

# Transfection

**T**ransfection of cells with DNA has become an important tool for studying the expression and function of genes, particularly mammalian genes, and their components. Historically, the most widely used transfection methods have included calcium phosphate coprecipitation, DEAE-dextran treatment, and electroporation. Success with these methods varies greatly with cell type.

## Cationic liposomal reagents improved DNA transfection

Cationic liposome-mediated transfection (*i.e.*, lipofection, cytofection) represented one of the most effective transfection methods developed in the past 10 years. Under carefully optimized conditions, liposome-mediated transfection methods yield high efficiencies and are much easier to use than earlier methods.

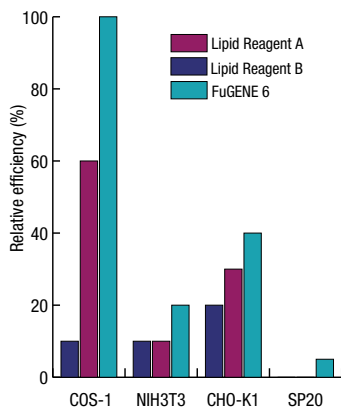
Boehringer Mannheim offers two liposome-based products for the transfection of DNA, oligonucleotides, RNA, and proteins: the monocationic DOTAP Liposomal Transfection Reagent and the polycationic DOSPER Liposomal Transfection Reagent. Once DNA/lipid ratios have been optimized, either DOTAP or DOSPER will transfect cells with high efficiency, function in the absence or presence of serum, and offer lower cytotoxicity than older methods.



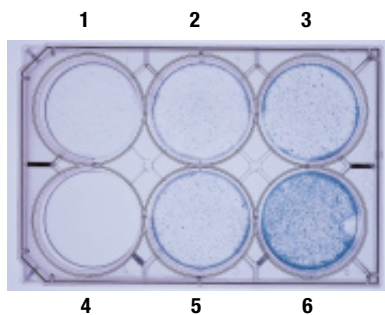
**Figure 2.** Boehringer Mannheim's transfection reagents.

## New generation of transfection reagents enhances transfection efficiency

Boehringer Mannheim is now taking transfection efficiency to higher levels with the introduction of FuGENE™ 6 Transfection Reagent. FuGENE 6 is an optimized blend of lipids and other, proprietary compounds. It produces higher transfection efficiencies and eliminates much of the optimization required by cationic liposome-based transfection reagents.



**Figure 3. Relative abilities of FuGENE 6 Transfection Reagent and two liposomal transfection reagents to transfect various cell lines.** Various cell lines were plated, grown to 60–80% confluency, and transfected with 2  $\mu$ g pCMV $\beta$ -Gal using 3  $\mu$ l FuGENE 6 Reagent. Expression of  $\beta$ -galactosidase activity was measured by scoring the ratio of transfected cells to total cell number; relative transfection efficiency was then calculated after assigning 100% as the highest ratio of transfected cells to total cells in the cell line transfected most efficiently (i.e., COS-1). Relative efficacies were then calculated based on this maximum.



**Figure 4. Comparison of transfections of COS-1 cell cultures performed with six commercially available transfection reagents.** Cells were subcultured in 6-well dishes on the afternoon prior to the experiment. Transfections were carried out under optimal conditions using the manufacturers' recommended procedures. Twenty-seven hours after transfection, cells were stained using the  $\beta$ -Gal Staining Set. Well 4 was transfected with a monocationic lipid transfection reagent. Wells 1, 2, and 5 were transfected with different polycationic lipid transfection reagents. Well 3 was transfected with a new non-liposomal spherical polycationic reagent. Well 6 was transfected with FuGENE 6 Transfection Reagent.

## NEW FuGENE™ 6 Transfection Reagent: For higher transfection efficiencies without optimization

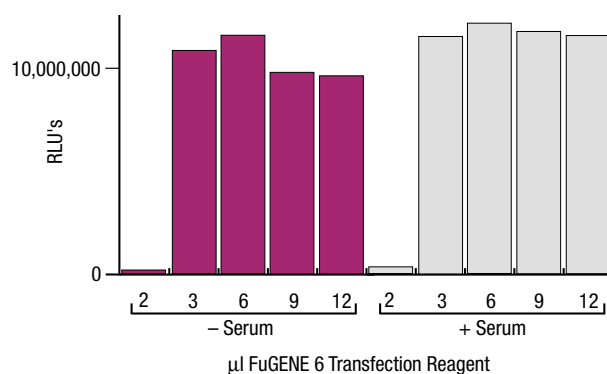
### Description and use

FuGENE™ 6 Transfection Reagent is a revolutionary blend of lipids and other, proprietary compounds for transfection of DNA into cells. It is supplied as a stable 1 ml solution dissolved in 80% ethanol.

