

# Optimization of Transfectam<sup>®</sup>-Mediated Transfection Using a Luciferase Reporter System

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*Transfectam reagent offers several advantages over standard techniques for transfection of many eukaryotic cell lines. Key factors for optimizing transfection efficiency with a given cell line include the duration of transfection, concentration of DNA, volume of media, and ratio of Transfectam to DNA. In this article, we describe the optimization of transfection efficiency for mouse NIH 3T3 cells using a luciferase reporter gene assay.*

The ability to introduce DNA into cells provides a powerful tool for studying the *in vivo* function and control of mammalian genes. Commonly used methods for gene transfection use calcium phosphate (1), DEAE-Dextran (2), electroporation (3) or liposomes (4) to facilitate the entry of DNA into cells. Transfectam reagent, a synthetic cationic lipopolyamine molecule, provides a new alternative to these transfection methods.

Transfectam reagent, developed at the University of Strasbourg in France (5), contains a spermine group with a strong affinity for DNA ( $K_d = 10^{-5}$ - $10^{-7}$  M) covalently attached through a peptide bond to a lipid moiety (Figure 1). It forms unilamellar vesicles of 800-1,000Å in size. The positively charged spermine headgroups bind to DNA, coating it with a lipid layer. In the presence of excess lipopolyamine, cationic lipid-coated plasmid DNA particles are formed. The lipid regions of these particles associate with the cell membrane and internalization of the DNA probably occurs by endocytosis.

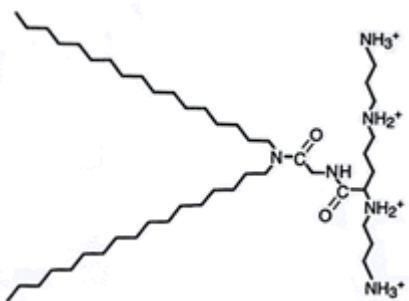


Figure 1. Structure of Transfectam reagent.

## Efficient and Rapid Transfection

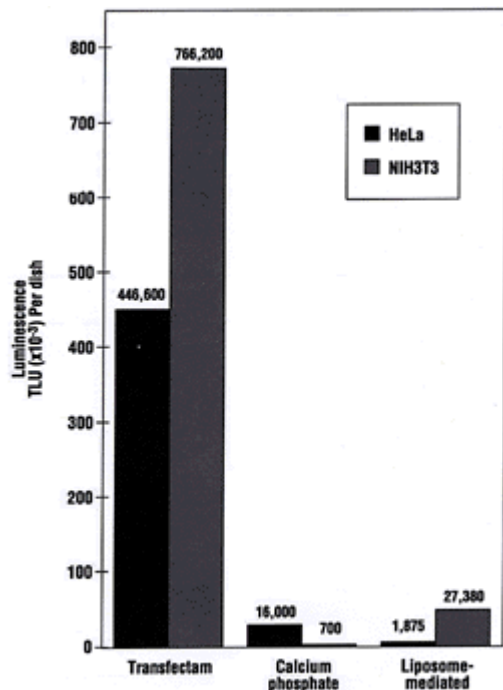
Transfectam-mediated transfection offers several advantages over other techniques for transfection. The transfection procedure is simple: Transfectam solution is mixed with the DNA solution and applied directly to cells. Maximum efficiency is obtained in serum-free medium. Due to the high affinity of the spermine headgroups for DNA, plasmid DNA is efficiently coated with the lipopolyamines. As a result, less DNA can be used per transfection than with other techniques, such as calcium phosphate coprecipitation.

Studies with primary melanotrope cells showed a rapid association of the DNA-lipospermine complex with the cells (5). Maximum transfection efficiency was obtained in 30 minutes with these cells. A short transfection time minimizes the risk of cell death, which is an important consideration for fragile cells that do not tolerate serum deprivation well.

## Successful Transfection with a Wide Range of Cell Types

Transfectam reagent has been used successfully in a variety of established cell lines, as well as several different primary cell cultures (6,7). It can be used for both transient expression or stable transformation studies. The use of this reagent has resulted in the successful transfection of many different cell types (8). Cell types that are difficult to transfect, such as neurons (9) and endocrine cells (5), have also been transfected successfully with Transfectam reagent.

The commonly used HeLa and mouse NIH 3T3 cell lines were used to compare the relative transfection efficiencies obtained with three different reagents (Figure 2). For both cell lines, the use of Transfectam led to at least 28-fold more expression of the luciferase reporter gene than with either calcium phosphate or liposome-mediated transfection.



