Plasmid DNA transfection of A549 human lung carcinoma cells with the Biontex K2® Transfection System

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Materials and methods

Cell culture

The human lung adenocarcinoma epithelial cell line A549 was obtained from the American Type Culture Collection (ATCC, Promochem, Germany). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Darmstadt, Germany) containing glutamax-I (L-alanyl-L-glutamine) and supplemented with 10% fetal calf serum (FCS; Life Technologies), 50 U/ml penicillin and 50 µg/ml streptomycin (Sigma-Aldrich, Munich, Germany) at 37 °C in a humidified atmosphere containing 5 % CO_2.

Transient transfection of cells and evaluation of transfection efficiency

For optimization of transfection conditions, 65,000 A549 cells per well were plated in a 48-well plate (Greiner Bio-One, Frickenhausen, Germany) 24 h before transfection. Cells were transfected with K2® Transfection System (Biontex), according to the optimization protocol provided by the manufacturer using the pGL3-Control luciferase expressing plasmid (Promega, Mannheim, Germany). Transfection efficiency was evaluated 48 h after transfection using the Luciferase Assay System (Promega) and an ELISA reader (Victor Wallac, Perkin-Elmer, Waltham, USA). For expression analysis of an Akt1 reporter plasmid (pEGFP-Akt1; cDNA encoding for human Akt1, inserted into KpnI/BamHI restriction sites of pEGFP-N1 (Clontech, Saint-Germain-en-Laye, France), 600,000 A549 cells per well were plated in 6-well plates (Greiner Bio-One) 24 h before transfection. The two best conditions from the optimization results were used for transfection of cells with pEGFP-N1 control vector and pEGFP-Akt1 plasmid according to the upscaling protocol of Biontex. Option A consisted of 1.6 µg plasmid DNA and 3.6 µl K2® Transfection Reagent with 22.5 µl K2® Multiplier treatment, while Option B included 0.8 µg plasmid DNA, 2.7 µl K2® Transfection Reagent and a 2-h pretreatment of the cells with 22.5 µl K2® Multiplier in 1.125 ml medium per well. In brief, cells were washed once with 2 ml PBS (Life Technologies) per well and 1.125 ml

medium, supplemented with FCS and antibiotics, were added per well. Cells were treated for 2 h with 22.5 µl K2® Multiplier prior transfection. K2® Transfection Reagent and plasmid DNA was diluted in 67.5 µl of Opti-MEM® I Reduced Serum Medium (Life Technologies). DNA solution was then added to the K2® Transfection Reagent solution, mixed gently by pipetting up and down and incubated at room temperature for 20 min. Immediately after the incubation step, DNA-transfection reagent solution was slowly added dropwise and cells were gently agitated in the cell culture plates. At 24 h after transfection, cells were trypsinized and plated either on microscopic slides (Becton Dickinson (BD), Heidelberg, Germany) for microscopic evaluation of transfection efficiency using an Axiovert 40 CFL microscope (Carl Zeiss, Göttingen, Germany) or on 6 well plates for assessment of recombinant protein expression by Western blotting 48 h after transfection using anti-Akt1 (Cell Signaling, Frankfurt, Germany, #9272S) or anti-GFP (Abcam, Cambridge, UK, #ab290) antibodies. β-actin (Sigma-Aldrich, #A5441-.2ML) served as loading control.

As a control, we transfected 3 x 10^5 cells in parallel with 5 µl transfection reagent x and 4 µg of each plasmid according to the manufacturer's recommendations, followed by microscopic evaluation and Western blotting as described above.

Results

Optimization of transfection conditions using a Luciferase reporter assay

Transfection of the human lung carcinoma cell line A549 was optimized using a Luciferase expression plasmid and the K2® Transfection System. Optimal transfection conditions were identified after analyzing Luciferase acitivity (Figure 1). The maximum transfection efficiency was achieved with 0.4 µg plasmid at a plasmid:Transfection Reagent ratio of 1:2 with 5 µl K2® Multiplier treatment (red bar; Option A) and with 0.2 µg plasmid at a plasmid:Transfection Reagent ratio of 1:3 with 5 µl K2® Multiplier pretreatment (blue bar; Option B).