### Advantages

Take advantage of this novel reagent's advantages over cationic liposome-based transfection reagents:

- Transfect cells with higher efficiency**  
 FuGENE 6 Transfection Reagent transfects cells with higher efficiency than liposomal and spherical polycationic transfection reagents (Figures 3,4).
- Avoid the extensive optimization required by liposomal transfection reagents**  
 Simply mix 0.5–2  $\mu$ g plasmid of interest with 3  $\mu$ l FuGENE 6 Transfection Reagent, and add the mixture to the cell sample. There is no need for extensive optimization of DNA:FuGENE 6 Transfection Reagent ratios (Figure 5).
- Save time and effort**  
 FuGENE 6 Transfection Reagent does not have to be removed from the cell culture after most applications (Table 2). It also functions both in the presence or absence of serum, so no serum-deprivation steps are required (Figure 5). These attributes of FuGENE 6 increase ease-of-use and also lower overall reagent cost.



**Figure 5. Ability of various concentrations of FuGENE 6 Transfection Reagent to transfect COS-1 cells with pCMV $\beta$ -Gal.** COS-1 cells were plated in 35-mm dishes. The next day, various amounts of FuGENE 6 Transfection Reagent were used to transfect the cells with 2  $\mu$ g pCMV $\beta$ -Gal in both serum-free (red) and serum-containing medium. After 27 hours, expression of  $\beta$ -galactosidase activity (in relative light units [RLU's]) was determined by analyzing 5  $\mu$ l lysate with the Boehringer Mannheim  $\beta$ -Gal Reporter Gene Assay, chemiluminescent. Note that amounts of FuGENE 6 greater than 3  $\mu$ l transfect cells with approximately equal efficiency.

■ **Protect your cells during transfection**

Relative to other methods, FuGENE 6 Transfection Reagent produces virtually no cytotoxic effects (Figure 6). This ensures that final results reflect true alterations in gene expression, not simply artifacts induced by toxic reagents.

■ **Transfect a wide variety of cell lines**

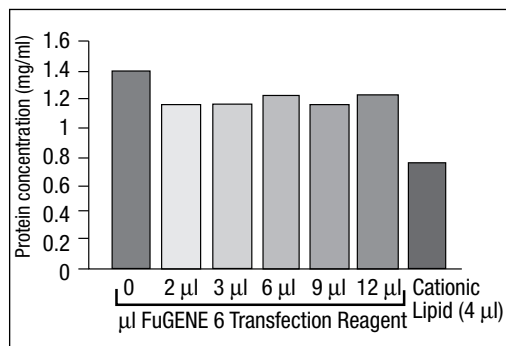
FuGENE 6 Transfection Reagent has successfully transfected many eukaryotic cell types, including those listed in Table 1. This robust reagent also functions over a wide reagent-concentration range (Figure 5).

■ **Take advantage of an extremely stable reagent**

FuGENE 6 Transfection Reagent is extremely stable. It can be stored at 4°C for 2 months or at -20°C for 24 months with no loss of activity. Repeatedly remove aliquots from the sample vial with no loss of efficacy.

COS-1
NIH 3T3
CHO-K1
SP20
HeLa
COS-7
HCT116
Calu-1
primary cultures of keratinocytes

**Table 1. Partial listing of cell types successfully transfected by FuGENE 6 Transfection Reagent.** For the most current list, visit <http://biochem.boehringer-mannheim.com.techserv/fugene.htm> on the Internet.



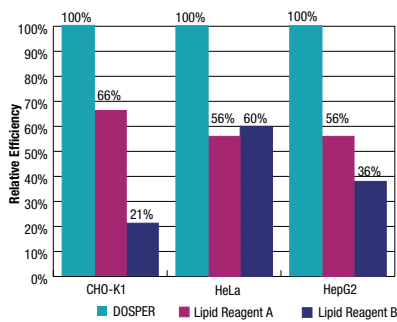
**Figure 6. Effect of FuGENE 6 Reagent concentration on transfected COS-1 cells.** COS-1 cells (130,000) were plated in 35-mm dishes. The next day, various amounts of FuGENE™ 6 Transfection Reagent were used to transfect the cells with 2 µg pCMVβ-Gal. The cells were further subcultured for 27 hours, and then total protein was assayed as an indicator of cellular viability.