**Figure 2. Transient expression of luciferase in HeLa and NIH 3T3 cells transfected by different methods.** Transfection was performed according to manufacturers' standard protocols for Transfectam reagent, the ProFection<sup>TM</sup> calcium phosphate system (Promega), and Lipofectin<sup>TM</sup> reagent (Life Technologies, Inc.), with the minor changes noted below.

Cells were exposed to Transfectam (in 1ml DMEM) or Lipofectin (in 1.5ml DMEM) for 24 hours, then replaced with 4ml DMEM containing 10% fetal bovine serum (FBS). The calcium phosphate precipitate, in DMEM with 10% FBS, was present for 48 hours. All cells were harvested 48 hours after initial exposure to DNA. Cell extract were assayed for luciferase, and the results were expressed in Turner Light Units (TLU) per dish of cells.

## Nontoxic and Stable

The use of Transfectam does not alter the physiological responses of neuroendocrine or peripheral neuronal cells (5,9). The absence of toxicity is especially important for the successful transfection of sensitive cells, such as primary cells. Transfectam reagent is supplied as a lyophilized powder, which is stable indefinitely at room temperature. After reconstitution with an ethanol solution, the reagent can be stored up to 6 months at 4°C.

## Optimization of Transfection Conditions

With any transfection method, it is important to optimize transfection conditions for each cell type studied. When using Transfectam reagent, key factors to optimize include the duration of transfection, concentration of DNA, and ratio of Transfectam to DNA.

It is technically difficult to assay directly for DNA uptake, but several easy assays exist for reporter gene products such as luciferase (10), chloramphenicol acetyltransferase (CAT) (11) and beta-galactosidase (12). The relative transfection efficiencies during the optimization studies can be inferred from the relative amounts of reporter protein activity measured in cell extracts. In this article, several transfection conditions were optimized for mouse NIH 3T3 cells using the luciferase reporter plasmid, pRSV-L (10).

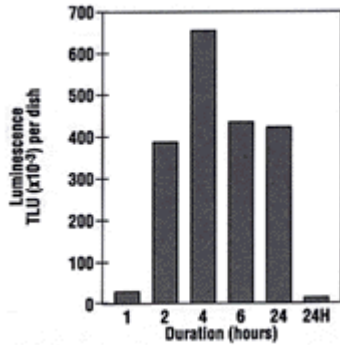
## Experimental Procedures

For these studies,  $5 \times 10^5$  cells were plated per 60mm dish the day before transfection, which resulted in approximately 75% confluency at the time of DNA exposure. Transfections were performed in serum-free Dulbecco's minimal essential medium (DMEM) at a Transfectam:DNA ratio of 2 $\mu$ l Transfectam to 1 $\mu$ g plasmid DNA. Unless indicated otherwise, 5 $\mu$ g of pRSV-L DNA was used per transfection and cells were harvested at 48 hours after the initial exposure to DNA. Promega luciferase assay reagents were used for cell lysis and for the assay of luciferase activity in cell extracts. Light emittance due to luciferase expression was measured with a Turner luminometer (Model TD-20e), and these data were expressed in Turner Light Units (TLU). Extracts prepared from control cells transfected with no pRSV-L DNA gave no detectable luciferase activity. Relative transfection efficiencies were inferred from the relative amounts of luciferase activity measured in cell extracts.

## Duration of Transfection

**Figure 3** shows the effect of transfection duration on the level of luciferase activity expressed in NIH 3T3 cells. Maximum luciferase activity was obtained from cells exposed to Transfectam for 4 hours. Exposure for longer periods, up to 24 hours, yielded a slightly lower, nearly constant amount of active luciferase per dish of cells. Cells transfected in the serum-free medium for 24 hours and then directly harvested expressed a low level of luciferase, 30-fold lower than that observed in cells allowed to recover in serum-containing medium for an additional 24 hours. A post-transfection recovery period in serum-containing media may be important for transient expression studies of other cell types, as well.

Monitoring the time course of transfection will be especially important for sensitive cell types that do not tolerate long exposures to serum-free media.



