



Diagnostics

Transfection Reagents

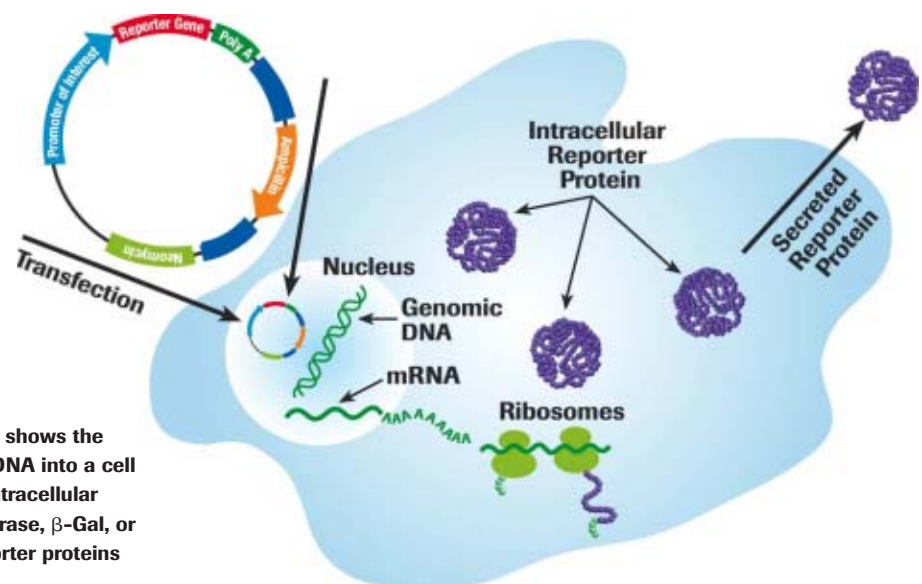
Powerful Solutions. Powerful Results.



www.roche-applied-science.com

Transfection is the introduction of cloned DNA into a cultured cell for expression of a gene, a technique essential to life sciences research. Routinely used in gene expression analysis as well as for the production of proteins, it is a critical link between the study of genomics and proteomics.

Introduction to Transfection	2-3
DNA Transfection	4-8
FuGENE® 6 Transfection Reagent	4-5
FuGENE® HD Transfection Reagent	6-7
Other Transfection Reagents and Related Products	8
siRNA Transfection	9
X-tremeGENE siRNA Dicer Kit	9
X-tremeGENE siRNA Transfection Reagent	9
Ordering Information	10-11



▶ **Figure 1:** The schematic shows the introduction of foreign DNA into a cell and the expression of intracellular reporter proteins (luciferase, β -Gal, or CAT), and secreted reporter proteins (hGH or SEAP).

Powerful Solutions. Powerful Results.

Roche Applied Science delivers a powerful family of advanced products for transfection. However, no single reagent or technique alone is suitable for the multitude of transfection applications being performed today. Experimental factors to consider in selecting a transfection reagent include:

- Whether gene expression or protein production is the desired end result
- The cell type to be used, such as adherent, suspension-adapted, or primary cells
- The molecule to be transfected (*i.e.*, DNA, RNA, siRNA, oligonucleotides, or protein)
- If this is a low-, medium-, or high-throughput application

Key performance issues to consider in selecting a transfection reagent are:

- Transfection efficiency and levels of protein expression
- Volume of reagent required per transfection
- Simplicity and reproducibility of method
- Cytotoxicity and non-target gene expression effects

Given all of these variables in experimental design and performance, a particular transfection reagent may possess advantages over others.

Select the Roche Applied Science transfection reagent that best meets your specific needs, and be confident you have selected a proven transfection reagent of the highest quality.

