

DNA quality for transfection

Transfection experiments yield best results when plasmid DNA of the highest purity is used (Table 1). DNA purified with QIAGEN[®] or QIAfilter[™] Plasmid Kits is ideally suited for transfection of most cell lines. For transfection of primary cells and endotoxin-sensitive cells, we recommend using DNA purified with EndoFree[™] Plasmid Kits. These kits efficiently remove bacterial lipopolysaccharide molecules during the plasmid purification procedure ensuring optimal transfection results (Table 1).

Table 1. Effect of DNA purification method on transfection efficiency

DNA Purification Method	Effectene Reagent	Liposome Reagent
EndoFree Plasmid Kits	21.0 ± 0.93%	9.2 ± 0.28%
QIAGEN Plasmid Kits	8.1 ± 0.57%	5.7 ± 0.4%
Silica Slurry	5.2 ± 0.74%	5.1 ± 0.5%

Primary rabbit gastric parietal cells were transfected with pEGFP-N2 (CLONTECH) prepared by different methods, and Effectene Reagent or a commonly used liposome reagent. The data represent the percentage of the cells expressing GFP determined by scoring the number of green fluorescent cells 48 h post-transfection. Data generated using Effectene Reagent and plasmid DNA purified with EndoFree Plasmid Kits correspond to images in Figure 9. Data kindly provided by Catherine Chew and John Parente, Medical College of Georgia, Augusta, Georgia, USA.

Trademarks

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pTracer is a trademark of Invitrogen Corporation.

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EndoFree DNA plus Effectene with Primary Cells

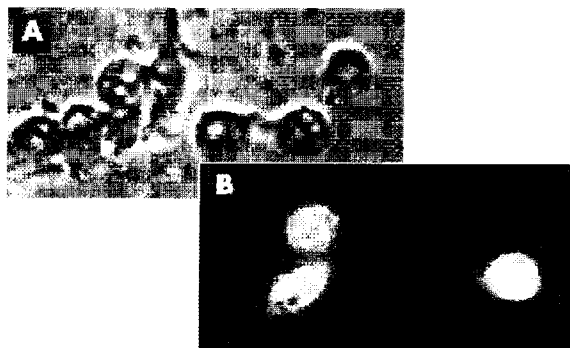


Figure 9. Transfection of primary rabbit gastric parietal cells using Effectene Reagent and plasmid DNA purified with EndoFree Plasmid Kits (see also data in Table 1). Transfection was performed in 35-mm dishes using 0.4 µg pEGFP-N2 (CLONTECH) and 4 µl Effectene Reagent. 1×10^6 cells were seeded per dish one day prior to transfection. On average 21% of the cells expressed GFP 48 h post-transfection. Images of cells were taken with a digital camera under (A) phase-contrast microscopy and (B) fluorescent microscopy. Data kindly provided by Catherine Chew and John Parente, Medical College of Georgia, Augusta, Georgia, USA.

Ready-to-use kits

Effectene Reagent and SuperFect Reagent come complete with a detailed handbook containing protocols for transient and stable transfection of adherent and suspension cell lines as well as valuable information for optimization of transfection.



Effectene Transfection Reagent

The next generation in lipid technology

Effectene[®] Transfection Reagent is a unique new non-liposomal lipid formulation combined with a special DNA-condensing enhancer. The Enhancer first condenses the DNA molecules and Effectene Reagent subsequently coats them with cationic lipids, providing a particularly effective way of transferring DNA into eukaryotic cells. Effectene Reagent offers significant advantages over many liposome reagents and other transfection methods.

Outstanding transfection efficiencies

Effectene Reagent yields significantly higher transfection efficiencies than many widely used liposome reagents. Effectene Reagent can be used for transient and stable transfection of a wide variety of cell lines (Figures 1, 2, and 3). For reagent recommendations on specific cell lines please refer to the table on page 6.

High efficiencies with many primary cells

Independent tests show that Effectene Reagent is particularly effective for transfection of many primary cells (Figure 2, and Figure 9, page 7).

Minimal cytotoxicity

The non-liposomal lipid Effectene Reagent is less toxic to cells than many other transfection reagents. Effectene Reagent enables transfection in the presence of serum without lowering transfection efficiencies, unlike many liposome reagents. This is an important consideration, particularly when primary cells or sensitive cell lines are used.

Comparison of Transfection Efficiencies

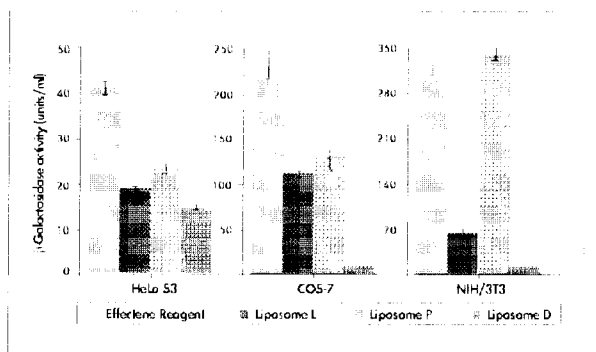


Figure 1. Comparison of transfection efficiencies obtained using Effectene Reagent and three of the most commonly used liposome reagents. HeLa S3, COS-7, and NIH/3T3 cells as indicated were transfected in 96-well format with pCMV β . 2×10^5 cells were seeded per well one day prior to transfection. Transfection efficiencies are given as β -galactosidase units per ml. Each bar represents the average efficiency from 4 replicates.

Effectene Reagent with Primary Cells

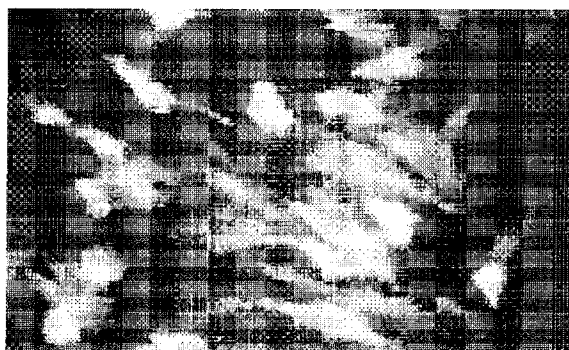


Figure 2. Expression of green fluorescent protein (GFP) from primary rabbit aortic smooth muscle cells transfected using Effectene Reagent. 1×10^5 cells were seeded 1 day prior to transfection, and transfections were performed in 6-well plates using 0.4 μ g pEGFP-N1 (CLONTECH) and 10 μ l Effectene Reagent. Cells were viewed 24 h post-transfection under fluorescence microscopy. Approximately 10% of the cells were transfected, as determined by FACS[®] analysis. Data kindly provided by Kerstin Veit, 2nd. Medical Clinic, Dept Clinical Pharmacology, Mainz, Germany.

No complex removal

Due to the low cytotoxicity of Effectene Reagent, removal of Effectene–DNA complexes after transfection is not necessary for most cell lines. This makes transfection with Effectene Reagent even faster and easier.

Significantly less DNA needed

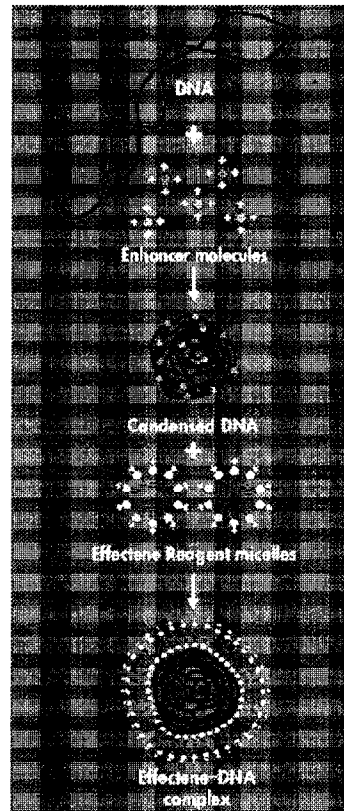
Effectene Reagent is very efficient in delivering DNA into cells. Typically, only one-fifth the quantity of DNA required for most liposome reagents is sufficient. This allows for more experiments with less DNA!

The Effectene Procedure

Effectene Reagent is used in conjunction with a special DNA-condensing enhancer and DNA buffer to achieve high transfection efficiencies. DNA is mixed with the buffer, which provides optimal salt conditions for strong DNA condensation by the enhancer. This step takes just 2–5 minutes. Effectene Reagent is then added and Effectene–DNA complexes form during a 5–10 minute incubation. The complexes are mixed with complete medium and added directly to the cells. The cells are then incubated until harvested and analyzed for gene expression.

Figure 3. Comparison of luciferase activities from M12.4 cells (a murine B cell line) transfected using Effectene Reagent and two liposome-based reagents. Transfections were performed in 24-well plates using 5×10^5 cells and 0.6 μg of a pGL3(Promega)-based reporter construct per well. Bars represent average relative light units (RLU) and standard deviations obtained from three replicates assayed 24 h post-transfection. Data kindly provided by Adrian Goldstein and Richard Scheuermann, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Model of Effectene Principle



Transfection of B cells

