

WHY TARGEFECT?

Highly efficient gene transfer accomplished using novel approaches to gene delivery!

- Use of unique reagents that escort genes directly to the nucleus and disrupt endosomes
- Use of simple to use lipid and non-lipid formulations that are extremely effective for gene delivery into many hard to transfect primary cells types
- Use of adenovirus -based enhancer reagents that exploit adenoviral receptors on the cell surface for efficient gene transfer. This method yields high transfection efficiencies without the requirement for constructing a recombinant virus.
- We provide optimized cell-specific protocol for most cell types.
- Simplified protocols provided for high throughput screening applications.

Targefect Reagents will give you high efficiencies of gene transfer even in hard to transfect primary cell types where other commercial reagents don't work. Some of the targefect reagents (Targefect F-2, Peptide enhancer, Virofect) directly take DNA to the nucleus because of their unique nuclear escorting properties. Since it is well documented that nuclear transfer enhances gene delivery, this usually results in higher transgene expression. The confocal image transfected with a green fluorescent protein expression vector shown below (Courtesy of Drs Steve Murphy and Dr J. Murphy, UT Southwestern Medical Center, Dallas, TX) clearly demonstrates efficient nuclear delivery

Targeting Systems focuses on the development of novel, efficient delivery systems for gene transfer:

Product Profile

Targefect F-1 is a formulation of cationic lipid vesicles in a special medium. The F-1 reagent has been effectively used in complex co-transfection experiments (co-delivery of up to 6 different plasmids).

Targefect F-2 is a non-lipid cationic polymers with many characteristics that make it an attractive transfection reagent, ability to deliver DNA into a wide range of cell types and low toxicity at the concentration required for optimal gene transfer. In addition targefect F-2 has DNA-condensing properties, which enhance cellular uptake of DNA.

Enhancer (Virofect): Virofect is an adenovirus-derived formulation, which has the ability to complex with unmodified plasmid DNA via the cationic targefect F-1, F-2 or other cationic lipid reagents. Virofect is likely to enhance gene transfer by increasing cellular uptake and enabling escape of the transfected DNA from lysosomal degradation because of the ability of Virofect to lyse the endosome. The Virofect enhancer formulation does not contain any replication-competent virus. The enhancer has been shown to significantly enhance cationic liposome-mediated gene transfer into a wide variety of cell types such as bovine and human endothelial cells, rat hepatocytes, bronchial epithelial cells, Hela cells, and cardiac myocytes.

This method yields high transfection efficiencies without the requirement for constructing a recombinant virus. Once the optimal DNA: targfect ratio has been determined add 5 μ l, 15 μ l or 20 μ l of Virofect per ml of transfection complex. Virofect should be added immediately after addition of targfect to DNA. Virofect* is a patented product, See reference below for more information.

Raja-Walia R, Weber J, Naftilan J, Chapman GD and Naftilan AJ. (1995) Gene Therapy, 2: 521-530)

Peptide enhancer: An endosmolytic peptide that enhances the efficiency of targfect-mediated gene transfer in certain cell types. The peptide enhancer complexes with DNA and targfect and escorts the transfection complex to the nucleus thus increasing the efficiency of gene expression.

General advantages of using Targfect reagents:

- Ability to transfect a wide range of cell types including many hard-to-transfect primary cells.
- High transfection efficiency compared to other commercial transfection reagents
- Low toxicity at concentrations required for optimal transfection.
- Extremely cost -effective compared to other commercial reagents both in terms of price and number of Transfections/kit. (0.5 ml of targfect is sufficient for 250-500 transfections performed in 24-well dishes).
- Simple 20 minute transfection protocol with serum compatibility especially well suited for High Throughput Screening.
- Optimized protocols provided for many cell types.
- Stability of transfection reagents (approx. 1 yr)
- Targfect reagents are well suited for complex co-transfection experiments. Up to 6 different plasmids have been simultaneously delivered to hepatocytes using Targfect F1