Product Selection Guide by Application

Application

Products (primary recommendation)

Plasmid DNA transfection of adherent cells for cellular analysis

- gene regulation
- pathway analysis
- protein interaction

FuGENE® 6 Transfection Reagent
FuGENE® HD Transfection Reagent
DOTAP Liposomal Transfection Reagent

Plasmid DNA transfection of adherent and suspension-adapted animal and insect cells for protein expression

FuGENE® HD Transfection Reagent
FuGENE® 6 Transfection Reagent

siRNA transfection

X-tremeGENE siRNA Transfection Reagent

Plasmid DNA transfection of K-562 or Jurkat cells

X-tremeGENE Q2 Transfection Reagent
FuGENE® HD Transfection Reagent

Transfection requiring reagents free of animal-derived components

FuGENE® HD Transfection Reagent

FuGENE® 6 Transfection Reagent

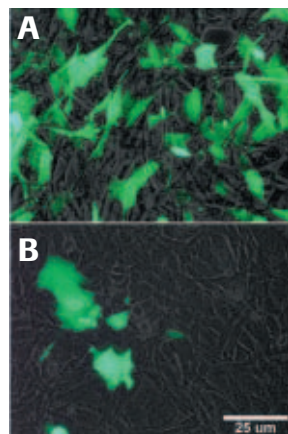
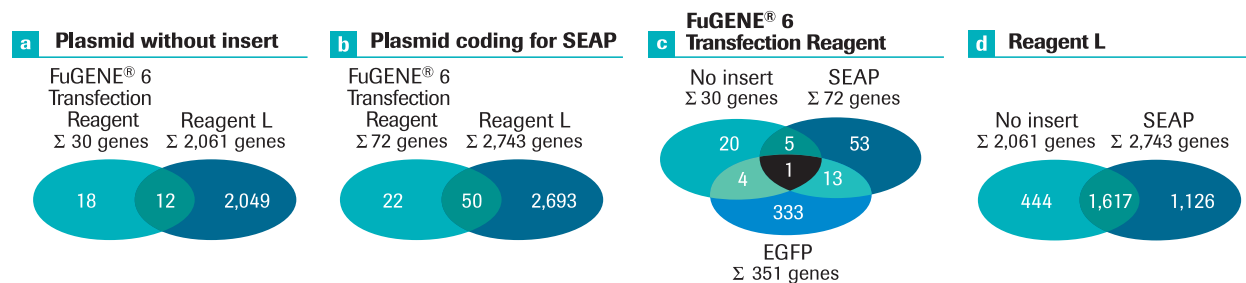
Easy on Your Cells. Easy on You.

FuGENE® 6 Transfection Reagent is a non-liposomal multicomponent reagent perfect for experiments involving cellular analysis due to its simple methodology, minimal optimization, low cytotoxicity, and ability to provide high transfection efficiency at low cell densities even in the presence of serum. With a mention in more than 6,000 publications, FuGENE® 6 Transfection Reagent has become one of the most widely used transfection reagents in the world. This popular reagent offers multiple benefits:

- **Avoid time-consuming optimization procedures** by relying on high transfection efficiency (Figure 3) proven in more than 700 cell types, including primary cell lines
- **Save time and effort by eliminating multiple handling steps** (Figure 5); simply dilute FuGENE® 6 Transfection Reagent, incubate it with plasmid DNA, and pipet the mixture directly onto your cells (with or without serum)

- **Generate physiologically relevant results with the low cytotoxicity of FuGENE® 6 Transfection Reagent.** A recent gene-chip study demonstrated that a leading competitor's transfection reagent significantly influenced the expression of more than 2,000 genes, compared to only 30 genes affected by FuGENE® 6 Transfection Reagent (Figure 2)
- **Use less reagent per transfection.** Perform more transfections with 1 ml of FuGENE® 6 Transfection Reagent than with 1 ml of competitive reagents
- **Obtain a higher yield of stable transfectants** through higher cell-survival rates

See if your cell type is among the more than 700 cell types successfully transfected with FuGENE® 6 Transfection Reagent by visiting www.roche-applied-science.com/transfection



▲ **Figure 2: Alterations in gene expression following transfection with FuGENE® 6 Transfection Reagent or Reagent L.** The number of affected genes after transfection with FuGENE® 6 Transfection Reagent (c) is compared with those after transfection using reagent L (d). The changes in expressed genes were compared between transfection with FuGENE® 6 Transfection Reagent and reagent L for the plasmid without insert (a) and the plasmid coding for secreted human placental alkaline phosphatase (SEAP) (b). Overlapping areas represent genes with changed expression levels for all the plasmids (c and d) or by both transfection reagents (a and b).

