# Transfection of human aortic endothelial cells (HAECs) with METAFECTENE™ PRO and comparison with Lipofectamine™ 2000 reagent

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## INTRODUCTION

In order to evaluate the feasibility of transfecting non-viral plasmids into human aortic endothelial cells, we compared the transfection efficiency of two commercial reagents METAFECTENE™ PRO and Lipofectamine™ 2000 to deliver a constitutive CMV-controlled plasmid, DsRed2-C1, to these cells.

## MATERIALS AND METHODS

Metafectene™ PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). Lipofectamine™ 2000, a cationic liposomal transfection reagent, and OptiMEM reduced serum media were obtained from Invitrogen (Carlsbad, CA). The DsRed2-C1 vector was obtained from Clontech, Inc. (Mountain View, CA). Primary HAEC cells and growth medium were obtained from the laboratory of Dr. Gary Nackman (UMDNJ, New Brunswick, NJ).

Cells were plated into a 24-well plate one day prior to transfection at 50% confluency. Cells were pre-washed with OptiMEM media and 300 µL of OptiMEM was added to each well.

The Lipofectamine™ 2000 transfection reagent was complexed with the DsRed2-C1 plasmid at a pre-optimized reagent:DNA ratios of 2 µl:0.8 µg. Both DNA and reagent were diluted in 50 µL of OptiMEM media, combined after 5 minutes of incubation at room temperaure, incubated for an additional 20 minutes, and then added to the cells for 3.5 hours at 37°C and replaced with regular growth medium. Fluorescent activity was assessed after 24 hours via flow cytometry analysis (10,000 cells counted).

The Metafectene™ PRO transfection reagent was complexed with the DsRed2-C1 plasmid at a reagent:DNA ratios of 1 µl:1 µg, 4 µl:1 µg or 6 µl:1 µg. DNA was diluted in 50 µl of OptiMEM media. Metafectene™ PRO was diluted in 50 µl of OptiMEM media. The DNA solution was added to the Metafectene™ PRO solution and incubated at room temperature for 20 minutes. The complexes were then added to the cells and incubated at 3.5 hours at 37°C and replaced with regular growth medium. Fluorescent activity was assessed after 24 hours via flow cytometry analysis (10,000 cells counted).

## RESULTS

As noted in Table I, the reagent:DNA ratio played a major role in optimizing transfection efficiency. Pre-optimized transfection reagent ratio for Lipofectamine™ 2000 yielded a maximum efficiency of 13.56%. The Metafectene™ PRO conditions yielded range of efficiencies from 10.61%-23.81%, with the maximum efficiency attained at the 1 µl:1 µg ratio.

Table I. Transfection conditions and expression.

| ***Transfection condition*** | ***Percentage of cells fluorescent (transfected)*** |
|---|---|
| Lipofectamine™ 2000 (2 µl:0.8 µg) | 13.56% |
| Metafectene™ PRO (1 µl:1 µg) | 23.81% |
| Metafectene™ PRO (4 µl:1 µg) | 10.61% |
| Metafectene™ PRO (6 µl:1 µg) | 11.82% |

## CONCLUSIONS

While challenges still exist in improving non-viral transfection efficiency to primary human aortic endothelial cells, Metafectene™ PRO transfection reagent shows an advantage over the Lipofectamine™ 2000 reagent in increasing gene delivery on a cell percentage / population basis.