

Efficient Delivery of siRNA in Human Renal Cell Carcinoma Cell Line Using NIMT[®]FeOfection|PURPLE

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Introduction

Short interfering RNA (siRNA) transfection is an exciting new method for introducing foreign genetic information by inducing RNA interference. siRNA works by silencing key sequences on messenger RNA, which turns off specific genes by cleaving them on the RNA strand. For this process to work efficiently one of the key steps is delivery of the siRNA into cells. Here, novel siRNA delivery agent NIMT[®]FeOfection|PURPLE is tested for its ability to deliver siRNA.

Materials and Methods

Cell Culture

786-O (Human Renal Cell Carcinoma cell line) cells were grown in DMEM supplemented with 10% FCS and Streptomycin/ Penicillin at 37°C and 5% CO₂. Cells were seeded into plates or dishes one day before transfection giving 60-80% confluence on the day of transfection (1,2).

Transfection using NIMT[®]FeOfection|PURPLE

siRNA used: *Silencer*[®]KIF11 (Eg5) siRNA, *Silencer*[®] GAPDH siRNA and *Silencer*[®] Negative Control #1 siRNA from Ambion, *siCONTROL* Non-target siRNA #1 and *siCONTROL* Lamin A/C from Dharmacon. NIMT[®]FeOfection|PURPLE was diluted in MQ H₂O and siRNA was also diluted in MQ H₂O before mixing with NIMT[®]FeOfection|PURPLE. The NIMT[®]FeOfection|PURPLE /siRNA solutions were incubated 10 min in room temperature before the solution was added to the cells. Other transfection reagents (Lipofectamine[™] RNAiMAX Invitrogen and DharmaFECT[®] 1 and 2 Dharmacon) were prepared according to manufacturers' protocol.

Knockdown of KIF11

Bright field images of cells were taken of cells 24 hours post transfection with KIF11 (Eg5) siRNA and Negative Control #1 siRNA (50 nM per well in a 96-well plate).

mRNA Measurements

Cells were transfected with Lamin siRNA and Non-target siRNA #1 in a 96-well plate with 100 nM siRNA per well. Analysis of mRNA levels were performed 48 hours post transfection with Panomics QuantiGene assay according to protocol. Knockdown was calculated using cells transfected with negative control siRNA as reference.

GAPDH Enzyme Assay

Cells were transfected with GAPDH and Negative Control siRNA #1 (50 nM per well in a 96-well plate). Analysis of GAPDH activity was performed 48 hours post transfection with Ambion KDalert GAPDH Assay Kit according to manufactures protocol.

Western Blot

Cells were transfected with GAPDH siRNA and Negative Control #1 siRNA in a 6-well plate with 50 nM siRNA per well. After 72 hours incubation cells were lysed with Cell Extraction Buffer (Biosource) supplemented 1:20 with Protease Inhibitor Cocktail (Sigma). Protein content in cell lysate was estimated using Pierce BCA Protein Assay according to manufacturer's protocol.

SDS-PAGE was performed using NuPAGE[®] Novex Bis-Tris 4-12% Gel 12 wells and NuPAGE[®] MES SDS running buffer supplemented with NuPAGE[®] Antioxidant according to manufacturers' protocol. Cell lysate was added to 0.1 µg total protein per well. Lysate was mixed with NuPAGE[®] Sample Reducing Agent and NuPAGE[®] LDS Sample Buffer according to protocol. SeeBlue[®] Plus2 Pre-stained Protein Standard and MagicMark[™] XP Western Protein Standard were used as markers (Invitrogen).

The blotting was performed with Invitrolon[™] PVDF/Filter Membrane Sandwiches and NuPAGE[®] Transfer Buffer supplemented with NuPAGE[®] Antioxidant (Invitrogen). WesternBreeze[®] Chromogenic Western Blot Immunodetection Kit (mouse) was used to detect

proteins on membrane. Mouse anti GAPDH monoclonal primary antibody (Zymed) was used at 1 µg/ml.

Results

Lamin knockdown using NIMT[®]FeOfection|PURPLE compared to other commercial available siRNA delivery agents was evaluated (Figure 1).