Reference: *Biochemica* (2004) 4:9-11. View online at www.roche-applied-science.com/publications/biochemica.htm

◀ **Figure 3: Expression of green fluorescent protein (GFP) in MC3T3-E1 cells, following transfection with vector phGFP-S65T using (A) FuGENE® 6 Transfection Reagent or (B) leading supplier's liposomal transfection reagent (L).**

(Data courtesy of S. Ritter, Univ. of Texas, Houston, TX, USA, see *Biochemica* 1998 (3): 47-49)

FuGENE® 6 Transfection Reagent

For High-Throughput Screening Applications

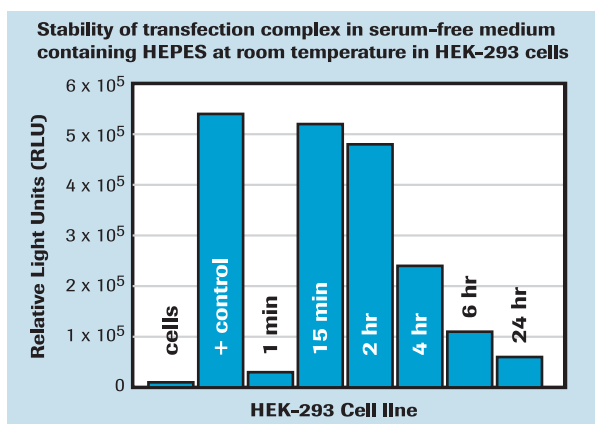
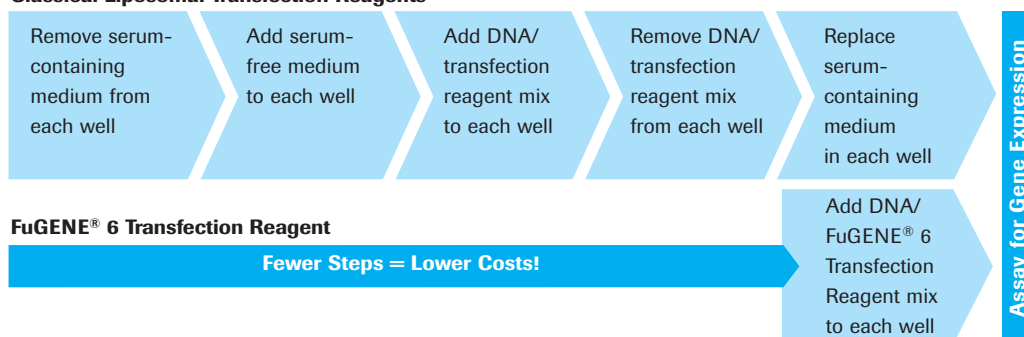
FuGENE® 6 Transfection Reagent has proven to be highly effective in automated high-throughput screening protocols due to the:

- Stability of the FuGENE® 6 Transfection Reagent:DNA complex for up to 2 hours (Figure 4)
- Low volume of the complex required for effective transfection
- Ability to transfect cells at low density
- Equally high effectiveness whether the complex is added to cells, or cells to the complex
- Ability to efficiently transfect cells immediately following trypsinization, prior to or just following plating

These factors, in combination with the simple and consistent protocol, effectiveness in the presence or absence of sera, and low cytotoxicity with minimal off-target gene expression effects make FuGENE® 6 Transfection Reagent the premier transfection reagent for high-throughput screening applications.

▼ **Figure 5: Transfection procedure.** Save time and effort by eliminating multiple handling steps; simply dilute FuGENE® 6 Transfection Reagent, incubate it with plasmid DNA, and pipet the mixture directly onto your cells (with or without serum).