Steps	FuGENE 6 Reagent	Cationic Liposomes	Calcium Phosphate
1 Prepare transfection solutions	not required	not required	✓
2 Plate cells	✓	✓	✓
3 Mix transfection reagent with DNA	✓	✓	✓
4 Prepare cells by changing culture media	not required	✓	✓
5 Add transfection reagent/DNA solution, and incubate	✓	✓	✓
6 Wash off transfection reagent to prevent cytotoxic effects	not required	✓	✓
7 Incubate and then assay for gene expression	✓	✓	✓
<b>TOTAL STEPS</b>	<b>4</b>	<b>6</b>	<b>7</b>

**Table 2. Comparison of steps required by various transfection methods.**

Product	Cat. No.	Pack Size
FuGENE™ 6 Transfection Reagent	1 814 443	1 ml

FuGENE™ is a trademark of Fugent, L.L.C.



**Figure 7. Correlation of relative transfection efficiency with cell viability when using DOSPER or other liposomal transfection reagents.** CHO-K1 cells were transfected in the absence of serum. Forty-eight hours after transfection, total protein per dish was measured as an indicator of cellular viability. In parallel, transfection efficiency was measured with the CAT ELISA.

CHO-K1
HeLa
HepG2
293
BHK 21
COS-1
Vero
primary fibroblasts

**Table 3. Partial listing of cell types successfully transfected with DOSPER.**

## DOSPER:

### Polycationic liposomal reagent for high transfection efficiencies

#### Description and use

Choose DOSPER Liposomal Transfection Reagent for efficient cationic liposome-based transfection. DOSPER's optimized polycationic structure effectively binds negatively charged DNA, creating stable transfection complexes that fuse with cell membranes and release the DNA into the cell. DOSPER is supplied as a solution in 0.4 ml aliquots.

#### Advantages over other liposomal reagents

- Achieve high-efficiency transfection**

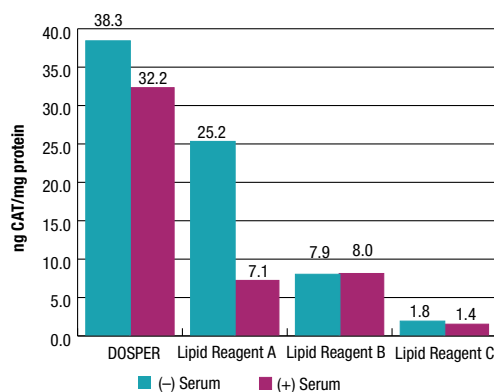
DOSPER's polycationic structure ensures higher transfection efficiency and lower cytotoxicity than other liposomal transfection reagents (Figure 7). Also, DOSPER is bottled in convenient aliquots under argon to prevent oxidation and the resulting reduction in transfection efficiency.

- Transfect a variety of cell types**

DOSPER successfully transfects a variety of common cell types (Table 3) and cell lines known to be difficult to transfect (*e.g.*, HepG2).

- Transfect in the presence or absence of serum**

DOSPER exhibits similar transfection efficiencies in the presence or absence of serum, eliminating the need for serum removal, a process that can reduce viability in some cells types (Figure 8).



**Figure 8. Transfection efficiencies in CHO-K1 cells in the absence and presence of serum.** DOSPER was compared to three other commercially available polycationic lipid preparations. Following each manufacturer's recommended conditions for use, the amounts of DNA and transfection reagent were optimized under serum-free conditions. Transfection efficiency was then determined both in the absence and presence of serum. Note that with and without serum, DOSPER delivered transfection efficiencies superior to those obtained with the other preparations.