Primary Cell types

HUVECc
HMVECs

Cell Lines

HAECs (human aortic endothelial cells)		
Porcine endothelial cells		
Bovine aortic endothelial cells	A549	HT108
Sheep endothelial cells	B lymphoma	Hep3B
Hepatocytes (rat , mouse, chimp and human)	(murine)	HO23
Rat liver stellate cells	BEAS	INS-1
Kupffer cells	C2C12	Jurkat
Rat aortic smooth muscle cells	Capan1	K562
Human aortic smooth muscle cells	CHO-K1	LNCaP
Mouse Embryo fibroblasts	Cos-1	3T3-L1
Human foreskin fibroblasts	Cos-7	preadipocytes
Human keratinocytes	CV-1	MCF-7
Human skeletal muscle cells	2-CF	MDA 468
Neonatal rat cardiomyocytes	DT-40	MDCK
Brochial epithelial cells	Fao hepatoma	NB-4
Human lens epithelial cells	HCT116	Neuro2A
Liver stem cells	HEK-293	NIH3T3
Human melanocytes	HEK-293t	NRK6
Procine Chondrocytes	HeLa	neuroblastoma
Osteoblasts	HeLa S3	OVCAR
Rat hippocampal neurons	HepG2	PC-12
Cardiac myocyte	HS-27	Raw-264.7
Human mesenchymal stem cells	HL60	U2OS
	SiHes2	Wet1-3B

Transfection protocol for transfection in presence of serum:

Set up cells to be transfected so that they are about 70-80% confluent at the time of the experiment. Store Targefect F-1 and Virofect at – 20 o C. Store Targefect F-2 and the peptide enhancer at 4 o C and mix well upon arrival as the reagents sometimes freeze during shipment. Do not vortex the F-2 reagent. Note that the Targefect F-1 reagent needs to be thawed and vortexed at full speed for 30 seconds once or twice just before use. Plate out or maintain cells to be transfected in media with 5%-10% serum (or charcoal stripped serum). This protocol has been optimized for cells maintained in media with 5% Serum.

Prepare transfection complexes as follows:

Note: It is important to use high glucose DMEM (DMEM containing 4500 mg/liter glucose for complex formation. Use clear plastic tubes for complex formation. Vortex the F-1 reagent thaw the reagent and vortex at full speed for 30 seconds once just before preparing the transfection complexes. Do not vortex the Targefect F-2 reagent.

Prepare transfection complexes as described below (transfection of cells in 6-well plates):

Tube #	DMEM	Plasmid DNA	Targefect-293
1	0.5 ml	6. µg	12 µl

Add DMEM 1 first, then add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add targefect next, mix well again by flicking the tube. Incubate the tubes at 37 o C for 20 minutes to form the transfection complexes. Add 250 µl of transfection complex to 2 ml of complete media per well of a 6-well dish. Swirl the dish to mix transfection complexes with complete media and incubate at 37oC overnight. Assay at 24-48 hrs post transfection.

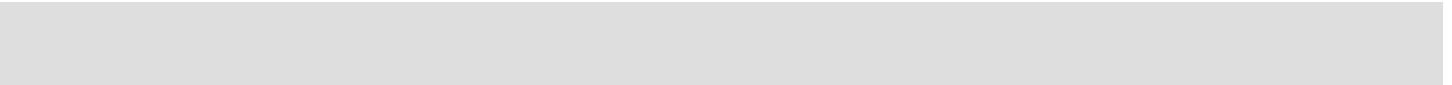
Recommended volumes of transfection complex for performing transfection in different size dishes:

Culture Vessel	Volume of plating medium	DNA (µg) in transfection complex volume (µl)	Targefect in transfection complex volume (µl)
96 well	100 µl	0.2 µg in 25µl	0.5 µl in 25µl
24 well	500µl	0.8 µg in 50µl	1.5 µl in 50 µl
6 well	2ml	3.0 µg in 250µl	6µl in 250µl
60mm	5ml	6.0 µg in 0.5ml	12µl in 0.5 ml
10cm	15ml	18 µg in 1.5ml	36µl in 1.5ml

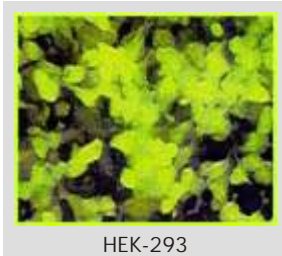
Complexing conditions using Targefect reagents plus Enhancers: Once the optimal DNA: targefect ratio has been determined for the cell type of interest, we recommend complexing 10 µl or 25 µl, 35 µl of Virofect enhancer per 0.5 ml of transfection complex. And then following the general protocol. Usually 25 µl of Virofect /0.5 ml transfection complex works best For transfection using the peptide enhancer please follow the guidelines recommended in the Targefect-endothelial protocol on our website

Note Most cell types can be efficiently transfected using either the F-1 or F-2 reagent alone. Please read through the Targefect brochure to find out which cell types transfect better using the peptide enhancer or Virofect.

