

DNA Transfection with High Transfection Efficiency and Low Toxicity Using NIMT[®]FeOfection

Sylvia Koivunen and Pontus Kjellman
Genovis AB

Introduction

Transfection of DNA is used to analyze and study functions of genes, regulatory sequences and metabolism in living cells (1). To study cells and the effects of the gene transfer researchers need methods that give high transfection efficiencies, during a period of time and with low toxicity. Lipid based transfection agents often generates good transfection rates but with high toxicity (2). Here the novel NIMT[®]FeOfection transfection agents are tested for its ability to transfect different cell types and cell lines with a high efficiency and low toxicity.

Materials and Methods

Cell Cultures

K562 (Human myeloid leukaemia) cells were grown in RPMI 1640 supplemented with 10% FCS and 5% streptomycin/penicillin (Invitrogen). 293 (Human Embryonic Kidney) and COS-7 (Green Monkey Kidney) were grown in DMEM (Invitrogen) supplemented with 10% FCS and 5% Streptomycin/Penicillin (Invitrogen). All cells were grown at 37°C and 5% CO₂. Adherent cell lines were seeded into plates or dishes one day before transfection giving 60-80% confluence on the day of transfection. Suspension cells were seeded into plates or dishes on the day of transfection.

Transfection Using NIMT[®] FeOfection

DNA used: pHRL-SV40 vector (Promega) and pEGFP-C1. NIMT[®]FeOfection|YELLOW (K562) or NIMT[®]FeOfection|PINK (COS-7 and HEK293) and DNA were diluted in double distilled (dd) H₂O before mixing. The FeOfection/DNA solution was incubated 15-30 min in room temperature before addition to cells. Other transfection agents (Lipofectamine[™] 2000 (Invitrogen), PolyFect (Qiagen) and HiFect (Amaya)) were prepared according to manufacturers protocol.

Evaluation of Transfection

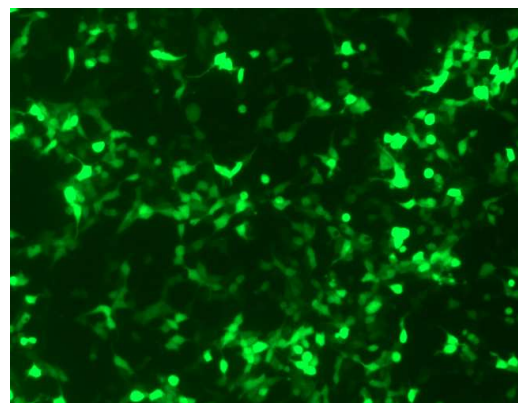
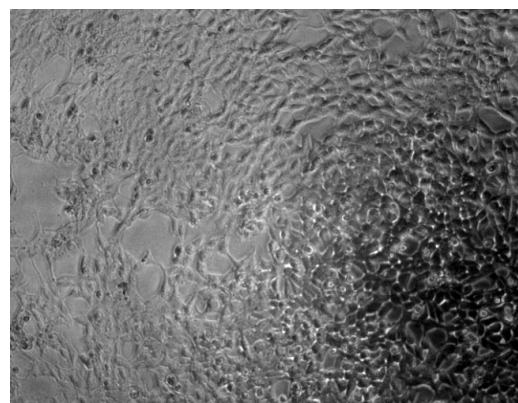
Bright field and fluorescence images were taken of HEK293 cells transfected with pEGFP-C1 48 hours post transfection. EnduRen[™] Live Cells Substrate (Promega) was prepared as described in manufactures protocol and then added to cells transfected with pHRL-SV40 vector 24 hours post transfection.

Cytotoxicity Assay

Cytotoxicity was measured 24-48 hours post transfection with MultiTox-Fluor Multiplex Cytotoxicity Assay (Promega). The assay was prepared and used as described in the protocol. The ratio between dead and live cells was calculated and the values represent the cytotoxicity.

Results and Discussion

48 hours post transfection with NIMT[®]FeOfection|PINK images were taken of HEK293 cells. The pictures show cells with a normal phenotype as well as high transfection efficiency (picture 1).



Picture 1: Bright Field and Fluorescence images of HEK293 cells taken 48 hours post transfection.

Luminescence was measured 24 hours post transfection on COS-7 cells and 48 hours post transfection on K562 cells to evaluate transfection efficiencies. Cytotoxicity was then measured on these cells and the ratio between gene expression and toxicity was calculated. Cells with high gene expression and a low toxicity will get a high ratio value (figure 2 and 3).

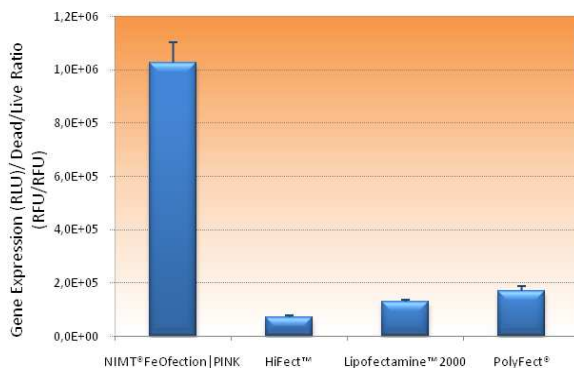


Figure 2: Ratio between transfection and cytotoxicity on COS-7 cells 24 hours post transfection. Cells were transfected with NIMT®FeOfection|PINK, Lipofectamine™ 2000, HiFect and PolyFect.

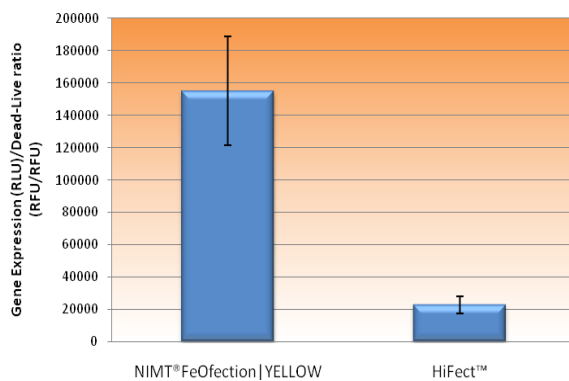


Figure 3: Ratio between transfection and cytotoxicity on K562 cells 48 hours post transfection. Cells were transfected with NIMT®FeOfection|YELLOW and HiFect.

Conclusions

NIMT®FeOfection was compared to other commercially available transfection reagents for DNA in regard to their transfection efficiency and toxicity. The results presented here show that NIMT®FeOfection generates high transfection rates and with low cytotoxicity on both adherent and suspension cell lines.

References

1. Freshney R.I, Culture of Animal Cells, A Manual of Basic Technique, 5th Ed., Wiley-Liss
2. Aramaki Y, Takano S, Tsuchiya S (1999) Induction of apoptosis in macrophages by cationic liposomes. FEBS Lett 460:472-476