K2 Transfection Kit Application note: Application for hard to transfect cells.

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Materials:

Plasmid pEGFP-NI (Clontech)

T75 cell culture plates and 24-well cell culture plates

ZB28 cell culture medium: 45% Ham's F12 Medium, 45% IMEM, 10 % fetal calf serum

WSL cells (Wild boar lung) cells

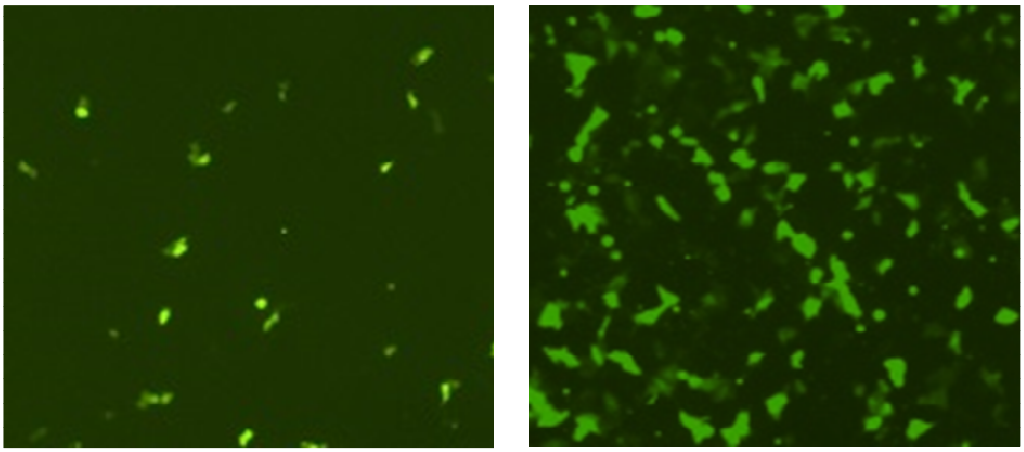
Transfection reagents:

Polyethyleneimmine (PEI; Sigma-Aldrich),

K2 transfection Kit

Optimization of the transfection for WSL cells:

WSL cells were grown in T75 ml cell culture flasks in ZB28 cell culture medium until confluency. Cultures were trypsinized 24 h prior transfections and seeded 1: 2 (about $1{,}25 \times 10^6$ cells/well) into 24 well tissue culture plates to reach 90 to 100 % confluency the next day. For transfection of WSL cells with PEI, 625 ng pEGFP-N1 DNA were mixed with 1,25 µg PEI (optimized ratio) in a final volume of 200 µl ZB28 without FCS. After 20 min incubation at room temperature, the DNA-PEI mixture was added to the cells. The inoculum was replaced by fresh medium 3.5 h later. Transfections with the K2 transfection kit were optimized according to the manual. Briefly, cukture medium was replaced by 0.5 ml fresh medium containing 10 µl multiplier per well. After 2 h incubation at 37°C K2 transfection reagent and 625 ng pEGFP-N1 DNA incubated at different ratios at 25 °C for 20 minutes were added dropwise to the cultures which were then further incubated at 37°C. Photography of the GFP-autofluorescence in the different cultures at 24 hours post transfection revealed an optimal DNA : K2 active ingredient ratio of 1:3 for WSL cells (see figure).



High efficacy of K2® transfection kit mediated gene transfer efficacy into WSL cells. WSL cells in 24 well plates were transfected with 0.625 µg pEGFP-N1 using PEI at a DNA:PEI ratio of 1:2 (A) or the K2® transfection kit at a DNA:active ingredient ratio of 1:3 (B). GFP autofluorescence was photographed 24 hours after transfection. Representative sections are shown.

As obvious from the number of autofluorescing WSL cells, in comparison to PEI the K2 kit mediated gene transfer resulted in an impressing increase of GFP expressing cells. Of note, viability of K2- transfected cells was not affected by the procedure and thus exchange of the inoculum by culture medium was not required.

K2-raised increases in gene transfer efficacy, although to varying extents, were observed using a number of mammalian and avian cell cultures. It should be noted that conditions for optimal transfection efficacy using the K2 kit need to determined for each cell line.