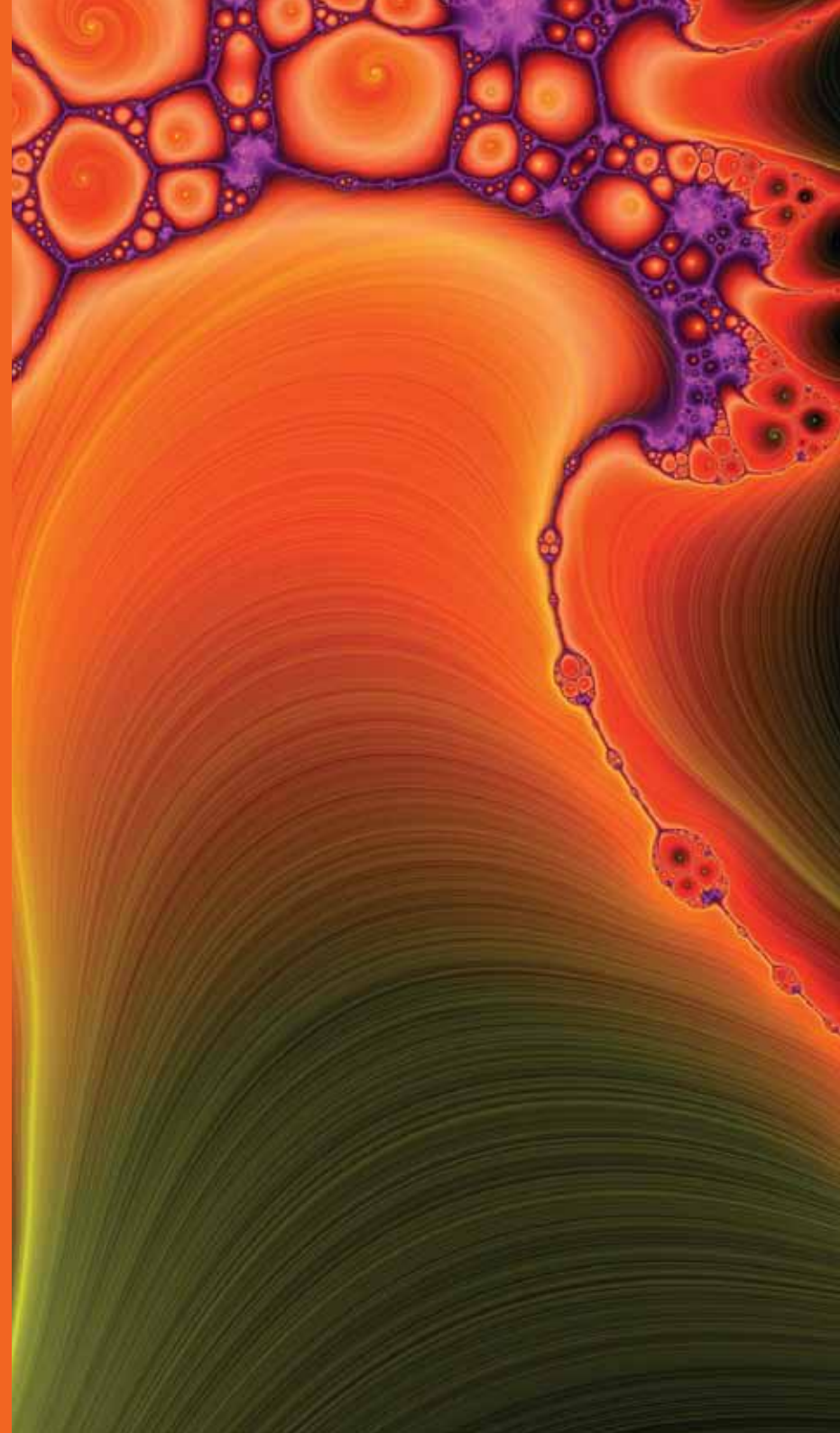


2

2.6 Mammalian cell transfection reagents

169 DNA transfection : ICAFectin™ 441

170 siRNA transfection : ICAFectin™ 442



Mammalian cell transfection reagents

DNA transfection : ICAFectin™ 441

ICAFectin™ 441 Transfection Reagent is based on an innovative synthetic derivative of a natural compound, and especially designed to provide the best transfection efficiency and protein expression level.