Classical Liposomal Transfection Reagents



▲ **Figure 4: Stability of transfection complex in serum-free medium.** The complex was formed by combining 50 µg plasmid DNA with 150 µl FuGENE® 6 Transfection Reagent in 5 ml serum-free growth medium. The complex was added to HEK-293 cells at various time points. SEAP expression (RLU) was measured at 48 hours post-transfection.

(Data courtesy of L.B. Jacobsen *et al.*, *Methods* 33 (2004) 104–112.)

Product	Cat. No.	Pack Size
FuGENE® 6 Transfection Reagent	11 815 091 001	0.4 ml (up to 120 transfections in 6-well plates)
	11 814 443 001	1 ml (up to 300 transfections in 6-well plates)
	11 988 387 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)

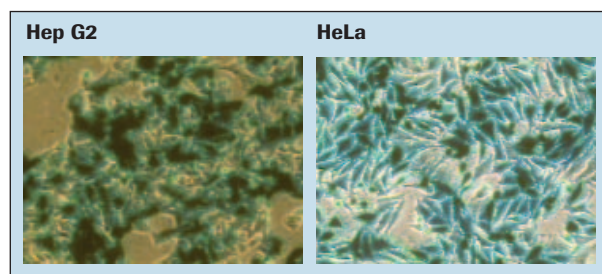
DNA Transfection

FuGENE® HD Transfection Reagent

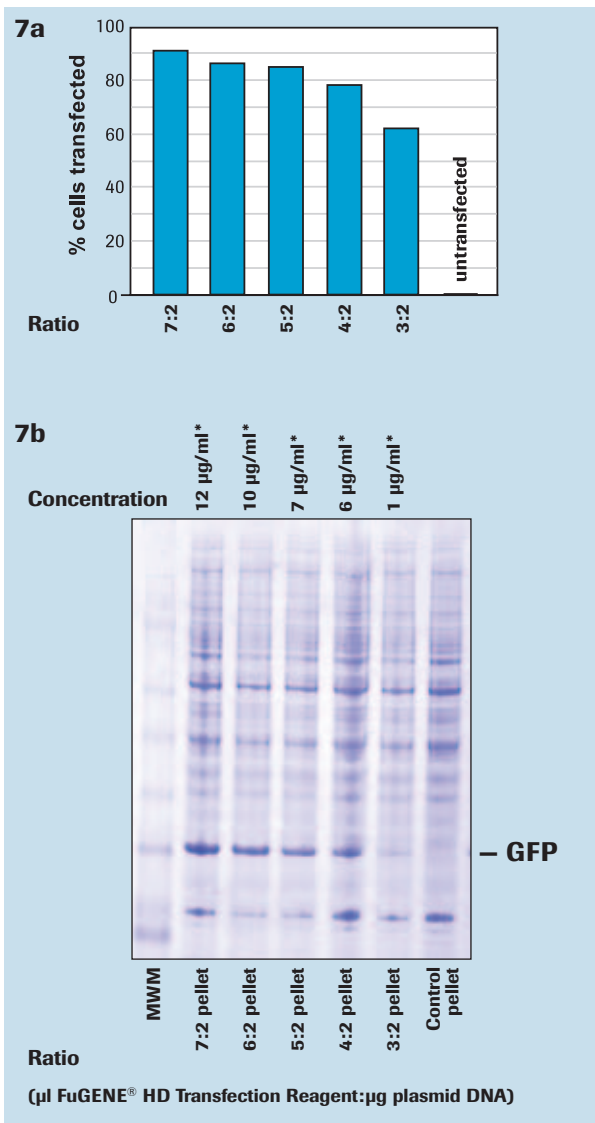
Powerful Protein Expression

FuGENE® HD Transfection Reagent is a non-liposomal multicomponent reagent suitable for transfection of animal and insect cells for protein expression. It is free of animal- or human-derived components. The combination of a rapid protocol, activity in up to 100% serum, and effectiveness with many adherent and suspension-adapted cell lines commonly used for protein expression makes it the product of choice for this application.

