QIAGEN News

Highly efficient stable and transient transfection using Effectene® Reagent

Catherine Smith

Molecular Toxicology, Division of Biomedical Sciences, Imperial College, London, UK

A series of optimization experiments was performed in order to maximize efficiency of both stable and transient transfection of Chinese hamster V79-MZ cells. Using Effectene® Reagent, transient transfection efficiencies of up to 40% and stable transfection efficiencies of up to 0.8% were obtained.

Therapies based on gene transfer require reproducible and efficient transfection procedures and development of standardized protocols to enable comparison of the effects of transfected genes. Development and optimization of these procedures play a vital role in determining the potential effectiveness and safety of nucleic acid-based therapies. We aimed to develop an optimized transfection procedure for Chinese hamster V79-MZ cells grown in flasks, using an enhanced green fluorescent protein (EGFP) reporter gene.

Materials and methods

For optimization studies, Chinese hamster V79-MZ cells were transfected in six-well plates with the plasmid vector pL3112BSKS, which codes for EGFP and incorporates a *neo* resistance gene. DNA (0.8 µg) was transfected into cells using 10:1, 25:1, and 50:1 ratios of Effectene Transfection Reagent to DNA (µl:µg) according to the protocol in the *Effectene Transfection Reagent Handbook*. Plasmids were purified using the QIAGEN® Plasmid Maxi Kit. For scaled-up experiments, V79-MZ cells were seeded at a density of 1.5 x 10^6 cells per T75 cell culture flask (Nunc). Cells were cultured in DMEM supplemented with 5% fetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin. Cells were cultured for 24 hours before transfection using 3.47 µg plasmid DNA and a range of transfection reagent:vector ratios. After transfection, cells were cultured at 37°C and 5% CO_2 and medium was changed after 16 hours. After a further 24 hours growth, cells were harvested and an aliquot analyzed by FACS® to determine transient transfection efficiency. To determine stable transfection efficiency, 100 and 1000 cells were plated using medium containing 0.8 mg/ml G418 (replaced every 4 days) and cultured for 2–3 weeks until colonies could be counted. All transfections were carried out in triplicate. Mock-transfected V79-MZ cells were used as a control. ▶

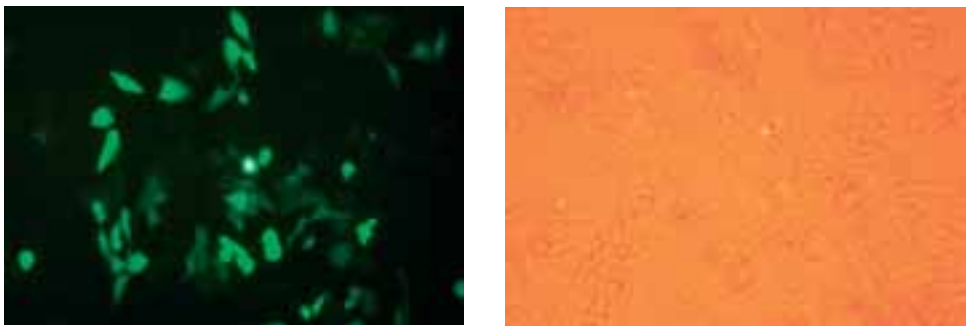

Figure 1 *Fluorescent and light micrograph images of transfected V79-MZ cells. Cells were transfected in T75 flasks with Effectene Reagent–DNA complexes for 16 hours and viewed 24 hours later.*

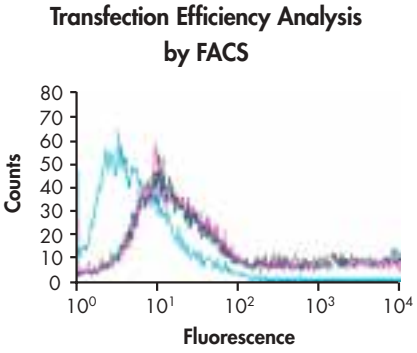

Figure 2 *FACS analysis of transfection efficiency. V79-MZ cells were treated with a 25:1 ratio (v/w) of Effectene Reagent:DNA. Experiments were carried out in triplicate. The cyan trace shows analysis of mock-transfected cells.*

Results

Four different ratios of Effectene Reagent:DNA were used. Figure 1 shows fluorescent and light microscope images of transfected cells. FACS traces from one set of experiments are shown in Figure 2. Figures 3 and 4 show the efficiencies of transient and stable transfection respectively. The most efficient transient and stable transfection was obtained using an Effectene Reagent to DNA (µl:µg) ratio of 25:1. Transient transfection efficiencies of up to 40% and stable transfection efficiencies of up to 0.8% were obtained.

Conclusions

By optimizing the ratio of Effectene Reagent to DNA used, high efficiencies were obtained in both transient and stable transfection experiments. By allowing transfection in the presence of serum, cytotoxicity is reduced. Transfection reactions can be easily scaled up using the simple procedure. The high efficiency and reproducibility of the procedure facilitates comparison of the effects of transfected genes and their gene-products on cells. ■

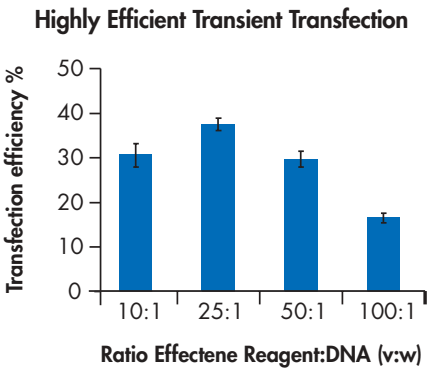

Figure 3 *Efficiency of transient transfection. V79-MZ cells were transfected in triplicate using the indicated ratios of Effectene Reagent to DNA. Cells were incubated for 16 h, the medium was changed, and the efficiency of transfection was analyzed by FACS after a further 24 hours of growth.*

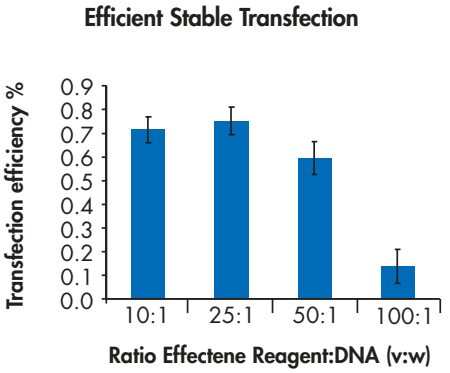

Figure 4 *Efficiency of stable transfection. V79-MZ cells were transfected in triplicate using the indicated ratios of Effectene Reagent to DNA. Efficiency of stable transfection was determined as described in "Materials and methods".*

Ordering Information

Product	Contents	Cat. No.
Effectene Transfection Reagent (1 ml)	1 ml Effectene Reagent, Enhancer, Buffer; for 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
Effectene Transfection Reagent (4 x 1 ml)	4 x 1 ml Effectene Reagent, Enhancer, Buffer; for 160 transfections in 60 mm dishes or 640 transfections in 12-well plates	301427