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TransFast^{TM} Transfection Reagent Update

By Jennifer Oler, B.S., and Elaine Schenborn, Ph.D.
Promega Corporation

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

TransFast^{TM} Transfection Reagent[a] is comprised of the synthetic cationic lipid, N,N [bis (2-hydroxyethyl)-N-methyl-N-[2,3-di (tetradecanoyloxy) propyl] ammonium iodide, and the neutral lipid, DOPE. The TransFast^{TM} Reagent is supplied as a dried lipid film that forms multilamellar vesicles upon hydration with water. The experiments described in this article address some commonly asked questions about the protocol for use of this reagent.

Introduction

This article examines several parameters associated with the transfection protocol for TransFast^{TM} Transfection Reagent. This reagent, which contains a mixture of cationic and neutral lipids, is incubated with nucleic acids and then exposed to eukaryotic cells (see Figure 1). Although the protocol for use of TransFast^{TM} Reagent is similar to that of other commercially available cationic lipid products, there are several differences. For example, TransFast^{TM} Reagent is supplied as a lipid film that is suspended with water and frozen before and after use. This differs from other cationic liposome products supplied as sonicated liposomes, which should never be frozen. The experiments described in this article help to answer questions about reagent preparation, lipid suspension storage temperatures and the effects of multiple freeze-thaw cycles on product performance. We also examined transfection conditions such as incubation time with the transfection mixture, changing media, effect of cell confluence and type of tube used to prepare the lipid and DNA complexes.

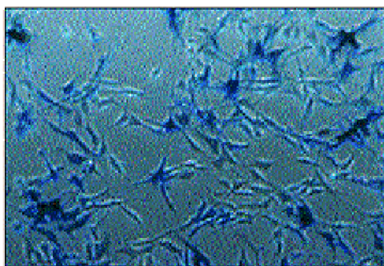

Figure 1. Histochemical staining of NIH3T3 cells for beta-galactosidase activity. Cells were plated in 24 well plates and transfected with 1µg DNA (per well) containing the beta-galactosidase gene under the control of the CMV promoter. TransFast^{TM} Transfection Reagent was used at a 1:1 reagent:DNA ratio. Cells were fixed with glutaraldehyde 2 days post-transfection and stained for beta-galactosidase activity using standard techniques. (See the *TransFast^{TM} Transfection Reagent Technical Bulletin* #TB260 as well as #TB097). Positive cells are stained blue.

The standard protocol for transfection of adherent eukaryotic cells with TransFast^{TM} Transfection Reagent is outlined in Figure 2.

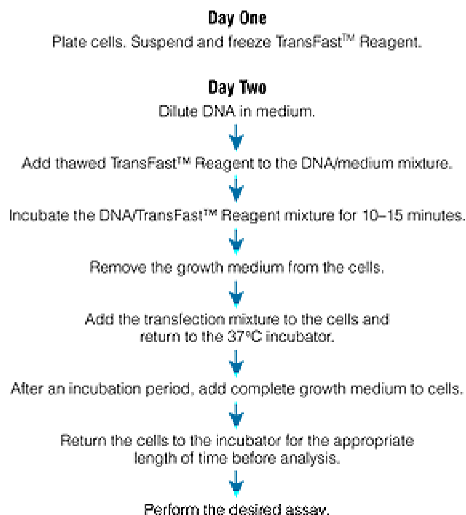

Figure 2. Overview of TransFast™ Transfection Reagent protocol with adherent cells.

The examples presented in this article use optimal transfection conditions that were previously determined for each cell type (see the legend to Figure 3). For all experiments, cells were transfected with pGL3-Control DNA[(b,c)] (Cat.# E1741), which contains the firefly luciferase gene under the control of the SV40 early promoter region. Cell lysates were prepared approximately 48 hours post-transfection and assayed for luciferase activity using a standard luminometer. Light unit output per well of cells was normalized to a 100% value for comparison in each figure.

The protocol provided in the* TransFast™ Transfection Reagent Technical Bulletin *(#TB260) recommends suspension of the lipid film with water followed by freezing overnight at 20°C before use. Can the reagent be used if the lipid suspension is left at room temperature overnight?