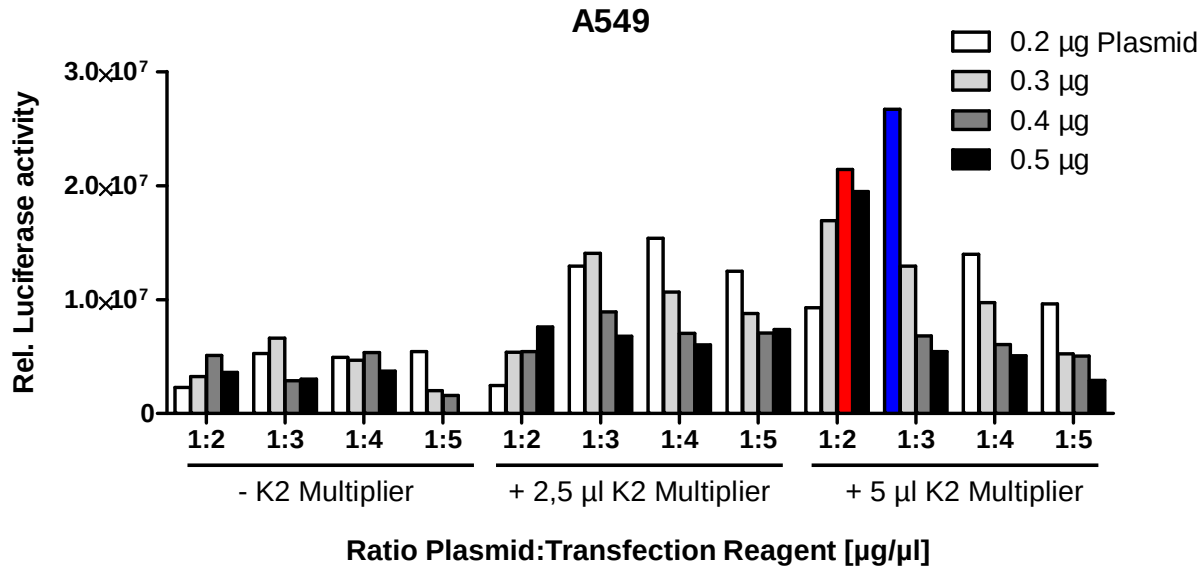

Figure 1: A549 lung carcinoma cells were transiently transfected with the pGL3-Control luciferase expression vector (Promega) using the K2® Transfection System (Biontex) according to the optimization protocol of the manufacturer in a 48-well scale. At 48 h after transfection, cells were subjected to a Luciferase Assay (Promega) as suggested by the manufacturer. Luciferase activity was measured in an ELISA reader (Victor Wallac, Perkin-Elmer, Waltham, USA).

Expression of a pEGFP-Akt1 reporter construct in A549 cells

Efficient expression of a pEGFP-Akt1 reporter and pEGFP-N1 control plasmid was monitored by fluorescence microscopy (Figure 2) and Western blotting (Figure 3) in comparison to a commercially available transfection reagent x using optimized K2® Transfection System conditions acquired with the optimization protocol and Luciferase reporter assays (Figure 1).

