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TECH NOTE

NIMT®FeOfection | YELLOW and K562; Incubation Times of Transfection Complexes

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Introduction

NIMT®FeOfection transfection reagents consist of iron oxide nanoparticles that have been coated in a way that will facilitate binding of DNA and uptake into cells. NIMT®Booster solution has been shown to improve transfection efficiencies and is added to the NIMT®FeOfection | YELLOW particles prior to mixing with DNA. Complexes of particles/Booster and DNA are formed and the complexes are then added to the cells. Here we have tested if the incubation times of the complexes have an influence on transfection efficiency and/or cell proliferation on K562 suspension cells.

Material and Methods

K562 cells were grown in RPMI 1640 supplemented with 10% fetal calf serum and 5% Streptomycin/ Penicillin at 37°C 5% CO_2. On the day of transfection 4×10^4 cells/well were seeded in a white 96-well plate with clear bottom (Corning Costar 3610) in 100 µl growth medium.

Cells were transfected with phRL-SV40 *Renilla* luciferase vector (Promega) using NIMT®FeOfection | YELLOW and NIMT®Booster. The transfection reagent was used at a ratio of 3 µl concentrated NIMT®FeOfection | YELLOW to 1 µg DNA. NIMT®Booster was used at a ratio of 2 µl Booster to 1 µg DNA. The transfection complexes were prepared as described in the protocol for suspension cells. Twelve identical samples were prepared in micro tubes, one for each incubation time. The transfection mixtures were incubated for 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 240 and 320 min before 20 µl was added per well in triplicates.

Cells were incubated 24 hours at normal growth conditions before measurements of luminescence and proliferation was preformed. Luminescence was measured after addition of Endure Live Cells Substrate (Promega), prepared as described in the protocol. Luminescence was measured after approximately two hours incubation at 37°C 5 % CO_2. Proliferation was measured after the luminescence measurement using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) according to protocol.

Results

Results of the transfection presented in figure 1. Luminescence values are shown as RLU (relative luminescence units) and proliferation as absorbance values compared to untreated cells.

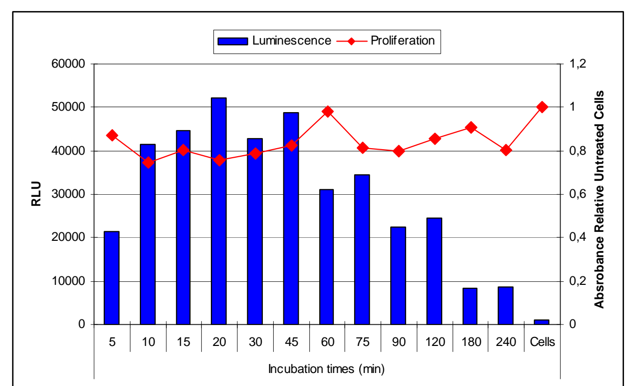

***Figure 1**: Luminescence and absorbance (relative untreated cells) on K562 cells transfected with DNA using NIMT®FeOfection | YELLOW and NIMT®Booster. Transfection complexes were incubated 5-320 min before addition to cells.*

Conclusions

When using NIMT®FeOfection | YELLOW and NIMT®Booster for DNA transfection on K562 cells, transfection efficiency becomes high if the transfection solution is incubated 10-45 min. Longer or shorter incubation times reduces the transfection efficiency. Best efficiency is achieved when incubating the complexes for 20 min. Proliferation does not seem to be influenced by the incubation time.