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An Efficient New Transfection Reagent for Eukaryotic Cells: TransFast™ Transfection Reagent

By Elaine Schenborn, Virginia Goiffon and Jennifer Oler
Promega Corporation

Corresponding author: e-mail to eschenbo@promega.com

The TransFast™ Transfection Reagent has recently been added to Promega's cationic lipid transfection reagent product line. TransFast™ Reagent performs well in many cell lines, has utility for transient and stable transfections and may be used in the presence or absence of serum. This new transfection reagent combines the advantages of cationic lipid-mediated transfection with the additional benefits of speed and ease-of-use.*

**Patent Pending.*

Introduction

Reagents and methods that facilitate the entry of nucleic acids into intact cells allow experiments to be performed today that could only be dreamed of 50 years ago. Chemical entities, such as DEAE-dextran and calcium phosphate, were among the first reagents developed for the transfer of RNA and DNA into intact mammalian cells (1,2). This process of nucleic acid delivery to cells is now commonly referred to as transfection. The development of molecular biology techniques provided the means to engineer recombinant nucleic acid sequences of choice and to prepare milligram amounts of DNA from *E. coli*. The combination of these advances in molecular biology and the development of effective transfection methods for delivery of recombinant DNA to cells enabled the advancement of knowledge in the areas of genetic regulation and protein function within eukaryotic cells, tissues and organisms.

By the 1980s, artificial liposomes were being used to deliver DNA to cells (3). Continual improvements have been incorporated into the development of liposomes since the early reports using liposomes for transfection (4). These include the synthesis of defined, "designer" cationic lipid components (5) and formulation with other lipids that facilitate the transfection process (6). The advantages of cationic liposome-mediated transfection of cells include the ability to transfer DNA of various sizes ranging from oligonucleotides to yeast artificial chromosomes (5,7,8) to transfer RNA (9), to support long-term stable transfection and to transfer nucleic acids under *in vitro* and *in vivo* conditions (10).

Properties ofTransFast™ Transfection Reagent

The newest member of the Promega family of transfection products is TransFast™ Transfection Reagent. This reagent is a cationic liposome formulation containing the synthetic, cationic lipid compound N,N [bis(2-hydroxyethyl)-N-methyl-N-[2,3-di (tetradecanoyloxy)propyl] ammonium iodide (Figure 1) together with the neutral lipid compound L-dioleoyl phosphatidylethanolamine (DOPE). The cationic lipid contains an amine head group that imparts a single positive charge at neutral pH. It is hypothesized that the positively charged lipid head group associates with the negatively charged phosphates of nucleic acids causing compaction of the nucleic acid and neutralization of the negative charges. This allows the nucleic acid:liposome complex to come into apposition with negatively charged cellular membranes. The complex may be taken up by the cell via endocytosis (6). The DOPE reportedly enhances release of the nucleic acid:liposome complex trapped within endosomes by a process of membrane destabilization within the vesicle (11).

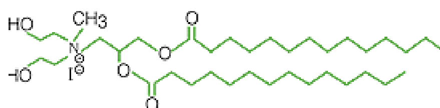

Figure 1. Structure of the cationic lipid component of the TransFast™ Transfection Reagent.

Performance characteristics of TransFast™ Transfection Reagent for transient, short-term studies (Figure 2) were tested using the pGL3-Control Vector**[(a)] (Cat.# E1741), which contains the firefly luciferase reporter gene under control of the SV40 enhancer and early promoter sequences. COS-7 cells were transfected with a reporter vector containing the cytomegalovirus (CMV) immediate-early

gene promoter driving transcription of the luciferase gene. This avoids replication effects of pGL3-Control DNA in COS-7 cells, which express the SV40 large T antigen. Cell lysates were assayed for luciferase activity using the Luciferase Assay System[b] (Cat.# E1500) to assess overall reporter gene expression dependent upon DNA delivery to cells.

