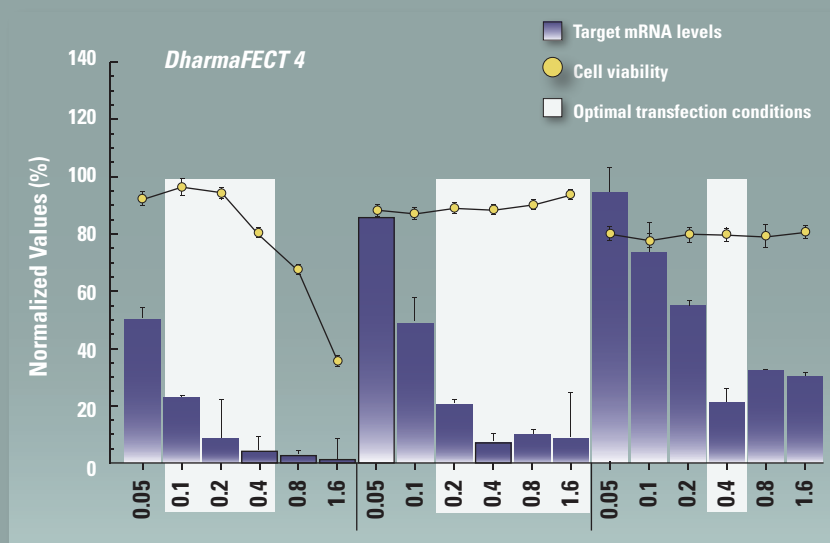
Effective siRNA delivery for reliable silencing

Experimental reproducibility is essential to RNAi studies, yet poor siRNA delivery or cellular toxicity may confuse the interpretation of results.

DharmaFECT siRNA transfection reagents ensure effective delivery across a broad range of cell densities and lipid volumes with minimal effect on cell viability.

- Fast and easy optimization producing reliable and reproducible RNAi experiments
- A broad window of optimal conditions provides experimental flexibility
- Well-suited for high-throughput RNAi screening

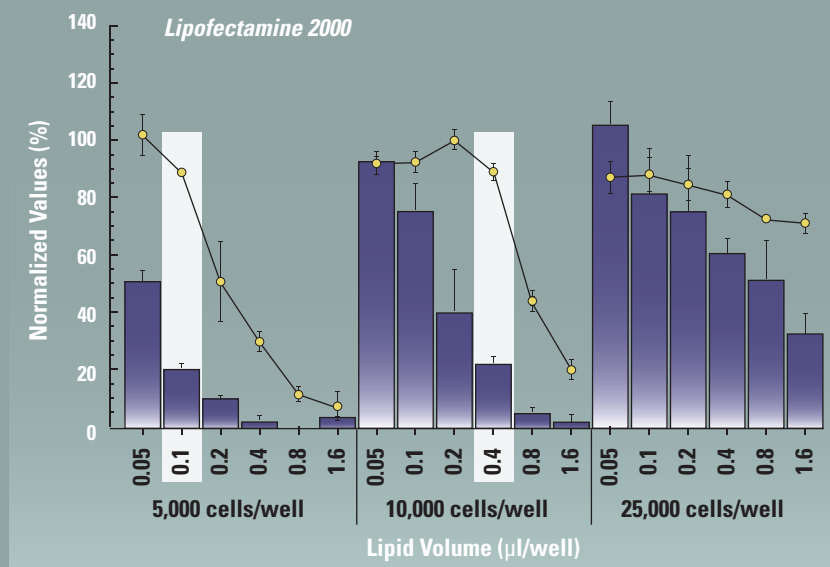
reliable and reproducible



DharmaFECT siRNA transfection reagents are effective across a wider range of conditions

DharmaFECT reagents are effective across a broader range of experimental conditions when compared to Lipofectamine 2000 (Invitrogen).

Several cell densities and lipid volumes were investigated to determine optimal transfection conditions, shown by the shaded boxes. Three cell densities of HepG2 cells were transfected with GAPD siRNA (100 nM) using a range of volumes (0.05 - 1.6 μl/well) of Lipofectamine 2000 and DharmaFECT 4 transfection reagents.



mRNA levels (bars) were assessed by branched DNA assay (Panomics Quantigene Reagent System) and cell viability (data points) was determined by alamarBlue (Biosource International).



DharmaFECT Duo reagent for RNAi molecules and plasmid delivery

Co-transfection of siRNA or miRNA reagents with a plasmid reporter system is increasingly common, yet few transfection reagents on the market will effectively deliver both molecules.

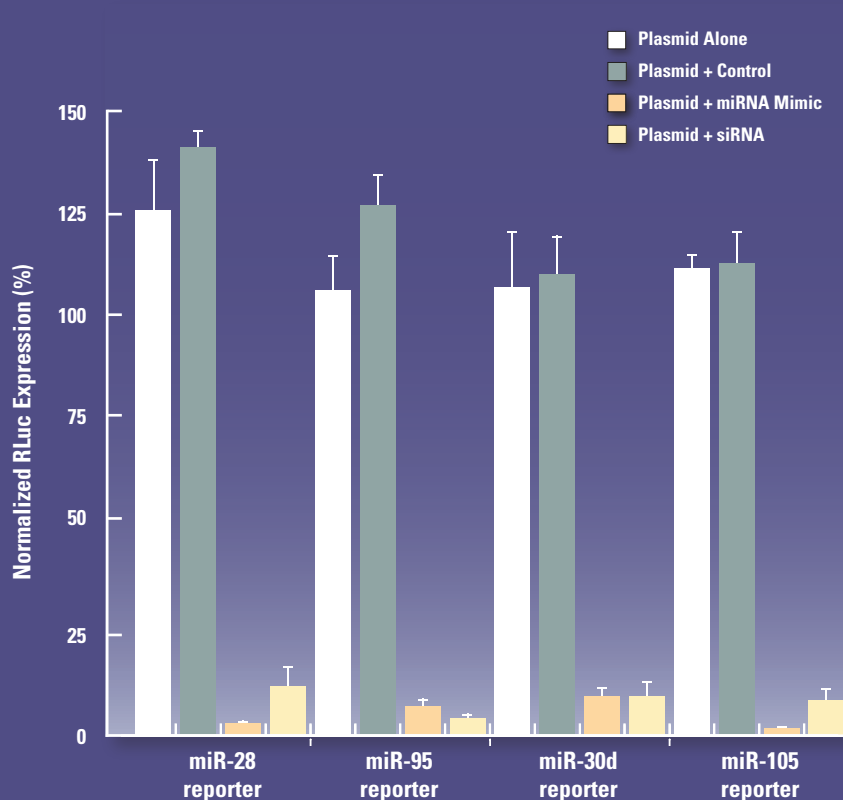
In co-transfection experiments optimized for cell viability, DharmaFECT Duo reagent resulted in both reliable plasmid expression and potent siRNA-mediated knockdown.

DharmaFECT Duo transfection reagent is specially formulated to provide:

- High co-transfection efficiency of plasmid and siRNA or miRNA reagents
- Efficient siRNA target knockdown plus plasmid expression
- Reliable delivery for reporter-based miRNA regulation experiments

co-transfect with confidence

DharmaFECT Duo is highly effective in co-transfection of plasmid with siRNA or miRNA mimics



DharmaFECT DUO transfection reagent achieves reliable co-transfection results for a variety of experimental goals. The graph demonstrates specific modulation of the Renilla luciferase (Rluc) gene:

- Rluc expression is similar for Plasmid Alone and Plasmid + Control
- Down-regulation of Rluc as a result of miRNA mimic (plasmid + miRNA mimic)
- Greater than 85% knockdown of Rluc expression by siRNA (Plasmid + siRNA)

The psiCHECK-2 vector (100ng/well; Promega) with cloned microRNA recognition sites for miR-28, miR-95, miR-30d and miR-105, respectively, were complexed alone or with RNAi reagents (10 nM) using DharmaFECT Duo (0.2uL/well) in MCF-7 cells at 10,000 cells/well (96-well plate). The RNAi reagents were Thermo Scientific Dharmacon miRIDIAN Mimics (Negative Control, miR-28, miR-95, miR-30d and miR-105, respectively) or siRNA (Renilla luciferase pool). Firefly and Renilla luciferase expression was assessed at 48 hours using the Dual-Glo Luciferase Assay System (Promega) and normalized to identically treated psiCHECK-2 empty vector.



Find the DharmaFECT formulation that's right for you

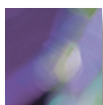
DharmaFECT 1 formulation is the most broadly-applicable lipid for effective siRNA delivery across cell lines. In a number of cases, another DharmaFECT formulation gave even better results – emphasizing the value of optimized reagents.

- Choose DharmaFECT formulation 1, 2, 3 or 4 for optimal siRNA transfection or DharmaFECT Duo reagent for effective plasmid and siRNA co-transfection
- Review validated recommendations to help you select the appropriate DharmaFECT formulation for your research (see table)

Cell line-specific DharmaFECT formulation recommendations (96-well format)

Cell line	Cell type	Recommended DharmaFECT formulation	DharmaFECT volume/well (μL)	Plating density/well	Additional Successful DharmaFECT formulations	Validated DharmaFECT Duo protocol available
Human						
A549	Lung carcinoma	1	0.2	1 × 10 ⁴	2, 3, 4	X
BxPC3	Pancreas adenocarcinoma	2	0.2	5 × 10 ³	1, 3, 4	
DU 145	Prostate carcinoma	1	0.2	1 × 10 ⁴	2, 3, 4	
HEK293	Kidney transformed embryonic cells	1	0.2	1 × 10 ⁴	2, 4	X
HeLa	Cervical epithelial adenocarcinoma	1	0.2	5 × 10 ³	2, 3, 4	X
HeLa S3	Cervical epithelial adenocarcinoma	4	0.4	5 × 10 ³	1, 2, 3	X
Hep G2	Hepatocellular carcinoma	4	0.4	1 × 10 ⁴	1, 2	X
H1299	Lung carcinoma	2	0.2	1 × 10 ⁴	4	
HT1080	Fibrosarcoma	4	0.2	5 × 10 ³	1, 2, 3	
HT - 29	Colorectal carcinoma	1	0.2	5 × 10 ³	2, 3, 4	
MCF7	Breast adenocarcinoma	1	0.2	1 × 10 ⁴	2, 4	X
MCF - 10a	Breast adenocarcinoma	1	0.2	1 × 10 ⁴	2	X
MDA - MB453	Breast adenocarcinoma	2	0.2	1 × 10 ⁴	1, 3, 4	
hMSC	Mesenchymal stem cells	1	0.4	5 × 10 ³	2, 3, 4	
PC - 3	Prostate carcinoma	2	0.2	1 × 10 ⁴	3	
SKBR3	Breast adenocarcinoma	2	0.2	1 × 10 ⁴	1, 3, 4	
786 - 0	Kidney adenocarcinoma	1	0.4	5 × 10 ³	2	
HCT-116	Colorectal carcinoma	2	0.1	5 × 10 ³	4	
MDA-MB-231	Breast adenocarcinoma	4	0.1	5 × 10 ³	1	
Huh7	Hepatology	4	0.05	5 × 10 ³	1, 2	X
SKOV-3	Ovarian adenocarcinoma	3	0.4	1 × 10 ⁴	1, 2, 4	
DLD-1	Colorectal adenocarcinoma	2	0.4	5 × 10 ³	1, 3	
OVCAR 3	Ovarian adenocarcinoma	1	0.1	5 × 10 ³	2, 3, 4	
HUVEC	Human umbilical vein endothelial	4	0.2	2 × 10 ⁴	1, 2	
u-87 MG	Brain glioblastoma	1	0.1	5 × 10 ³	2, 3, 4	
JEG-3	Placenta, choriocarcinoma, epithelial	3	0.2	1 × 10 ⁴	-	
LNCaP	Prostate carcinoma	2	0.2	1 × 10 ⁴	1, 3	
ARPE19	Retinal pigment epithelial	4	0.2	1 × 10 ⁴	1, 2	
Saos-2	Bone, osteosarcoma, epithelial	1	0.05	1 × 10 ⁴	2, 3, 4	
Rodent						
A7R5	Rat aortic smooth muscle	2	0.1	5 × 10 ³	1	
C2C12	Mouse myoblasts	3	0.4	5 × 10 ³	2	
CHO K1	Chinese hamster ovary	1	0.4	1 × 10 ⁴	2	
ES - D3	Mouse embryonic stem cells	1	0.2	2 × 10 ³	2	X
ES - E14TG2a	Mouse embryonic stem cells	1	0.2	2 × 10 ³	2	
H9C2	Rat heart myoblasts	1	0.2	1 × 10 ⁴	2, 3, 4	X
J774	Mouse macrophage	4	0.2	1 × 10 ⁴	-	
NIH / 3T3	Mouse embryonic fibroblast	1	0.2	1 × 10 ⁴	3	X
NRK - 49F	Rat kidney fibroblast	2	0.2	1 × 10 ⁴	1, 4	
RAT2	Rat fibroblast	1	0.2	2 × 10 ⁴	2	
3T3 L1	Mouse embryonic fibroblast	1	0.2	5 × 10 ³	3	
Other						
COS 7	African green monkey kidney	2	0.4	5 × 10 ³	1, 3, 4	

All conditions listed resulted in control gene silencing of 85% or better and viability of 80% or better in a 96-well format. X indicates cell lines where DharmaFECT Duo transfection reagent successfully delivered plasmid and siRNA with greater than 70% viability. Conditions should always be re-evaluated in the context of assay-specific requirements for cell density.



Ordering DharmaFECT transfection reagents

Our online resources for over 100 cell lines will help you determine the right DharmaFECT formulation for your research.

- Optimized, validated protocols available for over 25 cell lines
- Customer-provided recommendations for the optimal DharmaFECT formulation in over 80 cell lines
- DharmaFECT Set of 4 (one tube of each formulation) available for optimization of novel cell types

easy online ordering

DharmaFECT products

Product	Size	Catalog #.
siRNA Transfection		
DharmaFECT 1	0.2 mL	T-2001-01
	0.75 mL	T-2001-02
	1.5 mL	T-2001-03
	5 x 1.5 mL	T-2001-04
	2 x 10 mL	T-2001-07A
DharmaFECT 2	0.2 mL	T-2002-01
	0.75 mL	T-2002-02
	1.5 mL	T-2002-03
	5 x 1.5 mL	T-2002-04
DharmaFECT 3	2 x 10 mL	T-2002-07A
	0.2 mL	T-2003-01
	0.2 mL	T-2003-02
	0.2 mL	T-2003-03
	5 x 1.5 mL	T-2003-04
DharmaFECT 4	2 x 10 mL	T-2003-07A
	0.2 mL	T-2004-01
	0.2 mL	T-2004-02
	0.2 mL	T-2004-03
	5 x 1.5 mL	T-2004-04
DharmaFECT Set of 4	2 x 10 mL	T-2004-07A
	0.2 mL x 4	T-2005-01
	0.75 mL x 4	T-2005-02
DharmaFECT Duo	1.5 mL x 4	T-2005-03
siRNA / plasmid co-transfection		
DharmaFECT Duo	0.2 mL	T-2010-01
	0.75 mL	T-2010-02
	1.5 mL	T-2010-03
	5 x 1.5 mL	T-2010-04

General ordering guidelines

Plate type	Package size (ml)	Approximate number of transfections per package
96 well	0.02	200 - 1000
	0.75	750 - 3750
	1.50	1500 - 7500
24 well	0.02	200
	0.75	750
	1.50	1500
12 well	0.20	100
	0.75	375
	1.50	750
6 well	0.20	50
	0.75	187
	1.50	375

complete, validated protocols available online at
www.thermo.com/dharmaconprotocols

Thermo Scientific DharmaFECT Transfection Reagents



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Euro Literature # PB 2008 14

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