- **Obtain high levels of protein expression** in many common adherent and suspension-adapted animal cell lines, including HeLa, NIH/3T3, COS-1, COS-7, CHO-K1, Hep G2, HEK-293, MCF-7, and insect cell lines such as Sf9 (Figure 7a and b)
- **Achieve excellent transfection efficiency** in some cell lines (e.g., RAW) that are not transfected well by other reagents (Figure 6)
- **Minimize cytotoxicity** or changes in morphology by transfecting cells at high densities
- **Save time** by eliminating the need to change media — the reagent functions exceptionally well in the presence or absence of serum
- **Employ a reagent that is free of animal- or human-derived components**



▲ **Figure 6: FuGENE® HD Transfection Reagent effectively transfects many difficult-to-transfect cell lines.** Several cell types were transfected using FuGENE® HD Transfection Reagent to deliver the pM1-β-Gal Expression Vector and stained 24-48 hours later. Ratios between 2.5:1 and 3:1 (μl FuGENE® HD Transfection Reagent:μg plasmid DNA) were found to be most effective.



▲ **Figure 7a and b: FuGENE® HD Transfection Reagent provides high transfection efficiency and protein expression.** HEK-293 EBNA suspension-adapted cells were transfected with plasmid DNA for GFP following the recommended protocol, using ratios of 7:2, 6:2, 5:2, 4:2, and 3:2. The percentage of cells transfected was determined 28 hours post transfection, and quantity of GFP protein was estimated from the Coomassie-stained gel at 72 hours post transfection.

* estimated yield

FuGENE® HD Transfection Reagent

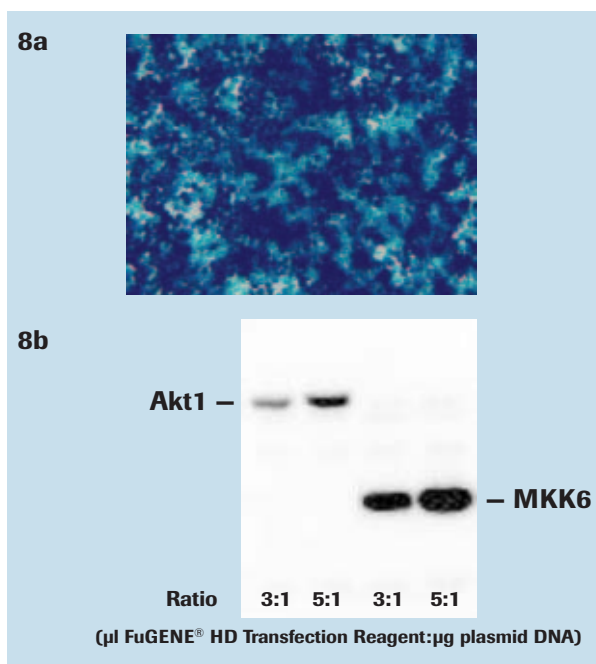
Transfection of Insect Cells for Protein Expression

FuGENE® HD Transfection Reagent has also proven highly effective for protein expression using insect cell lines such as Sf9, providing a replacement for the tedious and time-consuming baculovirus cell expression system. Employ FuGENE® HD Transfection Reagent to transfect insect cells and:

- **Save up to 18 days compared to baculovirus cell expression** from start of experiment to purification of protein (Figure 9)
- **Achieve high transfection efficiency** along with high levels of protein expression (Figure 8)
- **Reduce resources required and increase throughput** with a simple and consistent protocol

Product	Cat. No.	Pack Size
FuGENE® HD Transfection Reagent	04 709 691 001	0.4 ml (up to 120 transfections in 6-well plates)
	04 709 705 001	1 ml (up to 300 transfections in 6-well plates)
	04 709 713 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)