*Cell-specific protocols along with a list of published citations are provided for many commonly used cell types upon request. Please email us at targsys@aol.com to receive the recommended transfection protocol for your cell



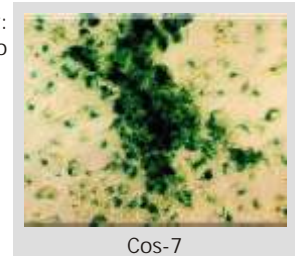
Transfection Of Different Cell Types With Targefect Reagents:



HEK-293

Fig. 1: HEK 293 cells transfected with targetfect F1:
(Data provided by Dr. Peter Ordentlick, Dr Ronald M Evans
' lab, Howard Hughes Medical Institute,
The Salk Institute for Biological Studies, La Jolla, CA.

Fig. 2: Cos-7 cells transfected with the Targetfect F-2 reagent:
Transfection efficiency approx 80%, Targeting Systems, San Diego



Cos-7



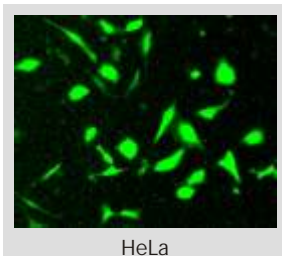
Mouse hepatocytes

Fig. 3: Mouse hepatocytes transfected with
the Targetfect F-1 reagent: Data courtesy of Dr Suzanne
Lyman, dr Behren's lab, University
of North Carolina at Chapel Hill, NC

Fig. 4: Neuro 2A cells transfected with Targetfect F-1 reagent:
transfection efficiency approx 60%



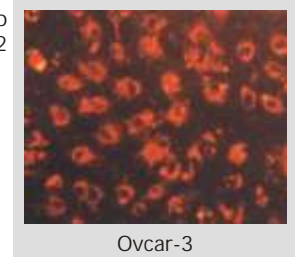
Neuro 2A



HeLa

Fig. 5: Hela cells transfected with the targetfect F-1 reagent:
Data courtesy of Dr Radu Stan-Vigil, University of California
at San Diego, Sand Diego, CA

Fig. 6: Delivery of fluorescent oligonucleotides into
OVAR-3 cells using Targetfect F-2



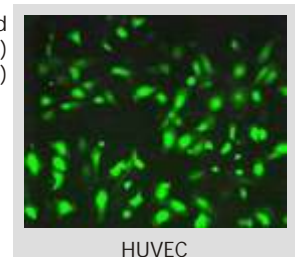
Ovcar-3



MCF-7

Fig. 7: MCF-7 cells transfected with the
Targetfect F-2 reagent: Transfection efficiency
approx- 70-80%, Targeting Systems, San Diego

Fig. 8: Human umbilical vein endothelial cells transfected
with targetfect -HUVEC (Targetfect F-2 plus virofect)
Targeting Systems, San Diego)



HUVEC

Pricing:

Catalog #	Product	Quantity	Price
#001	Targefect F-1	0.5 ml	\$115.00
#002	Targefect F-1	4X0.5 ml	\$420.00
#003	Targefect F-2	0.5 ml	\$115.00
#004	Targefect F-2	4X0.5 ml	\$420.00
#007	Virofect	.6 ml	\$100.00
#008	Peptide Enhancer	0.5 ml	\$100.00
#009	Variety pack	4X0.5 ml	\$420.00

Note: the number of reactions are approximated based on transfections in 35mm dishes.

Storage conditions and Stability:

Targefect F-1, Store at -20° C

Targefect F-2, Peptide enhancer, Targefect siRNA kit Store at 4° C

Virofect enhancer: Store at -20° C, Peptide Enhancer-Store at 4° C

To place an order: Please call us or send a fax indicating item #, purchase order code and your billing and shipping information

Related Products:

CELL-SPECIFIC TRANSFECTION REAGENTS:

These are transfection kits composed of the Targefect F-1 or F-2 reagents with or without enhancers and provided with optimized protocols for transfection of specific cell types. They are also more economical as they contain a lot more reagent.

Targefect-293

Targefect-Cos

Targefect-HUVEC

Targefect-Hepatocyte

Targefect-SMC

Targefect-BAC

Targefect-Hela

Targefect-Raw

Targefect-BAC

Targefect-Chondrocyte

Targefect-Osteoblast

Targefect- PCL

Targefect- HSC

Targefect-Keratinocyte

Targefect-Melanocyte

Profect: Reagents for efficient delivery of functionally active proteins.

Targefect siRNA kit: A transfection reagent kit for efficient delivery of siRNA into mammalian cells. For more info please go to www.targetingsystems.com/siRNA.pdf

Novel Gaussia Luciferase Assay system- 1000-fold brighter than firefly and renilla luciferases. Gaussia luciferase is a secreted luciferase which offers several advantages over both firefly and renilla luciferases. For more information please go to www.targetingsystems.com

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