

NIMT[®]FeOfection|PURPLE in siRNA Delivery into HEK293 Cells

Malin Mejáre
Genovis AB

Introduction

Short interfering RNA (siRNA) is used in many different applications to study gene silencing. For successful gene silencing the siRNA delivery method is crucial. An attentive delivery agent should stabilize the siRNA and give minimal background effects on cell viability and metabolism. NIMT[®]FeOfection|PURPLE is explored here for its siRNA delivery ability.

Materials and Methods

Cell culture

HEK293 (Human Embryonic Kidney) cells were cultured in DMEM (Invitrogen) supplemented with 10% FCS (Invitrogen) and 5% streptomycin/penicillin (Invitrogen) in 37°C at 5% CO₂. Cells were seeded in 96-well plates 24 hours before transfection giving 20-30% confluence on the day of transfection.

Transfection

siRNA used was *Silencer*[®] KIF11 (Eg5) siRNA, *Silencer*[®] GAPDH siRNA, *Silencer*[®] Negative control #1 siRNA and *Silencer*[®] Negative control #2 siRNA all from Ambion. siRNA was used at a concentration of 50nM.

NIMT[®]FeOfection|PURPLE and siRNA were diluted in ddH₂O to desired concentrations.

NIMT[®]FeOfection|PURPLE and siRNA were mixed in equal volumes and the samples were incubated for 15-20 min at room temperature. The siRNA/FeOfection complexes were then added to cells. Transfection with other commercial transfection agents, Lipofectamine[™] RNAiMax (Invitrogen) and DharmaFECT (Dharmacon), were performed according to their protocols.

Knockdown of KIF11 (Eg5)

The cells were studied in brightfield microscope 48 hours post transfection and brightfield images were taken. The cell proliferation was determined with Celltiter 96[®] Aqueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's protocol. The absorbance was read at 490 nm.

GAPDH Enzyme Assay

The GAPDH activity in cells transfected with *Silencer*[®] GAPDH, *Silencer*[®] Negative control #1 or *Silencer*[®] Negative control #2 was measured with Kdalert[™] GAPDH Assay Kit (Ambion).

Results and Discussion

Efficient Delivery with NIMT[®]FeOfection|PURPLE

Knockdown of KIF11 (Eg5) is typically used to optimize and evaluate transfection efficiency. Reduced levels of KIF11 (Eg5) mRNA results in mitotic arrest, the cells appear rounded and eventually they die. Successful delivery of Eg5 in HEK293 cells using NIMT[®]FeOfection|PURPLE is clearly demonstrated with brightfield pictures in figure 1.

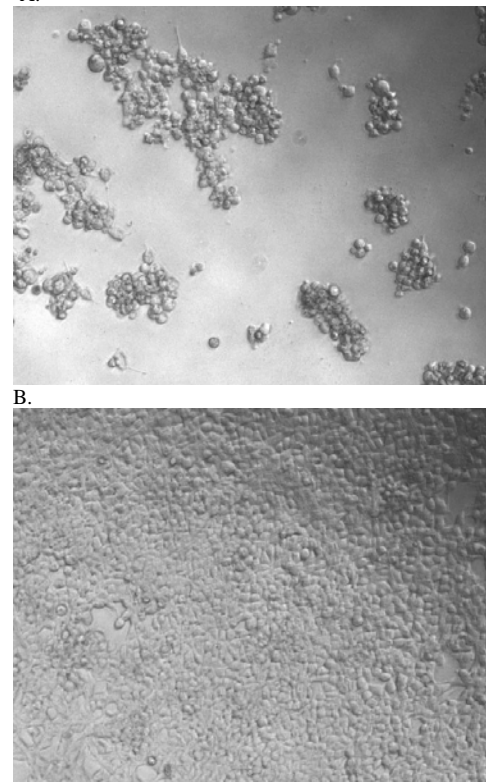


Figure 1: HEK293 cells 48 hours post transfection with (A) KIF11 (Eg5) siRNA and (B) Negative Control #1 siRNA.

Transfection Efficiency at Protein Level

Efficient siRNA delivery by NIMT[®]FeOfection|PURPLE was demonstrated at the protein level. Knockdown of the GAPDH enzyme activity in cells transfected with GAPDH siRNA is shown in figure 2.

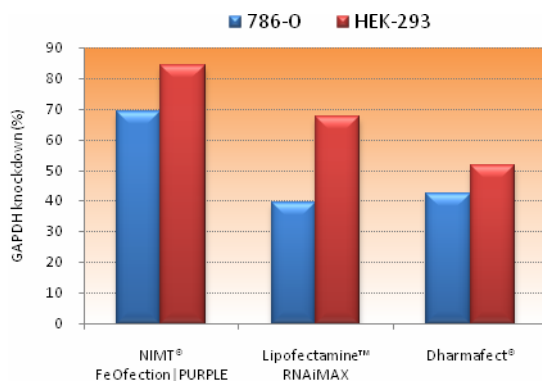


Figure 2: Knockdown of GAPDH in HEK293 cells. The GAPDH values are relative to cells treated with negative control siRNA #1 (blue bars) and #2 (red bars).

Optimization of Transfection Conditions

A number of parameters are crucial for effective gene silencing. One obstacle is that the delivery of RNAi reagents or the delivery agent itself can have an impact on cell viability. Usually, the highest levels of knockdown are strived for and using a higher amount of transfection agent will normally result in higher transfection efficiency. However, this will also increase the cytotoxicity. For each transfection a balance between the knockdown desired and the tolerable impact on cell viability must be taken into consideration. The

relation between toxicity and the amount of transfection agent is demonstrated in figure 3.

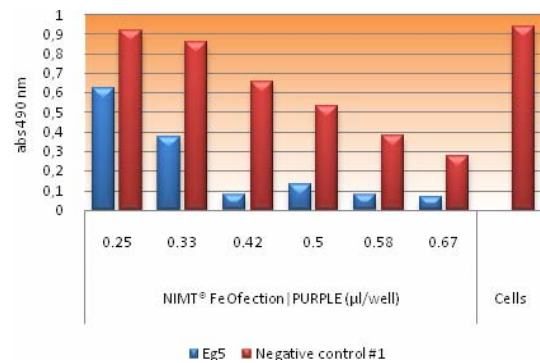


Figure 3: Relative transfection efficiency versus toxicity. The cell viability of cells transfected with 50 nM Eg5 (blue bars) and negative control (red bars) siRNA at different NIMT[®]FeOfection|PURPLE concentrations is shown.

Conclusions

NIMT[®]FeOfection|PURPLE is a new transfection agent based on nanoparticles with an iron oxide core. Here we have demonstrated the ability of NIMT[®]FeOfection|PURPLE to successfully deliver siRNA *in vitro*. The need to optimize the NIMT[®]FeOfection|PURPLE concentrations to maximize knockdown while minimizing toxicity was also shown.