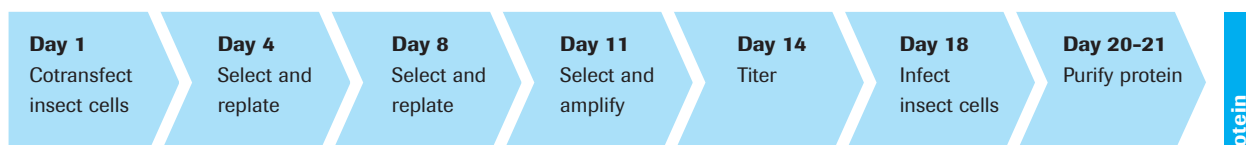
For more information about our transfection reagents, visit www.roche-applied-science.com/transfection



▲ **Figure 8a and b: Achieve high transfection efficiency and protein expression.** Transfection of a commonly used insect cell line using FuGENE® HD Transfection Reagent for expression of human kinases Akt1 and MKK6. X-Gal staining was performed 24 hours post transfection. Western blot from cell lysate at 72 hours post transfection.

▼ **Figure 9: Comparison of traditional baculovirus cell expression method versus FuGENE® HD Transfection Reagent.** Save up to 18 days from the start of procedure until recovery of purified protein by utilizing FuGENE® HD Transfection Reagent to transfect insect cells rather than baculovirus cell expression.

Traditional baculovirus method



FuGENE® HD Transfection Reagent



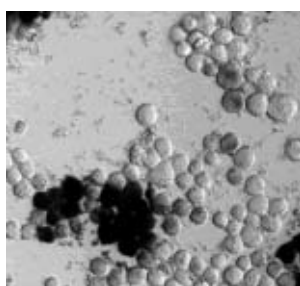
DNA
Transfection

Other Transfection Reagents and Additional Products

X-tremeGENE Q2 Transfection Reagent

X-tremeGENE Q2 Transfection Reagent was designed and optimized for transfection of **K-562 and Jurkat cells**, for high levels of protein expression with minimal optimization.

- Achieve **high transfection efficiency** in your K-562 and Jurkat cells (Figure 10)
- Obtain **high protein expression levels** without stimulation of Jurkat and K-562 cells with PHA and PMA



◀ **Figure 10: K-562 cells transfected with X-tremeGENE Q2 Transfection Reagent and X-tremeGENE pM1-β-Gal vector show strong β-galactosidase staining.** Cells were plated 1×10^5 cells/well in a 24-well plate and transfected with a complex of 2 μg of plasmid DNA and 4 μl of X-tremeGENE Q2 Reagent.

Forty-eight hours post transfection, cells were stained for β-Gal expression using the β-Gal Staining Set (Cat. No. 11 828 673 001).

Product	Cat. No.	Pack Size
X-tremeGENE Q2 Transfection Reagent	03 045 595 001	0.4 ml (up to 100 transfections in 6-well plates)
	03 036 421 001	1.8 ml (up to 450 transfections in 6-well plates)

DOTAP Liposomal Transfection Reagent

N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate

This time-tested, proven reagent has successfully transfected many established and primary cell lines *in vitro*. Additionally, DOTAP has been extensively used in gene-therapy research with non-human animal models, *in vivo*.

Product	Cat. No.	Pack Size
DOTAP Liposomal Transfection Reagent	11 811 177 001	0.4 ml
	11 202 375 001	2 ml (5 x 0.4 ml, 2 mg)

Antibiotics

High-quality antibiotics for selection and maintenance of stably transfected eukaryotic cells.

Product	Cat. No.	Pack Size
Geneticin (G 418)	11 464 981 001	1 g
	11 464 990 001	5 g
Hygromycin B from <i>Streptomyces hygroscopicus</i>	10 843 555 001	1 g (20 ml)

Prepare Transfection-Quality Plasmid DNA

Obtain ultrapure plasmid DNA, free of bacterial components and RNA contaminants. Avoid toxic materials and save time.

