

molecular biology

ESCORT™ V: An Extremely Efficient Reagent for Adherent Cell Transfection

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Application Notes

- Works effectively on a wide variety of cell lines – adherent or in suspension
- No toxic effect on tested cell line at the effective concentrations
- Stable reagent with a long shelf life
- Superior or comparable to commercially leading transfection reagents
- Easily scalable and provides highly reproducible results

Introduction

Following the rapid growth of knowledge in gene function and advances in gene therapy, gene delivery has become an indispensable tool for changing cell behavior, by modifying its gene expression profile. Foreign gene delivery is performed by utilizing different transfection methods that employ DNA carriers such as calcium phosphate, lipids, modified viruses and cationic polymers.

Polyethylenimine (PEI) cationic polymer, was introduced in 1995 as a versatile transfection reagent. It was successfully used for gene transfer and antisense oligonucleotide delivery both *in vitro* and *in vivo*. PEI binds tightly to DNA forming small globular or toroidal complexes that enter cells by endocytosis. Being in the endosome, PEI acts as a “proton sponge,” effectively buffering the endosomal interior, facilitating endosome disruption, and thus enabling the release of the complex into the cell cytoplasm. This intrinsic PEI endosomal release activity gives it an advantage over other polycationic polymers that require additional endosomal disrupting agents.

In this application note, we demonstrate an effective and reliable method for *in vitro* cell transfection using ESCORT™ V Transfection Reagent (Product Code [E 9778](#)), a specially processed polyethylenimine (PEI) that is highly efficient, stable, provides reproducible results, and is easy to use.

Highly efficient transfection reagent

Different cell lines — HCT 116, BHK, HeLa, HEK 293, HEK 293T, Vero, F9, PC-12, COS-1, COS-7, NIH 3T3, L929, CHO (adherent and in suspension), Jurkat, human foreskin fibroblasts (HFF) primary cells, and Bovine Endothelial Aorta Cells (BEAC) — were transfected with pCMV-gal using ESCORT V Transfection Reagent. The transfected cells were subjected to β -galactosidase staining using the β -Galactosidase Reporter Gene Staining Kit (Product Code [GAL-S](#)). Cells expressing the transfected β -galactosidase reporter gene were stained blue. The transfection yields following the use of ESCORT V Transfection Reagent were very high and could reach 60-100% depending on the cell line tested.

A specific example demonstrating the transfection efficiency of HEK 293T cells by ESCORT V Transfection Reagent using β -galactosidase reporter gene is demonstrated in Figure 1. Transfection efficiency measured by the β -galactosidase staining reached 90-100%.

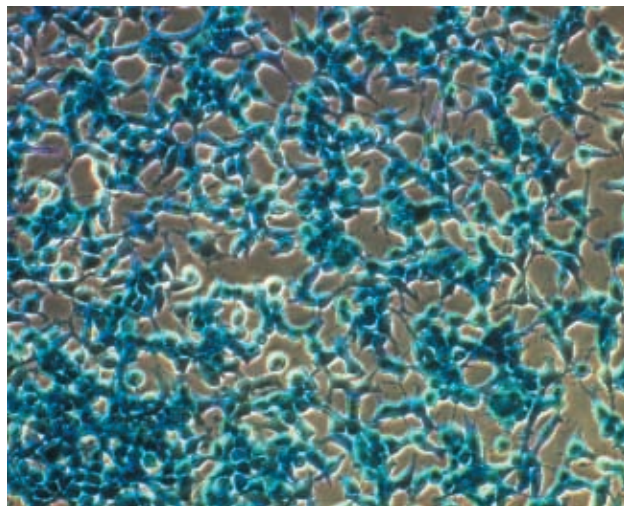


Figure 1. ESCORT V Transfection efficiency in HEK 293T. HEK 293T cells were transfected with pCMV-gal using ESCORT V Transfection Reagent according to the technical bulletin. Forty-eight hours following transfection the transfected cells were subjected to β -galactosidase staining using the β -Galactosidase Reporter Gene Staining Kit (Product Code [GAL-S](#)).

The transfection efficiency of ESCORT V was compared to that of two other benchmark competitors, Competitors G and R. Transfections were performed according to the technical bulletin of each transfection reagent. The cells were transfected with pCMV-gal plasmid and tested for β -galactosidase activity by a colorimetric assay, using Sigma's β -Galactosidase Reporter Gene Activity Detection Kit (Product Code [GAL-A](#)).

The transfection efficiency of each transfection reagent varied between cell lines. The comparison between the performance of ESCORT V Transfection Reagent and the competitors' products revealed that the transfection efficiency of ESCORT V is comparable or outperforms the competitors' reagents in the tested cell lines. A comparison between the performance of ESCORT V Transfection Reagent and the competitors', using HeLa, HEK 293 and NIH 3T3 cells, is demonstrated in Figure 2.

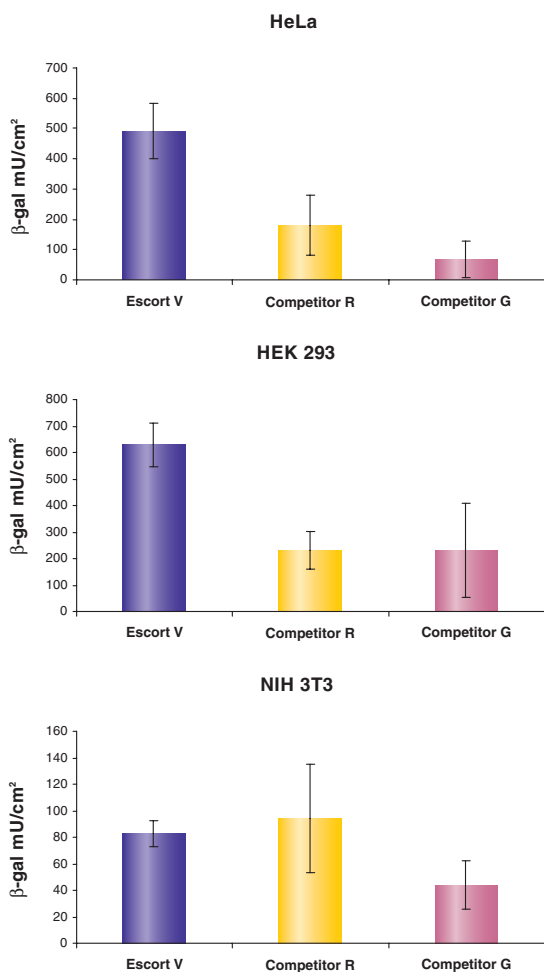


Figure 2. Performance comparison between ESCORT V Transfection Reagent and benchmark competitors. The plasmid pCMV-gal was introduced into HeLa, HEK 293 and NIH 3T3 cells using ESCORT V or Competitors G and R transfection reagents. On the day of transfection the cells were 60-80% confluent. Transfections were performed according to manufacturers' instructions. The cells were incubated for 44-48 hours under standard conditions and then lysed with the CellLytic M reagent (Product Code [C_2978](#)). Lysate samples were tested for β -galactosidase activity using Sigma's β -galactosidase Reporter Gene Activity Detection Kit (Product Code [Gal-A](#)). The data represents results obtained from 4 independent transfection experiments performed in triplicate.

In HeLa cells, ESCORT V outperformed Competitor R ($\alpha = 0.004$, T-test) as well as Competitor G ($\alpha = 0.005$, T-test). In HEK 293 cells, ESCORT V outperformed Competitor R ($\alpha = 0.001$, T-test) and Competitor G ($\alpha = 0.014$, T-test). The performance differences for all three reagents in NIH 3T3 cells were not statistically significant, but the reproducibility of the results for ESCORT V experiments reflected by the standard deviation, was higher than that of the competitors.

Nontoxic and outperforms the competitors

The cytotoxicity of ESCORT V Transfection Reagent was tested using the MTT assay and compared to Competitors G and R. ESCORT V was significantly less toxic than the competitors' reagents. At the effective concentrations, ESCORT V was not cytotoxic or had a minor toxic effect on the cell lines tested. The effect of ESCORT V on the viability of HeLa, HEK 292, and NIH 3T3 cells is shown in Figure 3.

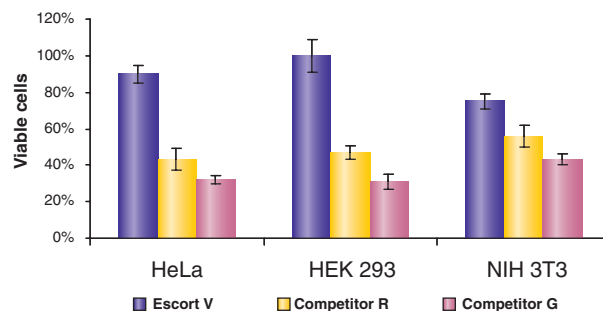


Figure 3. Cell viability following transfection using ESCORT V and competitors' transfection reagents. Cells were transfected with pFLAG-CMV2 vector containing promoter, but no expressible gene, thereby eliminating the possible effects of gene expression, using ESCORT V, Competitors G and R transfection reagents. The amount of transfection reagent used for this experiment was according to the manufacturers' instructions. HeLa, HEK 293 and NIH 3T3 cell lines (5×10^3 cells/well) were plated into 96-well tissue culture plates. Relative cell viability was determined 24 hours after complex addition using MTT assay.

In conclusion, ESCORT V Transfection Reagent is highly efficient in a variety of cell lines, stable, and nontoxic. As demonstrated by experimental results, Sigma's ESCORT V is a superior transfection reagent that outperforms the competition.

Ordering Information

Product	Description	Unit
E 9778	ESCORT V Transfection Reagent (Sufficient for 700-1000 transfections)	1.5 ml