Easy handling

- The ICAFectin™ 441 / DNA complex can be transfected in cells in the presence of serum.
- ICAFectin™ 441 is non-toxic, the removal of transfection complex is not needed.
- ICAFectin™ 441 / DNA complex may be kept at RT during transfection.

High-throughput compatible

The simple and rapid reverse transfection protocol of ICAFectin™ 441 makes it ideal for high-throughput transfections for transient protein expression and cDNA library screening. Transfection can easily be established for automated or robotic systems.

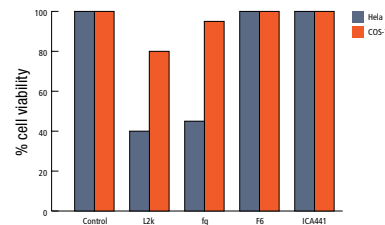


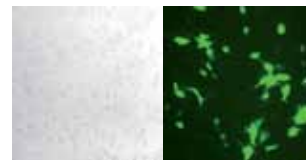
Figure 1: Cell viability analysis after transfection using ICAFectin™ 441 or competitors reagents

Outstanding transfection efficiency

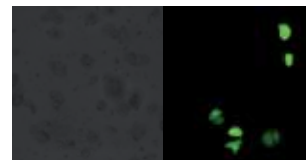
ICAFectin™ 441 Transfection Reagent surpasses the performance of competitors' reagents composed of other cationic lipid. It has been used successfully to transfect cell types derived from diverse species and tissues¹, including:

- Cancer cell lines
- Adherent and suspension cells
- Established cell lines
- Primary cells
- Stem cells

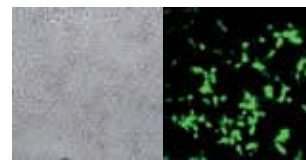
GFP microscopy visualisation



Mesenchymal stem cells (BMC9)



Primary Hepatocytes



COS-7 (Fibroblast-like cells)

Figure 2: Fluorescent microscopy visualisation of cells transfected with 0.5 µg DNA encoding the fluorescent protein GFP using the ICAFectin™ 441 reagent. The transfected cells were observed 24 hours after transfection using a FITC filter to visualise GFP fluorescence (right) or by light microscopy (left) to visualise cell morphology.

Luciferase activity after transfection

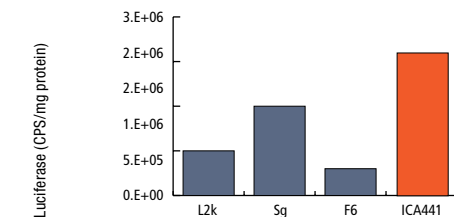
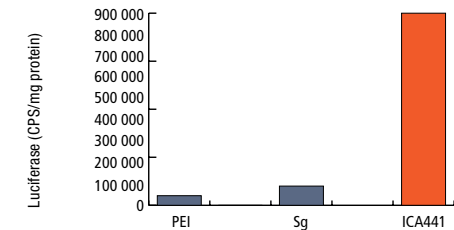
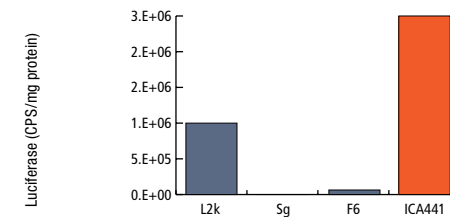


Figure 3: Luciferase activity 24 hours after transfection of cells with 0.5 µg DNA encoding luciferase complexed with ICAFectin™ 441 compared to that obtained with competitor reagents.