Product	Cat. No.	Pack Size
Genopure Plasmid Midi Kit	03 143 414 001	1 kit (up to 20 preparations)
Genopure Plasmid Maxi Kit	03 143 422 001	1 kit (up to 10 preparations)
Genopure Buffer Set	04 634 772 001	Buffer for 20 maxi preps or 60 midi preps

For more information about our transfection reagents, visit www.roche-applied-science.com/transfection

For more information about our nucleic acid purification kits, visit www.roche-applied-science.com/napure

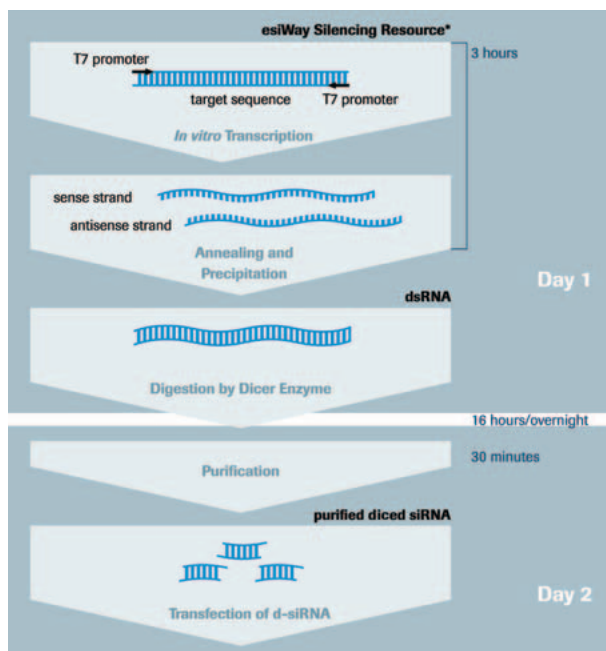
In vitro Synthesis of siRNA and siRNA Transfection

High Knockdown Efficiency and Low Cytotoxicity are Indispensable for Successful RNAi Applications

X-tremeGENE siRNA Dicer Kit

Use the new X-tremeGENE siRNA Dicer Kit to easily and efficiently prepare pure double-stranded small interfering RNA (siRNA) for gene silencing. Start with your cDNA and two PCR primers that each contain a gene-specific 3' portion and the T7 promoter sequence; or, employ ready-to-use target-gene-specific templates containing T7 promoters at both ends (*i.e.*, esiWay Silencing Resources*) (Figure 11).

- **Eliminate screening of different synthetic siRNAs** in initial target-gene silencing experiments by using a “natural” pool of siRNAs
- **Save time and resources** by using a cost-effective procedure to generate high yields of purified siRNA. Diced siRNA prepared using the X-tremeGENE siRNA Dicer Kit is as effective as synthetic siRNA for knockdown experiments



▲ **Figure 11: Overview of siRNA generation using X-tremeGENE siRNA Dicer Kit and an esiWay Silencing Resource as template.**

X-tremeGENE siRNA Transfection Reagent

The new X-tremeGENE siRNA Transfection Reagent delivers all the benefits of high knockdown efficiency, low cytotoxicity, and the capability of transfecting a broad range of cell types.

- **Achieve over 90% gene silencing** in many different cell types
- **Use a single reagent for cotransfection of siRNA and plasmid DNA**
- **Reduce off-target effects** — the reagent’s minimal cellular cytotoxicity ensures that observed cellular effects are only due to transfected siRNA

Product	Cat. No.	Pack Size
X-tremeGENE siRNA Dicer Kit	04 579 020 001	1 kit (10 reactions)
X-tremeGENE siRNA Transfection Reagent	04 476 093 001	1 ml (up to 400 transfections in 24-well plates)
	04 476 115 001	Multi-pack 5 x 1 ml (up to 2,000 transfections in 24-well plates)

* esiWay Silencing Resources are available from the RZPD German Resource Center for Genome Research. For further information, visit www.rzpd.de/products/esiRNA

For more information about siRNA generation and transfection:

Visit www.roche-applied-science.com/geneknockdown



Consult the *Gene Knockdown Integrated Solutions* brochure

Ordering Information

Products for DNA Transfection	Cat. No.	Pack Size
FuGENE® 6 Transfection Reagent	11 815 091 001	0.4 ml (up to 120 transfections in 6-well plates)
	11 814 443 001	1 ml (up to 300 transfections in 6-well plates)
	11 988 387 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)
	11 988 484 001	Custom pack (150 ml minimum)
FuGENE® HD Transfection Reagent	04 709 691 001	0.4 ml (up to 120 transfections in 6-well plates)
	04 709 705 001	1 ml (up to 300 transfections in 6-well plates)
	04 709 713 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)
X-tremeGENE Q2 Transfection Reagent	03 045 595 001	0.4 ml (up to 100 transfections in 6-well plates)
	03 036 421 001	1.8 ml (up to 450 transfections in 6-well plates)
DOTAP Liposomal Transfection Reagent	11 811 177 001	0.4 ml
	11 202 375 001	2 ml (5 x 0.4 ml)(2 mg)
DOSPER Liposomal Transfection Reagent	11 811 169 001	0.4 ml
	11 781 995 001	2 ml (5 x 0.4 ml)(2 mg)
Selection Antibiotics		
Geneticin (G 418)	11 464 981 001	1 g, non-sterile
	11 464 990 001	5 g, non-sterile
Hygromycin B	10 843 555 001	1 g (20 ml) sterile
DNA Preparation Kits		
Genopure Plasmid Midi Kit†	03 143 414 001	1 kit (up to 20 preparations)
Genopure Plasmid Maxi Kit†	03 143 422 001	1 kit (up to 10 preparations)
Genopure Buffer Set	04 634 772 001	Buffer for 20 maxi preps or 60 midi preps
Products for siRNA Transfection		
X-tremeGENE siRNA Dicer Kit†	04 579 020 001	1 kit (10 reactions)
X-tremeGENE siRNA Transfection Reagent	04 476 093 001	1 ml (up to 400 transfections in 24-well plates)
	04 476 115 001	Multi-pack 5 x 1 ml (up to 2,000 transfections in 24-well plates)

Reporter Gene Assays	Cat. No.	Pack Size
β-Gal ELISA	11 539 426 001	1 kit (192 tests)
β-Gal Reporter Gene Assay, chemiluminescent	11 758 241 001	1 kit (500 assays [microplate format] or 250 assays [tube format])
β-Gal Staining Set	11 828 673 001	1 set (for 100 tests in 3.5 cm dishes)
CAT ELISA	11 363 727 001	1 kit (192 tests)
hGH ELISA	11 585 878 001	1 kit (192 tests)
Luciferase Reporter Gene Assay, high sensitivity	11 669 893 001 11 814 036 001	200 assays 1,000 assays
SEAP Reporter Gene Assay, chemiluminescent	11 779 842 001	1 kit (500 assays [microplate format] or 250 assays [tube format])

Mycoplasma Detection and Elimination	Cat. No.	Pack Size
Mycoplasma PCR ELISA [†]	11 663 925 910	1 kit (96 reactions)
Mycoplasma Detection Kit	11 296 744 001	1 kit (25 tests)
BM-Cyclin	10 799 050 001	37.5 mg (for 2 x 2.5 l medium)

Trademarks and Disclaimers

GENOPURE and X-TREMEGENE are trademarks of Roche.

FuGENE is a registered trademark of Fugent, L.L.C., USA.

GENETICIN is a registered trademark of Life Technologies, Inc., Gaithersburg, MD, USA.

esiWay is a trademark of RZPD.

Other brands or product names are trademarks of their respective holders.

[†] This product is optimized for use in the Polymerase Chain Reaction (PCR) process covered by patents owned by F. Hoffmann-La Roche Ltd. No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product.

[‡] Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for the purchaser's life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, *i.e.*, an authorized thermal cycler.



www.roche-applied-science.com

For additional information, visit
www.roche-applied-science.com/transfection
or contact your local sales representative.



Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany