Product survey: siRNA Transfection

The 800-Pound siRNA Gorilla

Transfecting cells with small interfering RNAs (siRNAs) to silence genes via RNA interference has become a very popular and widely used technique. New transfection reagents may help to optimise the efficiency of siRNA delivery.

Knocking down genes by RNA interference (RNAi) is one of modern molecular biology's most stunning high-flyers. Since its discovery in 1998 by Andrew Fire and Greg Mellow, RNAi has taken the fast lane and conquered life science labs around the globe by storm. The fastest and easiest way to perform an RNAi is carried out by transfecting cells with a chemically synthesised, small interfering double-stranded RNA fragment (siRNA), complementary to a homologous stretch of the target gene DNA. As soon as the 21-23 nucloetide-long siRNA duplex enters the cytosol, the antisense and sense strand go their separate ways. The latter is cleaved and degraded whereas the antisense strand is incorporated into the RNA-inducing silencing complex (RISC) to guide RISC to complementary mRNA for subsequent endonucleolytic cleavage. This prevents translation of the target protein, i.e., the target gene is knocked down or silenced.

siRNA delivery is the 800-pound gorilla, that dictates the outcome of RNAi experiments.

The crucial step of gene silencing experiments performed with siRNAs is the uptake of the siRNA duplex into the cell. Small interfering RNAs have a molecular weight of approximately 13,000 Dalton and even worse, they are negatively charged. Hence, getting naked siRNAs across the lipid bilayer of cell membranes is like getting a camel through a needle-eye; however, molecular biologists have invented several tricks to facilitate the cellular uptake of siRNAs.

Lipid nanoparticles

The most popular vehicles for siRNA delivery are transfection reagents made of cationic lipids. Meanwhile, a whole bunch of cationic lipids or lipid formulas is commercially available and every company trying to get a piece of the RNAi-cake sells its own "magic" siRNA uptake recipe. Cationic lipids, such as DOTAP, DOTMA and DOPE are built of positively charged heads, usually quaternary ammonium groups, linked to a hydrophobic fatty acid tail, that form micellar structures called liposomes. Upon mixing liposomes and siRNAs, the latter become enclosed in the spherical liposomes to create lipoplexes that may fuse with the cell membrane. The siRNA-lipid nanoparticles are subsequently taken up by the cells via endocytosis and the siRNAs may escape from the endosomal vesicles, entering the cytosol during maturation of the endosomes into lysosomes.

siRNA lipofection via classical cationic lipids works for many cell lines and cell types but not for all. Typical examples of hard-to-transfect cells are adipocytes as well as some T-cell, fibroblast, epithelial and neuronal cell lines. To deliver siRNAs, even into these hard-to-transfect cells, researchers have coupled a lot of different substances to liposomes that may enhance the delivery properties of the formed lipoplex nanoparticles, including lipids, antibodies, aptamers, cell-targeting ligands, e.g., transferrin and cell penetrating peptides.

Some vendors also offer magnetic assisted transfection systems, often used in combination with cationic lipids and magnetic cell culture plates. The principle of magnetic siRNA transfection is pretty simple and straightforward. In the first step, siRNA oligos are mixed with magnetic nanoparticles made of biodegradable iron oxide, e.g., Fe_3O_4 or Fe_2O_3, that are coated with cationic molecules. The resulting magnetic beads-siRNA complexes are subsequently endocyted by the cells, supported by a magnetic field that is applied at the bottom side of the cell culture plate. Magnetic transfection is particularly suited for adherent cells; however, it also works, after an additional immobilisation step, for suspension cultures.

Cationic lipids or cationic polymers are very popular siRNA transfection reagents, they are, however, toxic to cells and may cause problems, especially if used for *in vivo* siRNA applications. A rather promising, recently developed alternative to traditional lipid transfecting is based on peptide transduction domains (PTDs). PTDs are short peptides, containing multiple positively charged amino acids such as arginine and lysine that are known for their ability to direct covalently attached macromolecules across membrane bilayers.

Masking the negative charge

Since electrostatic interactions between negatively charged siRNAs and positively charged PTDs would inevitably lead to precipitation of siRNA oligos, Steven Dowdy's group at the Howard Hughes Medical Institute in La Jolla, United States, has introduced a smart trick that enables PTD driven delivery of siRNAs (Eguchi et al., *Nat. Biotechnol.* 27, 567-571). The researchers added a small protein called double-stranded RNA-binding domain (DRBD) to the PTD peptide to get a bifunctional fusion protein. The DRBD part of the fusion has a high affinity to siRNA oligos and shields the negative charge of siRNA molecules upon binding to them, while the PTD domain interacts with the cell membrane and delivers the siRNAs into the cytosol. PTD-DRBD fusion proteins are not toxic to cells and may lend them especially to *in vivo* siRNA studies; one of their outstanding features, however, is their high transfection efficiency. According to the Dowdy group, the PTD-DRBD fusion protein was able to deliver siRNA in every single cell of a given cell population. Sounds almost too good to be true, however, it seems only a matter of time before PTD-DRBD siRNA transfection systems will enter the market.

Harald Zähringer

siRNA Transfection

Company/Distributor	Name of Product	Transfectable Cell Types	Miscellaneous, Specialities, Generally	Price [EUR]
5 Prime Hamburg, Germany www.5PRIME.com **Contact:** J. Tesdorpf Phone +49-40 3197927-0 info@5prime.com	RNotion Transfection Reagent	AGS, 293, HeLa S3, CHO, Huh-7, HUVEC, NIH/3T3, C6, ATDC5 and more	■ Superior transfection efficiency, low cytotoxicity ■ Convenient protocol: transfection in presence of serum ■ Consistent and reproducible results	On request
AMS Biotechnology (Europe) www.amsbio.com **Contact:** Peter Rettenberger Phone +49-01235 828200	TurboFectin Transfection Reagent	Commonly used established cell lines	■ 1 ml in 1 vial ■ 5 ml in 5 vial	300.- 1,190.-
	Magnetofection siRNA Starting Kit	n.a.		546.-
	GeneSilencer siRNA Transfection Reagent	n.a.	■ 50 reactions ■ 200 reactions	110.- 315.-
BioCat Heidelberg, Germany www.biocat.com **Contact:** Elke Gamer Phone: +49-6221-7141516 gamer@biocat.com	GeneSilencer siRNA Transfection Reagent	Wide variety of mammalian cells including primary cells: Aortic muscle cells; Aveolar type II cells; Bone marrow; Cardiac fibroblasts; Cardiac myocytes; Cerebellar neurons; etc.	■ High siRNA transfection efficiency, low cytotoxicity ■ Functional gene silencing post siRNA delivery ■ Compatibility with diverse growth conditions (with and without serum) ■ Easy-to-use protocols for both adherent and suspension cells ■ Different pack sizes, e.g. in a 96-well format for high-throughput siRNA screening projects	141.- (0.2 ml, 40 transfections in 6-well plates) 340.- (0.75 ml, 150 transfect. in 6-well plates)
Genovis AB www.genovis.com Germany and Austria: Biozym Scientific, www.biozym.com **Contact:** Monika Burbach Phone: +49(0)5152-9020 support@biozym.com	NIMT FeOfection / Purple	Common adherent cell lines, suspension cells, difficult to transfect cells and mesenchymal stem cells	■ Combined delivery and imaging ■ High efficiency ■ Low toxicity ■ Easy and quick handling	On request
Carl Roth Karlsruhe, Germany www.carlroth.de **Contact:** Stefanie Seipp Phone: +49-721-56061038 s.seipp@carlroth.de	Roti-Fect Plus	Eucaryotic primary cells and cell lines	■ Particularly low cell toxicity ■ Especially designed for siRNA transfections and other live assays ■ Superior transfection efficiency with all cell lines and primary cells ■ Detailed manual ■ For regular need special prices available	68.15 (0.2 ml) 258.65 (1 ml; larger amounts on request)
	Roti-Fect	Eucaryotic cell lines	■ No serum-inhibition ■ High and wide operating plateau ■ Low cell toxicity ■ Detailed manual ■ For regular need special prices available	51.70 (0.2 ml) 119.05 (0.5 ml) 185.30 (1 ml) (5 x 1 ml: on request)
Chemicell Berlin, Germany www.chemicell.com **Contact:** Cengiz Öztürk Phone: +49-30-2141481 info@chemicell.com	Magnetofection PolyMAG-200 CombiMAG-200 MagnetoFACTOR -96 or -24 plate	Magnetofection has been successfully used on a broad range of cell lines of hard-to-transfect cells and primary cells (see transfected cells in the downloadable document on *www.chemicell.com*)	■ Simple and highly efficient non-viral transfection system ■ Magnetic fields are used to concentrate siRNA conjugated magnetic nanoparticles into target cells ■ Extremely short process time	350.-
	PolyMAG-100 PolyMAG-500 PolyMAG-1000	Universally applicable magnetic particle preparation for high efficiency siRNA delivery.	■ PolyMAG is mixed in a one-step procedure with the siRNA	95.- 365.- 665.-
	CombiMAG-100 CombiMAG-500 CombiMAG-1000	Magnetic particle preparation designed to be combined with any commercially available transfection reagent for siRNA delivery.	■ CombiMAG can be combined with any commercially available transfection reagents ■ Allows creating your own magnetic gene vector based on your favourite transfection reagent	50.- 180.- 350.-
	MagnetoFACTOR -96 plate	Especially designed for Magnetofection with the 96-well, 12-well, 6-well plate and T-75 flasks	■ Optimal transfection when using the appropriate plate format	350.-
	MagnetoFACTOR -24 plate	Especially designed for Magnetofection with a 24-well plate	■ Optimal transfection for the 24-well plate format	350.-
Eppendorf Hamburg, Germany www.eppendorf.com **Contact:** Heide Niesalla Phone: +49(0)4053801198 niesalla.h@eppendorf.de	Eppendorf Eporator	Bacteria, yeast (siehe *www.eppendorf.com/eporator)*	■ Time-saving ■ Compact ■ GLP-compliant documentation	2,550.-
	Multiporator	Bacteria, yeast, animal cells (siehe *www.eppendorf.com/multiporator)*	■ Compact ■ Flexible system	Starting from 6,218.-
Fermentas St. Leon-Rot, Germany www.fermentas.com **Contact:** Wiebke Wetzel Phone: +49(0)6227356790 wiebke.wetzel@fermentas.de	TurboFect siRNA Transfection Reagent	Permanently growing cell lines (HeLa, HR5-CL11, CHO,NIH3T3) and primary cell cultures (HLF)	■ High efficiency ■ Effective gene silencing ■ siRNA delivery into a variety of cells ■ Compatible with serum and antibiotics ■ 0,5 ml sufficient for 500 transfections in a 24-well plate	185.- /0,5 ml (500 transfections in a 24-well plate)