[**]*The method of recombinant expression of* Coleoptera *luciferase is covered by U.S. Pat. No. 5,583,024 assigned to The Regents of the University of California.*

[a]*U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.*

[b]*U.S. Pat. Nos. 5,283,179, 5,641,641 and 5,650,289 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay.*

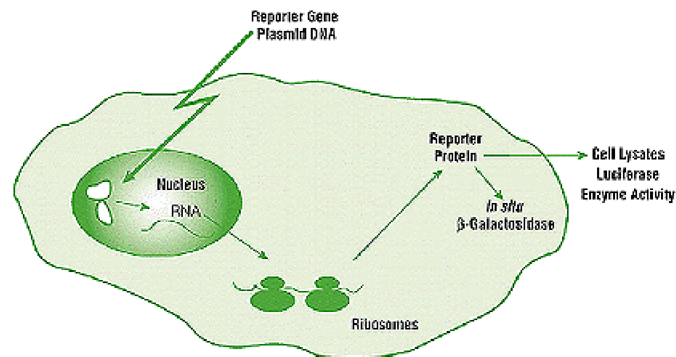

Figure 2. Transfection using reporter gene systems. For transient assays, cells were transfected with reporter gene plasmid DNA. Two days post-transfection, cell lysates were assayed for luciferase enzyme activity, or cells were stained to observe beta-galactosidase enzyme activity *in situ*.

Standard *in situ* staining protocols were used to assess the percentage of cells transfected by the beta-galactosidase reporter gene under transcriptional control of the CMV promoter (12). For long-term, stable transfection, the pCI-neo Mammalian Expression Vector[c,d] (Cat.# E1841) was used for transfection, and cells expressing the transferred neomycin phosphotransferase gene were selected using the drug Geneticin® (G418) in the culture medium. A flow diagram of the transfection procedure is shown in Figure 3.

[c]*The CMV vector technology is the subject of U.S. Pat. No. 5,168,062 assigned to the University of Iowa Research Foundation.*

[d]*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.*

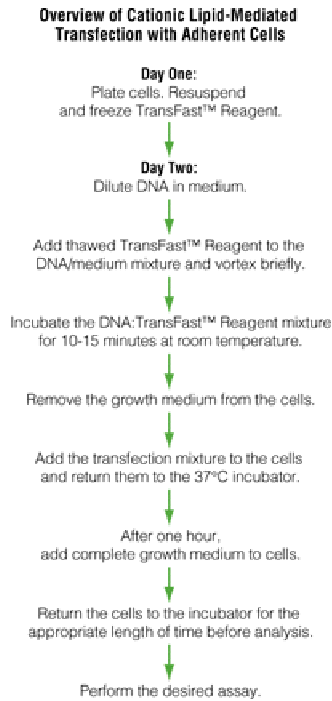

Figure 3. Flow diagram for transfection experiments using TransFast™ Reagent.

Optimization parameters

To achieve maximum transfection efficiency for a particular cell type, it is essential to optimize several parameters that are common for liposome reagents developed for transfection (13). The two most critical parameters for TransFast™ Transfection Reagent optimization are the DNA amount and the lipid:DNA charge ratio. Exposure to serum and time of the transfection interval will also be discussed in this article.

DNA

The optimal amount of DNA per transfection is the first critical parameter to determine. This will vary among cell types. It can be seen from Figure 4, Panel A, that increasing the amount of DNA may not necessarily result in higher expression levels of the transfected luciferase reporter gene. For 293 cells, 0.25µg of DNA per well was the optimal amount of pGL3-Control Vector to use per transfection; however, with BHK cells, the optimal amount of DNA was 1µg per well.

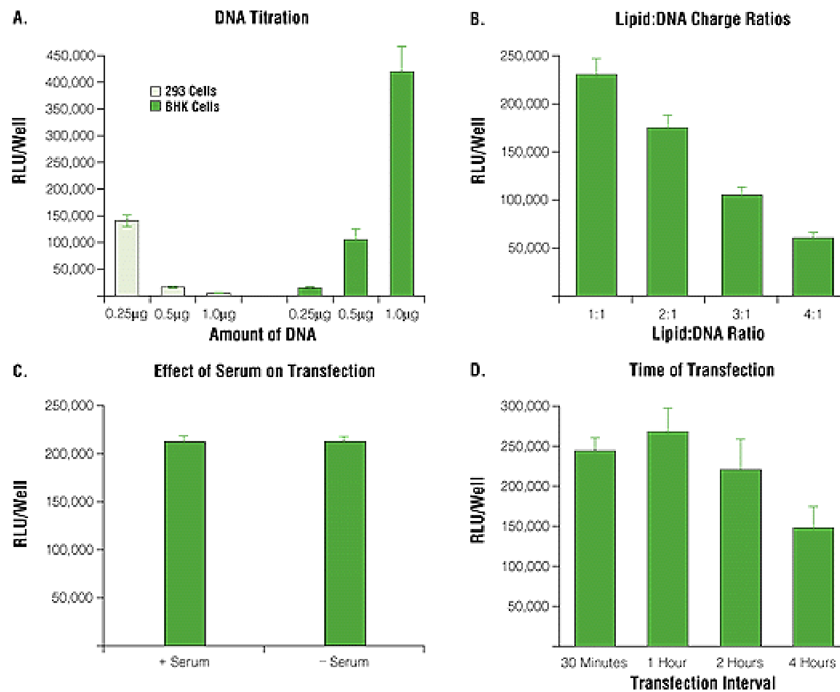

Figure 4. Relative levels of luciferase expression as a function of DNA amount, lipid:DNA charge ratio, serum and transfection time. All transfections were performed in 24 well plates and cell lysates were harvested 2 days post-transfection. The results represent the mean of six replicates plus standard deviation and are expressed as relative light units per well of cells. **Panel A:** 293 and BHK cells were transfected with various amounts of DNA per well at a 2:1 lipid:DNA charge ratio in serum-free medium. **Panel B:** NIH3T3 cells were transfected with 1µg of pGL3-Control Vector at various lipid:DNA charge ratios for one hour in serum-free medium. **Panel C:** COS-7 cells were transfected at a 1:1 charge ratio with 0.5µg DNA containing the luciferase gene under the control of the cytomegalovirus promoter. The lipid:DNA complexes were formed in the absence or presence of 10% serum. The transfection interval was one hour. **Panel D:** CHO cells were transfected with 0.25µg of pGL3-Control Vector at a 2:1 lipid:DNA charge ratio for various times in the absence of serum.

Lipid:DNA charge ratio

The optimal lipid:DNA charge ratio to use for transfection must also be determined. Charge ratios from 1:1 (i.e., 1 positive charge contributed by the cationic lipid for each negative charge contributed by the DNA) to 4:1 (i.e., 4 positive charges contributed by the cationic lipid for each negative charge contributed by the DNA) were tested with a constant amount of DNA. The results for NIH3T3 cells are depicted in Figure 4, Panel B. Reporter expression was highest when the DNA was transfected at a 1:1 lipid:DNA charge ratio. In general, TransFast™ Transfection Reagent performed best with lipid:DNA charge ratios of 1:1 and 2:1 in the established cell lines that we tested.

Serum

The TransFast™ Transfection Reagent performs in the presence of serum, as shown in Figure 4, Panel C. In this experiment, COS-7 cells were transfected with lipid:DNA complexes formed in the presence or absence of serum. The results indicate that, for COS-7 cells, TransFast™ Reagent performed well in the presence of 10% serum. Reporter activity was equivalent regardless of the presence or absence of serum. However, the effect of the presence of serum on transfection is dependent upon cell type. Generally, with the established cell lines we tested, optimal transfection efficacy occurred in the absence of serum during the transfection interval. The ability to perform in the presence of serum is expected to be especially beneficial for primary cells dependent upon the presence of serum for survival.

Time

The transfection interval for the TransFast™ Transfection Reagent is quite short compared with that recommended for other commercially available transfection reagents. Figure 4, Panel D, shows the effect of transfection times ranging from 30 minutes to four hours for CHO cells. A transfection interval as short as 30 minutes provided good transfection efficiency. For other standard cell types, a one-hour interval worked very well.

Performance features

Comparative studies were performed using Promega Tfx™ Reagents[(e)] and the TransFast™ Transfection Reagent with cell lines routinely used for transfection experiments. Cells were transfected with the pGL3-Control Vector under conditions optimized for each cell type. The results (shown in Table 1) indicate that TransFast™ Transfection Reagent provides an excellent transfection method for many commonly used established cell lines. However, one transfection reagent is not necessarily the best reagent to transfect all cell types. For example, Tfx™-20 Reagent is the transfection reagent of choice for HeLa cells, but not for NIH3T3 cells. The phenomenon of differential transfection efficacy of reagents with various cell types has been previously reported (14) and forms the basis for providing a set of reagents for the transfection of eukaryotic cells. A choice of reagents, such as is provided by TransFast™ Reagent and the Tfx™ Reagents, enables the user to choose the reagent that gives superior performance in any given cell type.

[(e)]*The cationic lipid component of the Tfx™ Reagents is covered by U.S. Pat. No. 5,527,928 assigned to The Regents of the University of California and pending foreign patents.*

Table 1. Suggested Lipid Reagent to Use With Established Cell Lines[*].

Cell Line	Origin	Cell Type	Tfx™-10 Reagent	Tfx™-20 Reagent	Tfx™-50 Reagent	TransFast™ Reagent
HeLa	Human	Epithelial		X		
Hep G2	Human	Hepatocyte		X		
293	Human	Kidney transformed				X
K-562	Human	Lymphoblast				X
Jurkat	Human	T-cell leukemia				X
COS-7	Monkey	Fibroblast				X
CV-1	Monkey	Fibroblast	X	X	X	
NIH3T3	Mouse	Fibroblast				X
BHK	Hamster	Fibroblast	X			
CHO	Hamster	Epithelial-like				X
PC12	Rat	Pheochromocytoma		X		X
Sf9	Insect	Ovary		X		X

[*]*Data were obtained from cells transiently transfected with plasmid DNA. Higher transfection efficiencies were generally obtained with reagent:DNA complexes incubated with the cells in serum-free medium. Some cells exhibit similar transfection efficiencies with several different reagents; thus, more than one suggested reagent is indicated for these cell lines.*

Stable transfection

Stable transfection was also tested using the TransFast™ Transfection Reagent and several different cell lines. Cells were transfected with the pCI-neo Mammalian Expression Vector using DNA amounts and lipid:DNA charge ratios previously optimized in transient transfection studies using a luciferase reporter gene. Stable transfectants that were resistant to the drug Geneticin® were produced in NIH3T3, CHO, COS-7 and HeLa cell lines (data not shown).

Transfection efficiency

Percentages of transfected cells were assayed by *in situ* staining for beta-galactosidase in CHO, NIH3T3 and COS-7 cells transfected with plasmid DNA containing the *lac*Z gene under transcriptional control of the CMV promoter. High transfection efficiencies were obtained, as evidenced by the result that 34 to >95% of the cell populations stained positive for beta-galactosidase activity (Table 2). An example of the histochemical staining results with NIH3T3 cells is shown in Figure 5.

Table 2. Percentage of Cells Stained Blue After Transfection With a Beta-Galactosidase Reporter Vector and TransFast™ Transfection Reagent.		
Cell Line		**% Cells With Detectable Beta-Galactosidase Expression**
CHO		>95%
NIH3T3		88%
COS-7		34%
Cells were transfected with a plasmid DNA containing the lac*Z gene driven by a cytomegalovirus promoter. The percentage of cells with detectable beta-galactosidase expression was compared between transfected cells and nontransfected controls.*		

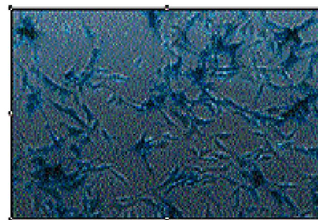

Figure 5. Histochemical staining of NIH3T3 cells for beta-galactosidase activity. NIH3T3 cells were plated in 24 well plates and transfected, per well, with 1µg DNA containing the *lac*Z gene under the control of the CMV promoter and TransFast™ Transfection Reagent at a 1:1 charge ratio. Cells were fixed with glutaraldehyde two days post-transfection and stained for beta-galactosidase activity using standard techniques (12). The cells expressing beta-galactosidase are stained blue.