In general, peak performance of TransFast™ Transfection Reagent occurs with material treated according to the standard protocol. Material stored overnight at room temperature shows reduced transfection activity, to as little as 50% of the peak activity, depending upon the transfected cell line (Figure 3). Reagent that had been stored overnight at room temperature and frozen for one hour on the day of transfection exhibited no significant improvement in transfection efficacy for the cell lines tested.

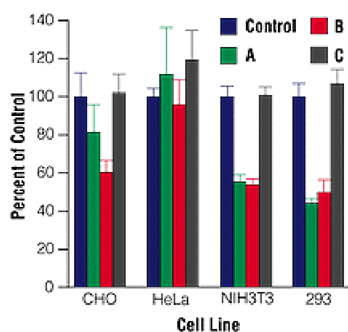

Figure 3. Effect of storage conditions on performance of TransFast™ Transfection Reagent. TransFast™ Transfection Reagent was suspended in water the day prior to transfection and stored at 20°C overnight as recommended in the TransFast™ Reagent protocol (Control), at room temperature overnight (A), at room temperature overnight and then frozen at 20°C for 1 hour before transfection (B), or suspended with water and frozen at 20°C for 1 hour on the day of transfection (C). The indicated cells were transfected with TransFast™ Reagent according to the standard protocol given below*. Each bar on the graph represents the mean normalized light units +/- S.D. of cell lysates, with 6 replicates. Control samples were set at 100%. Values given for A, B and C are relative to the 100% Control value.

Transfection conditions were as follows:* Unless otherwise specified, cells were plated at a density of 5 x 10^4 cells per well approximately 24 hours prior to transfection. The transfection interval was one hour. For all but CHO cells, serum-free media was used to make up the transfection mixture; for CHO cells, media containing 10% fetal bovine serum was used. pGL3-Control DNA was used in all transfections. TransFast™ Reagent and DNA amounts per 24 well plate (in a total volume of 200µl of media per well) for each cell line were as follows: **CHO cells: 1µg of DNA and 3µl of TransFast™ Reagent. **HeLa cells:** 0.5µg of DNA and 1.5µl of TransFast™ Reagent. **NIH3T3 cells:** 1µg of DNA and 3µl of TransFast™ Reagent. **293 cells:** 0.25µg of DNA and 0.75µl of TransFast™ Reagent. Cells were lysed approximately 48 hours post-transfection with Cell Culture Lysis Reagent (Cat.# E1531) and assayed for luciferase activity using a standard luminometer.

Can TransFast™ Reagent be resuspended and frozen on the day of the transfection?

Yes. As depicted in Figure 3, the lipid film maintained high activity when suspended and frozen on the day of the experiment. Similar results for transfection efficacy were obtained for reagent samples that were frozen at 20°C or 70°C (data not shown).

Is TransFast™ Reagent stable to multiple freeze-thaw cycles?

We subjected TransFast™ Transfection Reagent to 10 freeze-thaw cycles and measured transfection efficacy (Figure 4, Panel A). Performance of the reagent was not significantly affected by the freeze-thaw cycles (Student's t test paired comparisons, $p < 0.05$).

How stable is TransFast™ Reagent after suspension?

We assayed the transfection activity of TransFast™ Reagent suspended with water and stored at 20°C for up to 8 weeks (Figure 4, Panel B). All samples were assayed with NIH3T3 cells in the same experiment to eliminate interassay variability. The results indicated a slight decrease in activity at 2 weeks, and then no significant loss of activity over the remaining 6 weeks of the study (Student's t test paired comparisons, $p < 0.05$).