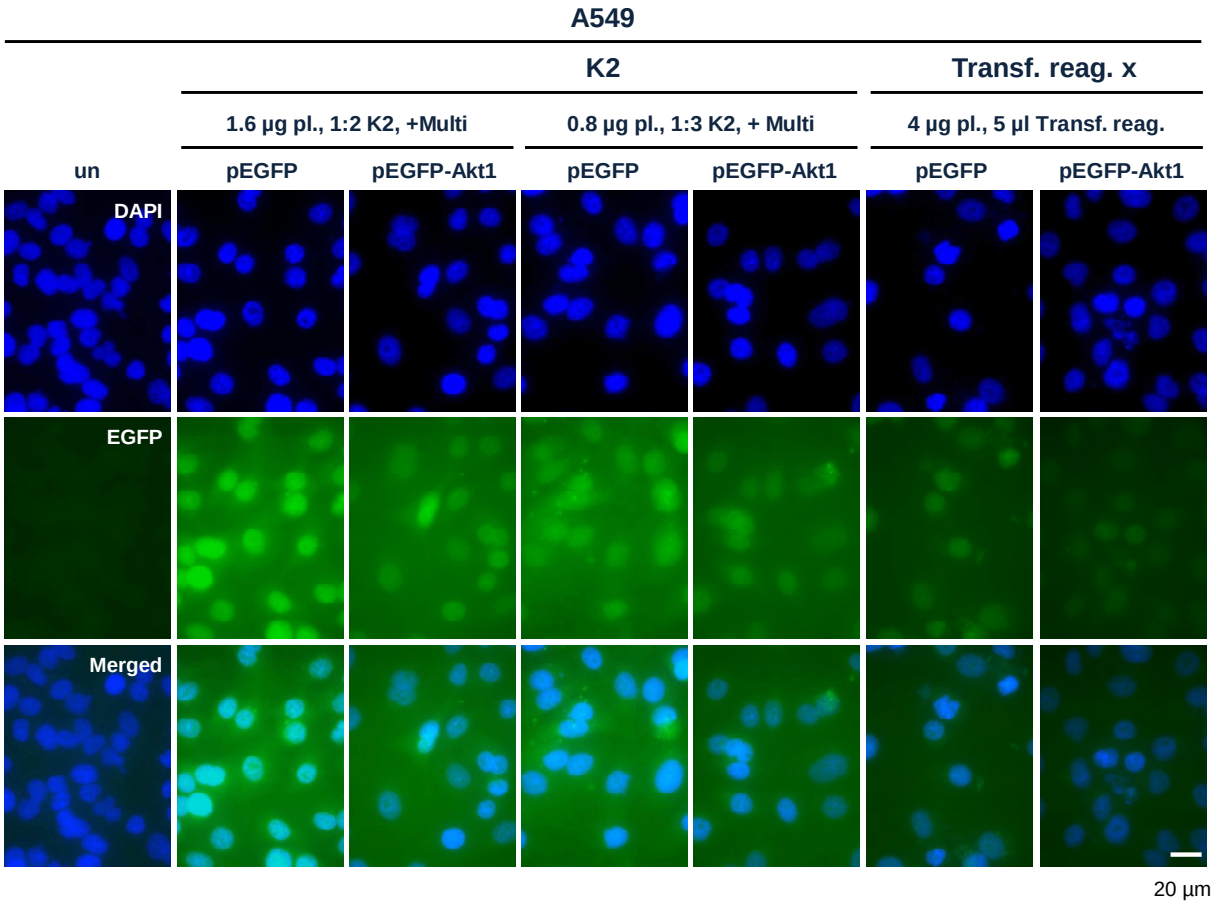

Figure 2: A549 cells were transfected using 1.6 µg or 0.8 µg plasmid (pl.) at a plasmid:transfection reagent ratio of 1:2 or 1:3 with K2® Multiplier treatment (+ Multi). Photographs were acquired with an Axiovert 40 CFL microscope (Carl Zeiss, Göttingen, Germany).