VENDOR COMPARISONS

Vendor comparisons were conducted using TransFast™ Transfection Reagent and a popular commercially available liposome reagent, "Reagent L". We performed extensive optimization studies with both reagents to determine the optimal DNA amount, reagent volumes and transfection intervals to use for each of the cell lines tested, according to the manufacturer's technical recommendations. Figure 6 summarizes the results of transfection experiments for several cell lines using the luciferase reporter and the optimized transfection conditions for each reagent. TransFast™ Transfection Reagent was found to be a superior transfection reagent in many cell lines.

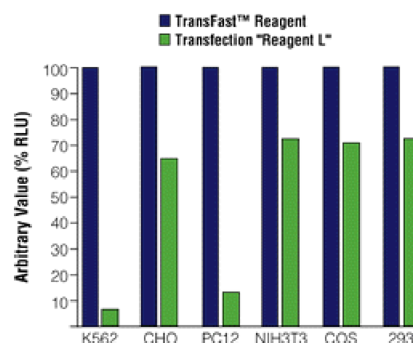

Figure 6. Comparative transfection experiments with TransFast™ Transfection Reagent and another commercial liposome reagent. In these comparative studies, the optimal DNA amount, volume of reagent and transfection interval to use per transfection were determined for both TransFast™ Transfection Reagent and "Reagent L" using the manufacturer's recommended protocols. Transfections were performed with cells plated in 24 well plates. pGL3-Control Vector was used in all cases except for COS cells, which were transfected with a luciferase gene under the control of the CMV promoter/enhancer. Cell lysates were harvested two days post-transfection and assayed for luciferase activity. The results were expressed as relative light units per well. The

mean of six replicate samples was calculated and the results were normalized for each cell type, with 100% reflecting the mean maximum light unit value.

Similarities and differences between TransFast™ Reagent and the Tfx™ Reagents

The properties and uses of the TransFast™ Transfection Reagent are similar to those of the Tfx™ Reagents in several respects. For example, each of the reagents is supplied as a lipid film that is resuspended in 400µl of water prior to use. The final concentration of the cationic lipid is 1mM for all the reagents, and each contains the neutral lipid, DOPE. Optimal performance of the liposome suspensions requires freezing before use. Procedures for using the liposome reagents are standardized for all these products and involve an easy, single-tube mixing of the lipid with DNA diluted in culture medium. The lipid and DNA associate during a 10- to 15-minute incubation at room temperature, prior to addition to the cells. After the transfection interval, lipid:DNA complexes remain in the tissue culture dish and complete, serum-containing medium is added.

There are several structural differences in the synthetic cationic lipid component between the TransFast™ and Tfx™ Reagents. The cationic lipid component of the TransFast™ Reagent contains dimyristyl lipid chains, whereas the Tfx™ Reagents contain a cationic lipid with dioleoyl lipid chains. The amine head group of the TransFast™ Reagent has a single positive charge, while the cationic lipid component of the Tfx™ Reagents provides two positive charges per molecule. Therefore, to achieve the same lipid:DNA charge ratio, twice as much TransFast™ Transfection Reagent must be added compared to the Tfx™ Reagents. However, the TransFast™ Transfection Reagent performs best with established cell lines at 1:1 and 2:1 charge ratios, compared to 2:1, 3:1 or 4:1 charge ratios for the Tfx™ Reagents.

Summary

The TransFast™ Transfection Reagent is an efficient, easy-to-use cationic liposome reagent formulated for gene transfer into eukaryotic cells. This new reagent complements the existing Promega Tfx™ Reagent line to provide efficient nucleic acid delivery to a wide variety of cell types. TransFast™ Reagent supports transient and stable transfection paradigms. Protocols are similar to procedures used with the Tfx™ Reagents and include a convenient single-tube method for mixing lipid and DNA and a fast, one-hour transfection interval of cells with the liposome:DNA complex prior to adding complete cell culture medium.

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Ordering Information		
Product	**Size**	**Cat.#**
TransFast™ Transfection Reagent	1.2mg	E2431

Contains sufficient reagent to transfect 400µg of DNA (at a 1:1 TransFast™ Reagent:DNA ratio), and 2ml Nuclease-Free Water (2 x 1ml). TransFast™ Transfection Reagent is also available in a trial size from your Field Applications Specialist, Branch Office or Distributor, or by contacting Promega Technical Services, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399 USA. E-mail: techserv@promega.com; *telephone: 800-356-*

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