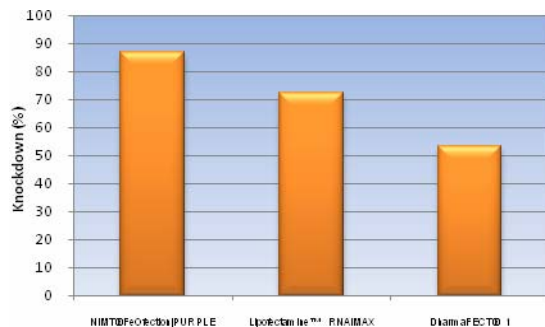


Figure 1: 786-O cells 48 hours post transfection using NIMT[®]FeOfection|PURPLE together with Lipofectamine[™] RNAiMAX and DharmafECT[®] 1.

To prove efficient knockdown by NIMT[®]FeOfection|PURPLE, GAPDH knockdown was evaluated and compared to other transfection agents. Knockdown was confirmed by enzyme assay (Figure 2) and western blot (Figure 3).

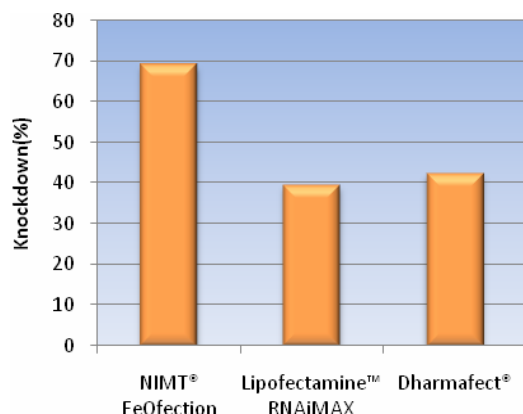


Figure 2: GAPDH knockdown in 786-O cells. From left: NIMT[®]FeOfection|PURPLE, Lipofectamine[™] RNAiMAX and DharmafECT[®] 2.

Knockdown of GAPDH on protein level was also confirmed by SDS-PAGE followed by western blotting (Fig.3).

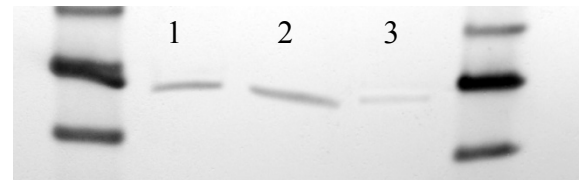


Figure 3: Western blot of 786-O cell lysate 72 hours post transfection using NIMT[®]FeOfection|PURPLE. Lane (1) untreated cells, (2) siCONTROL non-target siRNA #1 and (3) GAPDH siRNA.

To highlight the above results on the phenotype of 786-O cells, transfection was also performed using KIF11 (Eg5) siRNA (Figure 4).

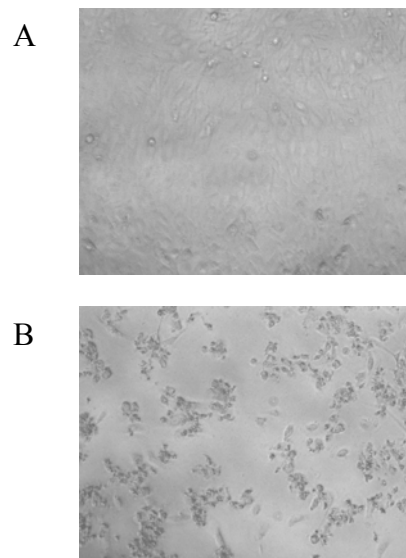


Figure 4: 786-O cells 24 hours post transfection using NIMT[®]FeOfection|PURPLE with (A) siCONTROL non-target siRNA #1 and (B) KIF11 (Eg5) siRNA.

Conclusions

NIMT[®]FeOfection|PURPLE was evaluated for *in vitro* delivery of siRNA in Human Renal Carcinom Cells 786-O and compared to other commercial available transfection agents. NIMT[®]FeOfection|PURPLE shows high

efficiency knockdown both at mRNA level and protein level. Also cytotoxicity was reduced compared to other transfection agents.

References

1. Williams RD, et al. In vivo cultivation of human renal cell cancer. I Establishment of cells in culture. *In vitro* 12:623-627, 1976
2. Williams RD, et al In vivo cultivation of human renal cell cancer. II Characterization of cell lines. *In Vitro* 14. 779-786