¹ Expression of the transfected gene depends on the cell type, the promoter used, the nature of the expressed protein and the amounts of ICAFectin™ 441 and DNA. Therefore transfection conditions should be optimized for every new cell type.

siRNA transfection : ICAFectin™ 442

ICAFectin™ 442 Transfection Reagent is based on an innovative synthetic derivative of a natural compound, and is used to transfect siRNA for RNA interference applications into a wide range of cell types such as HeLa, HEK 293, HepG2, MCF-7, HUVEC, HaCaT cells and many others.

Easy handling

- The ICAFectin™ 442 / siRNA complex can be transfected in cells in the presence of serum.
- ICAFectin™ 442 is non-toxic, the removal of transfection complex is not needed.
- ICAFectin™ 442 / siRNA complex may be kept at RT during transfection.

High-throughput compatible

The simple and rapid reverse transfection protocol of ICAFectin™ 442 makes it ideal for high-throughput transfections for transient protein expression and cDNA library screening. Transfection can easily be established for automated or robotic systems.

Outstanding transfection efficiency No off-target effect

ICAFectin™ 442 leads to a high release of siRNA within the cell and allows to use low concentration of siRNA. Reducing the amount of siRNAs used for transfections down to 1-20nM minimizes changes in gene expression in mammalian cultured cells (off-target effects). The knockdown efficiency of ICAFectin™ 442 was compared to other products available on the market. ICAFectin™ 442 shows the highest efficiency with a variety of cell lines.

Gene silencing in rat primary hepatocytes

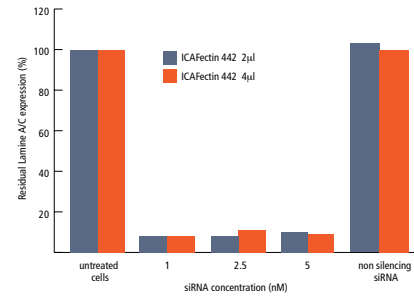


Figure 4: In each condition, residual Lamin A/C expression was determined to be 8-10 % of the control cells. Interestingly, identical Lamin A/C expression was obtained in cells transfected with non-silencing siRNA and in untransfected cells. This indicates that the transfection with ICAFectin™ 442 did not affect the endogenous Lamin A/C expression and is devoid of any “off-target effect”.

Lamin A/C silencing efficiency using ICAFectin™ 442 reagent or competitor reagents

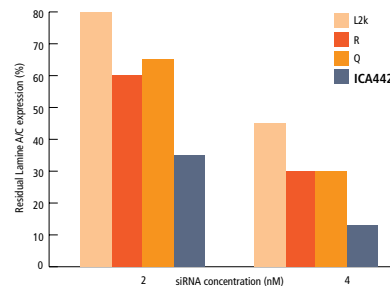


Figure 5: Anti-LaminA/C siRNA at 2 and 4nM (18.75 and 37.5 ng/well) were formulated with ICAFectin™ 442 or commercial reagents. Results of real-time quantitative RT-PCR analysis of human Lamin A/C mRNA after transfection of HeLa cells showed that ICAFectin™ 442 reagent was more efficient than the top leader products on the market.