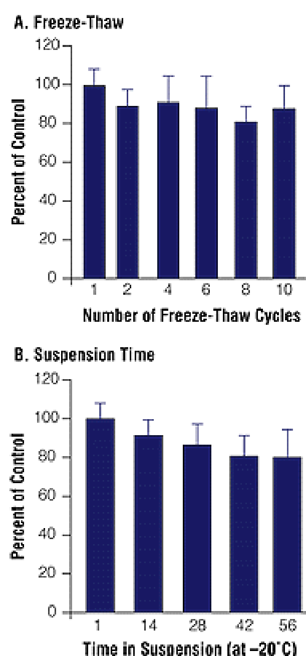

Figure 4. Stability of TransFast™ Transfection Reagent. Panel A: Freeze-thaw study of TransFast™ Transfection Reagent. Three vials of TransFast™ Transfection Reagent were used for this experiment, and each was suspended with water and stored overnight at 20°C prior to transfection. Subsequent freezing and thawing cycles were accomplished by freezing each vial of reagent at 20°C and thawing at room temperature. Each vial was frozen and thawed up to 10 times. At given cycle numbers, reagent was removed, complexed with DNA and used to transfect NIH3T3 cells using the conditions described for this cell line in the legend to Figure 3. Each bar on the graph represents the mean normalized light units +/- S.D. of cell lysates, with 6 replicates per vial of TransFast™ Reagent, for a total of 18 samples per cycle number. Samples that were thawed once were chosen as the 100% control value. **Panel B: Stability of frozen TransFast™ Reagent lipid suspensions over time**. Three vials of TransFast™ Reagent were suspended in water, consolidated into a single vial and frozen at 20°C at each time point prior to transfection. Vials of TransFast™ Reagent were suspended 1 day, and 2, 4, 6 and 8 weeks prior to transfection. On the day of transfection, the reagents were thawed and used to transfect NIH3T3 cells using the standard protocol described in the legend to Figure 3. Each bar on the graph represents the mean normalized light units +/- S.D. of cell lysates, with 4 replicates per time point. The one-day sample was chosen as the 100% control value.

The protocol recommends transfecting adherent cells that are approximately 80% confluent. What is the effect of cell number on overall transfection activity with TransFast™ Reagent?

The protocol recommends plating approximately 0.5×10^5 cells per well in a 24 well plate. We tested four different cell lines and plated four different concentrations of cells, ranging from 0.252×10^5 cells per well. The cells were transfected approximately 24 hours later using TransFast™ Reagent according to the standard procedure. As shown in Figure 5, highest luciferase activity per well of transfected cells tended to correspond with an original plating of 0.51×10^5 cells per well. Transfection efficacy, as monitored by total luciferase activity, decreased at lower (0.25×10^5) and higher (2×10^5) cell concentrations. The results also illustrate that cell lines differ in their response to TransFast™ Reagent when plated at different densities prior to transfection. For example, HeLa cells respond

better to transfection when plated at a higher density (1 x 10^5 cells per well). In contrast, CHO cells respond optimally at 0.5 x 10^5 cells per well. For other cell lines, the effect of cell plating density can be tested to identify optimal conditions for transfection efficacy.

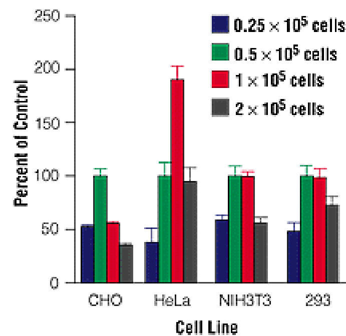

Figure 5. Cell density and transfection efficacy of TransFast^{TM} Transfection Reagent. CHO, HeLa, NIH3T3 and 293 cells were plated the day prior to transfection in 24 well plates at 0.252 x 10^5 cells per well. Cells were transfected according to the standard procedure described in the legend to Figure 3. Each bar on the graph represents the mean normalized light units +/- S.D. of cell lysates, with 6 replicates. The 0.5 x 10^5 cell per well sample was chosen as the 100% control value.

Should polypropylene or polystyrene tubes be used for preparing the TransFast^{TM} Reagent and DNA complexes?

A comparison of polypropylene and polystyrene tubes indicated that the type of tube had no significant effect on subsequent transfection performance. Either polypropylene or polystyrene tubes can be used with TransFast^{TM} Transfection Reagent. Transfections were tested with CHO, HeLa, NIH3T3 and 293 cells (data not shown).

The one-hour incubation interval recommended for TransFast^{TM} Reagent is relatively short compared to other commercial liposome protocols. What is the effect of lengthening the transfection interval? The overlay method recommended also differs from that of most other transfection protocols in that medium is added directly to the cells and the transfection mixture is not removed. What is the effect of removing the lipid and DNA solution after the transfection interval?

In general, a one- to two-hour transfection interval is optimal for transfection activity (Figure 6). A 24-hour transfection interval under serum-free conditions was detrimental, but was well tolerated in serum-containing media (e.g., with CHO cells). With the exception of HeLa cells, removing the transfection mixture did not result in increased transfection efficiency compared with the overlay method. Removing the transfection mixture and replacing it with fresh media is more tedious to perform than the overlay method, which simply involves adding serum-containing media to the transfection mixture. HeLa cells tend to be quite sensitive to the effects of TransFast^{TM} Reagent, and a short exposure to the transfection mixture (1 hour), combined with replacing the transfection mixture with complete media, appeared to be optimal for this cell line. This approach may be appropriate for other cell types that are especially sensitive to the effects of transfection reagents.

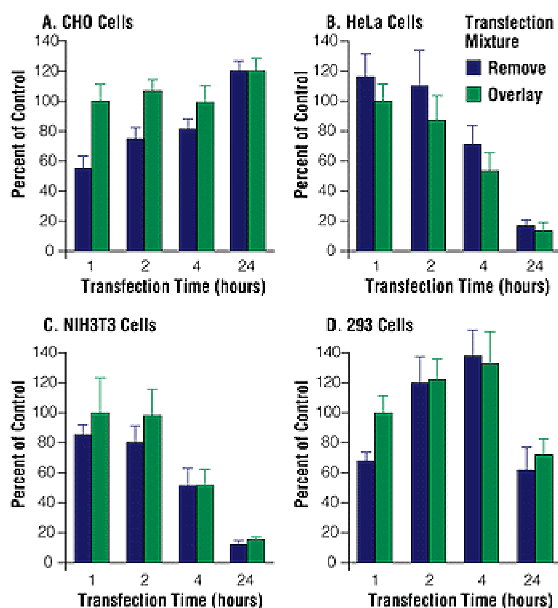

Figure 6. Effects of transfection interval and media replacement on transfection efficacy. CHO, HeLa, NIH3T3 and 293 cells were transfected with TransFast™ Reagent that was suspended with water and stored at 20°C overnight. The lipid/DNA complexes were prepared under standard conditions as described in the legend to Figure 3, and 200µl were applied to cultured cells from which the media had been removed. The complexes remained on the cells for 1, 2, 4 or 24 hours. At the end of these intervals, 1ml of complete media was either added to the cells without removing the lipid/DNA complex (Overlay Transfection Mixture), or added after removal of the lipid/DNA complex (Remove Transfection Mixture). Each bar on the graph represents the mean normalized light units +/- S.D. of cell lysates, with 6 replicates. The samples overlaid with complete media after 1 hour were chosen as the 100% control value for each cell line.

Additional technical information on TransFast™ Transfection Reagent and other Promega transfection reagents, such as the Tfx™ Reagents[(d)], is available in the *Transfection Guide* (#BR041). References for cell lines that were used with Promega transfection reagents, as well as example transfection conditions, are available from the online resource Transfection Assistant located on the Promega Internet site at www.promega.com. This information may also be obtained on request from Promega Technical Services.

ACKNOWLEDGEMENT

We wish to thank Virginia Goiffon for her contributions to the characterization of the TransFast™ Transfection Reagent.

Ordering Information

Product	Size	Cat.#
TransFast™ Transfection Reagent	1.2mg	E2431
Tfx™-50 Reagent[(d)]	2.1mg	E1811
Tfx™-20 Reagent[(d)]	4.8mg	E2391
Tfx™-10 Reagent[(d)]	9.3mg	E2381
Tfx™ Reagents Transfection Trio	5.4mg	E2400
pGL3 Control DNA	20µg	E1741
Cell Culture Lysis Reagent	30ml	E1531
Luciferase Assay System[(e)]	100 assays	E1500

[(a)]The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. No. 5,824,812 and pending foreign patents.

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

[(c)]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

[d]The cationic lipid component of the Tfx™ Reagents is covered by U.S. Pat. Nos. 5,527,928, 5,744,625 and pending foreign patents.

[e]U.S. Pat. No. 5,283,179 and other patents.

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