Product	Cat. No.	Pack Size
DOSPER Liposomal Transfection Reagent	1 811 169	0.4 ml
	1 781 995	2 ml (5 x 0.4 ml)

## DOTAP:

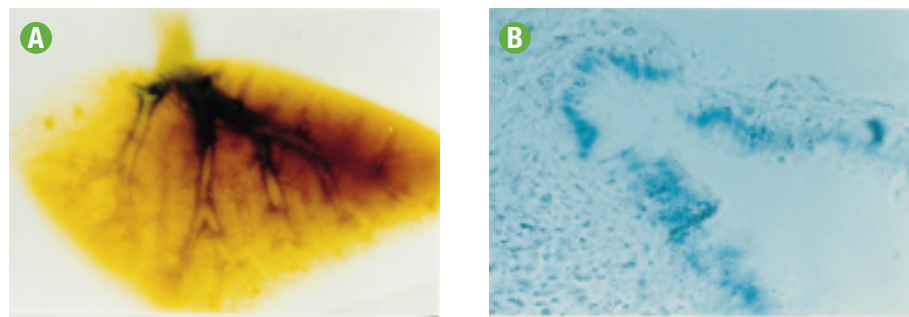
### Time-tested monocationic liposomal transfection reagent

#### Description and use

For successful transfections with a time-tested cationic liposome-based reagent, try DOTAP Liposomal Transfection Reagent. This monocationic reagent has been used to successfully transfect plasmid DNA, RNA, oligonucleotides, or peptides into more than 70 distinct cell types, including both primary cells and cell lines (see Table 4 for a partial listing). It also has been used successfully in gene therapy research. DOTAP is supplied as a solution in 0.4 ml aliquots.

#### Advantages over other liposomal reagents

- Achieve high efficiencies in the presence or absence of serum**  
 Functional in the presence or absence of serum, DOTAP is more efficient than other monocationic lipids (Figure 9) and 5–100 times more efficient than calcium phosphate- or DEAE-dextran-based transfection methods.
- Reduce cytotoxicity**  
 DOTAP is less cytotoxic than most other transfection reagents, increasing overall transfection efficiency because more cells survive the transfection process.
- Transfect *in vitro* and *in vivo***  
 In addition to *in vitro* applications, DOTAP has been employed in gene therapy research on non-human models (Figure 10). DOTAP has successfully introduced eukaryotic DNA into at least seven different *in vivo* models.



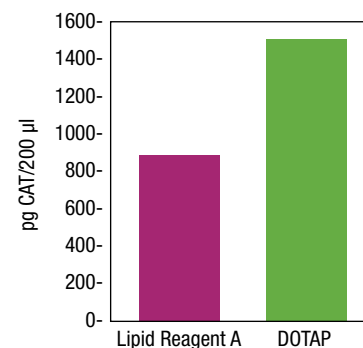
**Figure 10. Reporter gene expression *in vivo* after nebulized delivery of DNA/DOTAP.**

A standard nebulizer was used to expose mice to a mixture of DOTAP and pCMV $\beta$ -gal. After 4–10 days, the mice were sacrificed, and the lungs inflated, fixed, and stained with X-gal. **Panel A:** a whole lung; **Panel B:** a section through secondary bronchus showing comprehensive staining of the epithelium.

Product	Cat. No.	Pack Size
DOTAP Liposomal Transfection Reagent	1 811 177	0.4 ml
	1 202 375	2 ml (5 x 0.4 ml)

DNA	Oligonucleotides
Balb/3T3	CCRF-CEM/VLB
BHK	HepSV40
COS-1	LoVo/Dx
COS-7	MCF-7
ES cells	SK-BR-3
HeLa	<b>RNA</b>
HepG2	SW-480
Jurkat	Jurkat
MCF-7	MDA-MB-435
Raji	PFX 1118
Sf-9	<b>Protein/Glycoprotein</b>
SL-3	EL-4
Vero	I-3
Fibroblasts	BalbSV
(primary rat dermis)	MC57
Lymphocytes(primary human PBL)	

**Table 4. Partial listing of cell types transfected with DOTAP.** For a complete listing, contact your local Boehringer Mannheim representative, or find us on the Internet at [http://biochem.boehringer-mannheim.com/prod\\_inf/biblios/dotapoc.htm](http://biochem.boehringer-mannheim.com/prod_inf/biblios/dotapoc.htm)



**Figure 9. Comparison of DOTAP and another supplier's liposomal transfection reagent on COS-1 cells.**

Cells in serum-free media were transfected with pSV2CAT DNA using DOTAP or another supplier's lipid reagent according to each product's package insert. CAT expression was measured with the CAT ELISA.