siRNA Transfection

Company/Distributor	Name of Product	Transfectable Cell Types	Miscellaneous, Specialities, Generally	Price [EUR]
IBA Goettingen, Germany www.magnet-assisted-transfection.com **Contact:** Bettina Renker Phone: +49-551-50672-0 info@iba-go.com	MATra-si Transfection reagent	Successfully used on many and also critical cell lines (almost 200 cell types tested positively so far). For suspension cells: MATra-S Immobilizer is required in addition	■ Greatly improved transfection rates ■ Very gentle method with almost no toxicity ■ Membranes remain intact ■ No transfection related side effects on your model system and read out ■ Easy protocol	170.-
	MA Lipofection Enhancer	Successfully used on many and also critical cell lines, including adherent, suspension andprimary cells. For suspension cells: MATra-S Immobilizer is required in addition	■ Transfection with common lipidic or polycationic reagents can be enhanced bymagnetic assistance ■ Can be combined with IBAfect (by IBA) or any other commercially available lipofection reagent ■ Also suitable to enhance the efficiency of viral transfections ■ Universal magnet plate required	--
	Universal Magnet Plate (8 x 13 cm)	n.a.	■ Required for magnet assisted transfection or magnet assisted lipofection with MATra-si or MA Lipofection Enhancer ■ Further special magnet plate sizes available for e.g. 96-wells or larger culture plates	495.-
	MATra Starter Set 5: Test Set for Magnet Assisted siRNA Transfection	See above	■ Contains MATra-si, MA Lipofection Enhancer and a 4-week rental phase of the Universal Magnet Plate	130.- (please request; limited availability)
Integrated DNA Technologies www.idtdna.com **Contact:** Lynette Brown Phone: 01-800-328-2661 lbrown@idtdna.com	Transductin	All cell types	■ For hard to transfect cell lines	280.- (2 mg) 1,245.- (10 mg) 210.- (Trial kit)
	TriFECTin	All cell types	■ Minimal toxicity	115.- (0.5 ml) 170.- (1 ml) 765.- (5 x 1ml)
Merck Chemicals Calbiochem, Novabiochem, Novagen www.merck4biosciences.com **Contact:** Technical support Phone: 0800100 3496 techservice@merckbio.eu	RiboJuice siRNA Transfection Reagent	A549, BHK, CHO, COS-7, HEK293, HeLa, HepG2, HONE-1, L6, L428, L591, MCF-7, Neuro-2A, NIH 3T3, primary or first passage rat HSC, primary aortic smooth muscle, primary keratinocytes	■ Efficient delivery of siRNA for targeted gene suppression ■ Minimal cellular toxicity ■ Compatible with both serum-containing and serum-free media ■ Compatible with GeneJuice Transfection Reagent for cotransfection of siRNA and plasmid DNA	179.- (0.3 ml) 426.- (1 ml)
Millipore Bioscience Division Schwalbach, Germany www.millipore.com **Contact:** Phone: +49-6196-494-299 (DE) +41-433994049 (CH) +43-820874464 (AUT) technischerservice@millipore.com	siIMPORTER	Wide variety of mammalian cell lines	■ Cationic lipid formulation for efficient transfection of siRNA ■ Extremely low cytotoxicity ■ Includes siRNA diluent ■ Perform at least 200 transfections ■ Sample pack available	279.-
	SMARTpool siRNA reagent	For use in human cell lines	■ Contains siRNA SMARTpools and Control Pools ■ Available for several targets, e.g. Cdk2, Ras, Bcl2 ... ■ Targeted message level is reduced by more than 70% 24 hours after transfection	459.-
	siRNA/siAB Assay Kits	For use in mammalian cell lines	■ Contains siRNA SMARTpools and Control Pools and antibody against the targeted protein ■ Available for several targets, e.g. Abl, Akt3, c-Jun ... ■ Targeted message level is reduced by more than 70% 24 hours after transfection	749.-
PAA Laboratories Coelbe, Germany www.paa.com **Contact:** Manuel Klang Phone +49(0)6421 17539-0 techservice@paa.com	Nanofectin siRNA	Epithelial-like cells, Fibroblast-like cells, Endothelial-like cells, Hepatocyte cells, Tumor cells, Primary cells, other cells	■ Over 85% gene silencing ■ Excellent cell viability ■ Transfection in the presence and/or absence of serum ■ Highly reproducible ■ Easy protocol for use	46.- (200 µl) 225.- (1 ml)
PromoCell Heidelberg, Germany www.promokine.info **Contact:** Technical Support info@promokine.info Phone: +49-6221 64934-0	PromoFectin-siRNA	Numerous cell lines and primary cells (also hard-to-transfect cell types)	■ High transfection efficieny ■ Low cytotoxicity ■ Efficient gene suppression ■ Compatible with and without serum-containing media ■ Easy protocols for adherent and suspension cells	119.- (50 transfections) 269.- (200 tr.) 999.- (1000 tr.)
	MATra-siRNA	Numerous cell lines and primary cells (also hard-to-transfect cell types)	■ Extremely fast and efficient magnet-assisted transfection procedure ■ Low cytotoxicity ■ Functional with and without serum and antibiotics ■ Can be used with adherent and suspension cells ■ Suited well for high-throughput transfections	50.- (50 µl) 170.- (200 µl) 680.- (1000 µl)
Sigma-Aldrich Chemie Taufkirchen, Germany www.sigma.com **Contact:** Rainer Ebel Phone: +49-89 6513-1452 Rainer.ebel@sial.com	N-TER, Nanoparticle siRNA Transfection System	Mammalian standard cell as well as difficult to transfect cells lines (incl. primary, neuronal, differentiated and non-dividing cells)	■ Peptide-based system, non-liposomal, non-endosomal delivery ■ High transfection efficiency, even at low concentrations ■ The N-TER Peptide binds siRNAs non-covalently, forming a nanoparticle ■ N-TER/siRNA Nanoparticle can be stored up to one year ■ Low toxicity	79.20,- (120 µ) 198.- (400 µl) 374.- (1 ml)