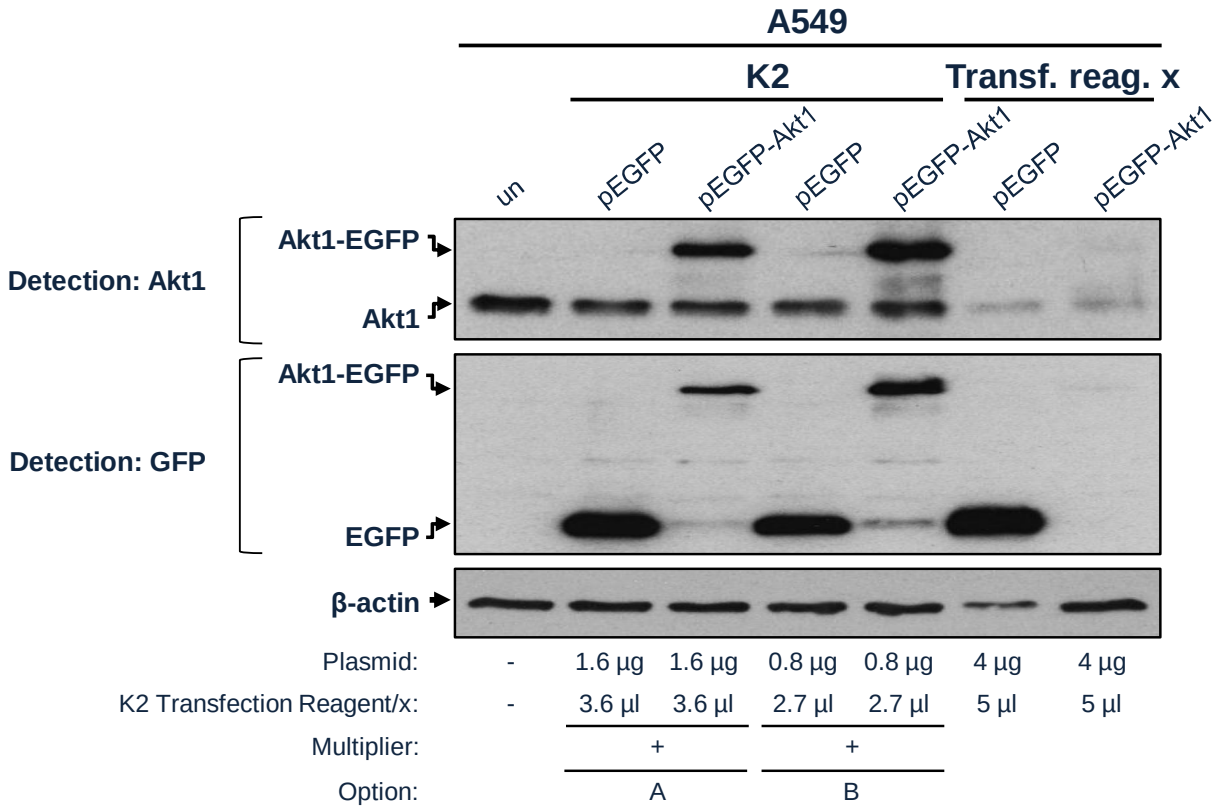

Figure 3: A549 cells were transfected using 1.6 µg (Option A) or 0.8 µg plasmid (Option B) at a plasmid:transfection reagent ratio of 1:2 (3.6 µl K2® Transfection Reagent) or 1:3 (2.7 µl K2® Transfection Reagent) with K2® Multiplier treatment (+ Multiplier). Expression of recombinant Akt1-EGFP or endogenous Akt1 was detected by Western blotting using an Akt1-specific antibody. Additional detection of recombinant EGFP and Akt1-EGFP was performed using an anti-GFP antibody. β-actin served as loading control.

Summary

Transfection of A549 lung carcinoma cells was highly efficient with transfection efficiencies up to 90 % using optimized conditions of K2 Transfection System (Biontex). Transfection efficiency and expression were higher than that of a commercially available transfection reagent x, commonly used in our lab, while a lower amount of plasmid DNA was sufficient. Cytotoxicity was significantly lower in the K2 Transfection Reagent samples while a higher confluency during transfection ensures sufficient numbers of cells for further experiments. Taken together, the Biontex K2® Transfection System is a useful tool for transfection of A549 human lung tumor cells with high efficiency.