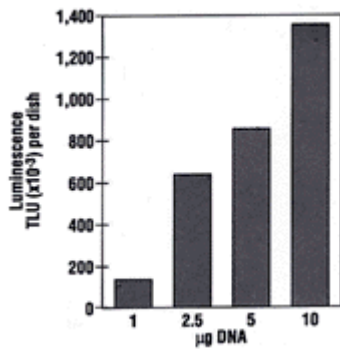
**Figure 3. Effect of transfection duration on transient expression of a luciferase reporter gene.** NIH 3T3 cells were exposed to Transfectam reagent in DMEM for the indicated time intervals, the medium was replaced with 4ml DMEM containing 10% FBS, and cells were harvested at 48 hours after initial exposure to DNA. Sample 24H was transfected for 24 hours and then directly harvested. Cell extracts were assayed for luciferase, and the results were expressed in Turner Light Units (TLU) per dish of cells.

The effect of the post-transfection recovery period described above illustrates the point that reporter gene activity is only an indirect measure of the initial transfection efficiency. Presumably the transfection efficiency, defined as the percentage of cells that take up exogenous DNA, was identical in the two cultures transfected for 24 hours. Yet, the luciferase activity increased 30-fold within the next 24 hours in cells exposed to serum-containing medium.

The levels of expressed reporter proteins measured at the time of cell harvest also are affected by many factors that influence gene expression. The general status of the cells (i.e., proliferating or contact-inhibited), the rates of transcription and translation, and the stability of reporter mRNA and protein products in the cells will each have an impact.

## Concentration of DNA

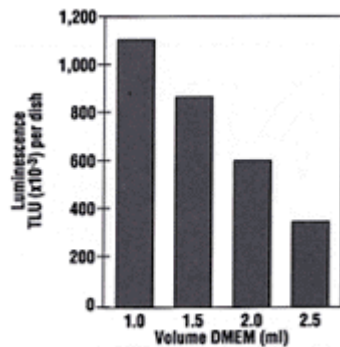
The relative level of luciferase expression increased with increasing DNA concentration ([Figure 4](#)) in a roughly linear pattern between 1 and 10 $\mu$ g DNA ( $r=0.96$ ). Because the conditions during and after transfection were constant for these samples, the observed increase in activity reflects an increased transfection efficiency. Other factors that could affect the efficiency of transfection include the size of the plasmid DNA and the purity of the specific DNA preparation used.



**Figure 4. Effect of DNA concentration on transient expression of a luciferase reporter gene.** NIH 3T3 cells were transfected with the indicated amounts of pRSV-L plasmid DNA. The ratio of Transfectam to DNA was constant, at 2µl Transfectam per 1µg DNA. Cells were transfected for 24 hours in 1.5ml DMEM, the media was replaced with 4ml DMEM containing 10% FBS, and cells were harvested 24 hours later. Cell extracts were assayed for luciferase, and the results were expressed in Turner Light Units (TLU) per dish of cells.

## Volume of Media During Transfection

The volume of media on the cells during the transfection also influenced the transfection efficiency with NIH 3T3 cells. A minimal volume of media (1ml per 60mm dish) led to the highest transient expression of luciferase (Figure 5). The observed decrease in expression of luciferase was proportional to the extent of dilution of the cationic lipid-coated plasmid DNA in the cell medium ( $r=-0.99$ ).



**Figure 5. Effect of media volume during transfection.** NIH 3T3 cells were transfected with 5µg pRSV-L plasmid DNA and 10µl Transfectam reagent for 24 hours in the indicated volumes of DMEM. After 24 hours, the medium was changed to 4ml DMEM with 10% FBS, and the cells were harvested 24 hours later. Cell extracts were assayed for luciferase, and the results were expressed in Turner Light Units (TLU) per dish of cells.

## Transfectam:DNA Ratio

Another parameter that can be optimized is the Transfectam:DNA ratio. For each µg of DNA, between 1.5 and 5µl of Transfectam can be added to determine the best ratio for subsequent experiments.

When optimizing transfection conditions for an untested cell line, we recommend initially trying the following conditions: 5µg plasmid DNA with 10µl Transfectam per 60mm dish of cells, a final volume

of 1.5ml serum-free DMEM, and overnight incubation with the cells. Each of these factors can then be further optimized.

## Summary

Transfectam reagent yields transfection efficiencies superior to other methods for many primary and established cell lines. Its ease of handling, relatively short transfection durations and applicability to either transient expression or stable transformation studies make this reagent useful for both the novice and expert performing cell transfections. For mouse NIH 3T3 cells, increased transfection efficiencies of the pRSV-L vector were obtained with increased DNA concentration, a minimal volume of media, and a 4-hour transfection duration.

## References:

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## Ordering Information:

Product	Size	Cat.#
Transfectam <sup>®</sup> Reagent	1mg powder	E1231

Includes sufficient reagents for 50 transfections.

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