GFP silencing efficiency using ICAFectin™ 442

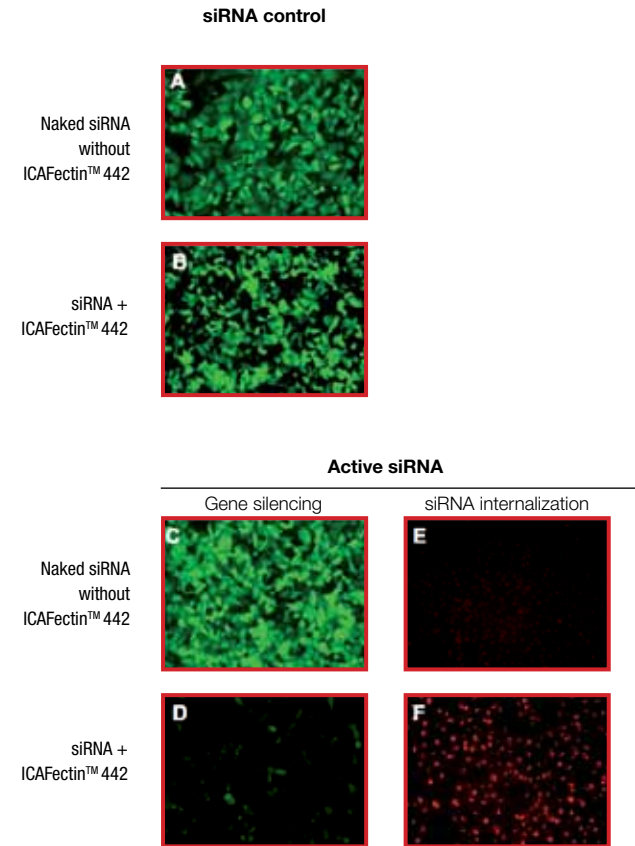


Figure 6: Fluorescence microscopy visualization of GFP silencing and siRNA internalization. The GFP-expressing human lung cancer H1299 cells were transfected with control siRNA (A,B) or 3'-rhodamine labeled anti-GFP siRNA (C-F). The siRNA molecules were formulated in the absence (“naked” siRNA in A,C,E) or in the presence of ICAFectin™ 442 reagent (B,D,F). The transfected H1299 cells were observed using a FITC filter to visualize GFP fluorescence (A-B) or a rhodamine filter to visualize siRNA internalization (E,F).

ICAFectin™ transfection reagents

Description	RXNs	Volume	References
ICAFectin™ 441 DNA transfection	375	0.5 ml	TR-ICA-441
ICAFectin™ 442 siRNA transfection	250	0.5 ml	TR-ICA-442
Free ICAFectin™ 441 sample	38	50 µl	TR-ICA-441SA
Free ICAFectin™ 442 sample	25	50 µl	TR-ICA-442SA

Related products

Control siRNA Duplexes

Description	Species	Synthesis scale	Purification	References
Control siRNA duplex LaminB1	Human	5 nmol	PAGE	SR-CL001-005
Control siRNA duplex Vimentin	Human	5 nmol	PAGE	SR-CL002-005
Control siRNA duplex NuMA	Human	5 nmol	PAGE	SR-CL003-005
Control siRNA duplex Beta-actin	Human	5 nmol	PAGE	SR-CL004-005
Control siRNA duplex Eg-5	Human	5 nmol	PAGE	SR-CL005-005
Control siRNA duplex Cdk-1	Human	5 nmol	PAGE	SR-CL006-005
Control siRNA duplex pGL2 luciferase	Firefly	5 nmol	PAGE	SR-CL010-005
Control siRNA duplex pGL3 luciferase	Firefly	5 nmol	PAGE	SR-CL011-005
Control siRNA duplex GFP	Jellyfish	5 nmol	PAGE	SR-CL020-005
Control siRNA duplex negative control	–	5 nmol	PAGE	SR-CL000-005

RNAi Oligonucleotides



See chapter 2.1

Product Citations

ICAFectin™ 441

Bernot D. *et al.* (2010) *J. Biol. Chem.* (285) 6508-6514
 Zychlinski D. *et al.* (2009) *Nucleic Acids Research*, 1-12

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Chèvre R. *et al.* (2010) *Nucleic Acids Research* 1-13
 Journo C. *et al.* (2009) *PLoS Pathogens*, (5) 7 e1000521
 Schirrmeyer W. *et al.* (2009) *Experimental cell research* (315) 3500-3508