Plasmid DNA-transfection of human keratinocytes (HaCat) using Biontex-K2®Transfection System

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Materials & Methods

Cell culture

The human keratinocyte cell line HaCat was cultured in antibiotic-free high glucose Dulbecco's modified eagle medium (DMEM, Lonza) containing 10% fetal bovine serum. For transfection 0.75 × 10^5 cells/well were seeded in a 48-well plate, with a culture volume of 0.25 ml per well. Cells were incubated 24 h at 37°C in a CO_2-incubator. Transfection was performed at a cell confluence of 90-100%.

Biontex-K2® Transfection

An optimization protocol was performed to find the optimal DNA/K2®Transfection Reagent ratio for transfecting HaCat cells. All reagents (plasmid DNA, K2® Multiplier and K2®Transfection Reagent) were brought to room temperature before the experiment. To test whether the K2®Multiplier has an influence on transfection efficiency either 0 µl (rows A and B), 2.5 µl (rows C and D) or 5 µl (rows E and F) of the K2®Multiplier was carefully dropped in the respective wells two hours before transfection (Table 1), mixing was achieved by gently agitating the culture plate. The plasmid used to monitor the transfection efficiency was a simple GFP vector under the control of a CMV-promoter. 20 µg of the CMV-GFP plasmid was mixed with 1 ml of DMEM (Solution A) by a single up- and down pipetting step. To determine the ideal DNA/K2®Transfection Reagent ratios, four solutions were prepared by mixing 225 µl DMEM with 9 µl (Solution B 1:2), 13.5 µl (Solution B 1:3), 18 µl (Solution B 1:4) and 22.5 µl (Solution B 1:5) K2®Transfection Reagent, respectively and mixed by a single up- and down pipetting step. 225 µl of the DNA/DMEM solution (A) was mixed with the 225 µl of the four solutions (B) and incubated for 20 at room temperature. After this incubation step 20 µl, 30 µl, 40 µ and 50 µl of these four DNA-lipid-complex solutions were carefully dropped onto the corresponding well indicated in Table 1.

Table 1 Overview of DNA-lipid-complex application of K2®Transfection Reagent in a 48-well plate containing HaCat cells.

		1	2	3	4	5	6	7	8
0 µl K2® Multiplier	A	20 µl A+B 1:2	30 µl A+B 1:2	40 µl A+B 1:2	50 µl A+B 1:2	20 µl A+B 1:3	30 µl A+B 1:3	40 µl A+B 1:3	50 µl A+B 1:3
	B	20 µl A+B 1:4	30 µl A+B 1:4	40 µl A+B 1:4	50 µl A+B 1:4	20 µl A+B 1:5	30 µl A+B 1:5	40 µl A+B 1:5	50 µl A+B 1:5
2.5 µl K2® Multiplier	C	20 µl A+B 1:2	30 µl A+B 1:2	40 µl A+B 1:2	50 µl A+B 1:2	20 µl A+B 1:3	30 µl A+B 1:3	40 µl A+B 1:3	50 µl A+B 1:3
	D	20 µl A+B 1:4	30 µl A+B 1:4	40 µl A+B 1:4	50 µl A+B 1:4	20 µl A+B 1:5	30 µl A+B 1:5	40 µl A+B 1:5	50 µl A+B 1:5
5 µl K2® Multiplier	E	20 µl A+B 1:2	30 µl A+B 1:2	40 µl A+B 1:2	50 µl A+B 1:2	20 µl A+B 1:3	30 µl A+B 1:3	40 µl A+B 1:3	50 µl A+B 1:3
	F	20 µl A+B 1:4	30 µl A+B 1:4	40 µl A+B 1:4	50 µl A+B 1:4	20 µl A+B 1:5	30 µl A+B 1:5	40 µl A+B 1:5	50 µl A+B 1:5

The volumes applied equal the amount of transfected DNA in [µg], for instance 20 µl corresponds to 0.2 µg of total DNA, etc. The ratios are equal to DNA/K2®Transfection Reagent given in [µg/µl]. The cells were incubated again at 37°C in a CO_2 incubator for 24-48 hours. Here, the medium was replaced with fresh DMEM+10%FBS after 19 hours. Transfection efficiency was estimated by fluorescence microscopy after 24 and 48 hours.

Results

After 24 hours the cells were first inspected under the light microscope for viability. Generally, the cell viability decreased with increasing amount of DNA and higher ratios of K2®Transfection Reagent from lane A to B, C to D and E to F, shown with blue bars in Figure 1. Assessment of the transfection efficiency was achieved by an inverse fluorescent microscope. It was obvious that the application of 5 µl K2® Multiplier yielded the highest transfection efficiency compared to 0 µl and 2.5 µl, whereas this equals approximately 30 % of the cells. The other effect that could be noticed was that the transfection efficiency increased with decreasing cell viability, green bars in Figure 1.

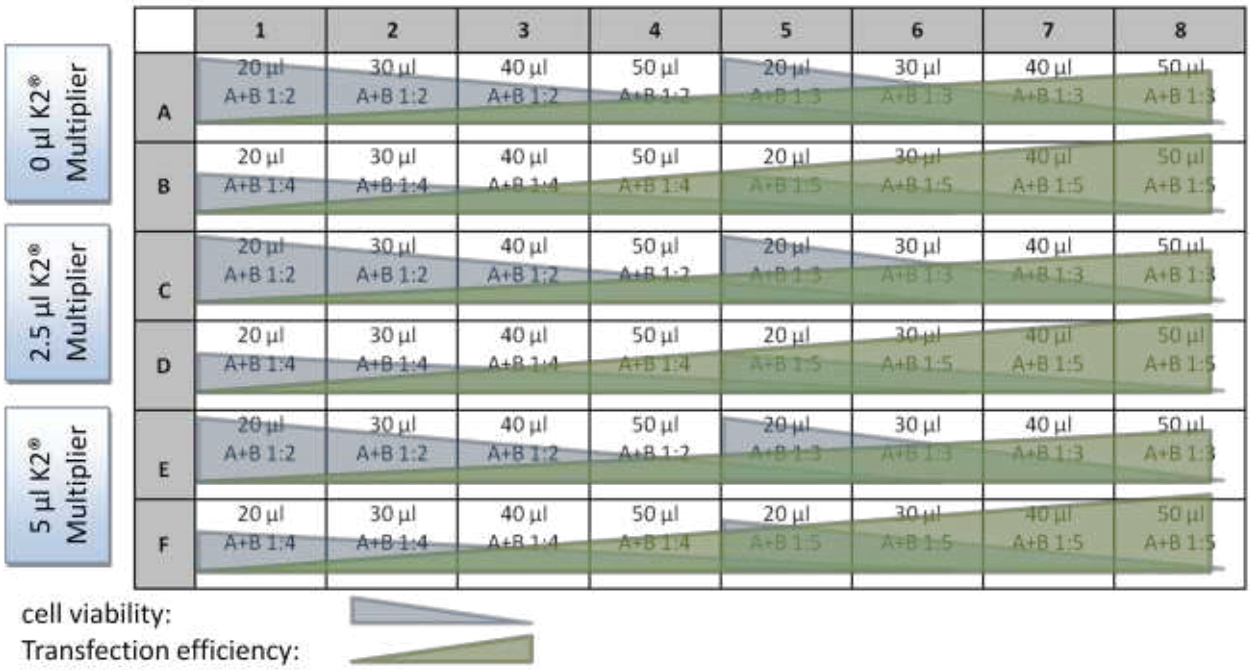

Figure 1. Results of an optimization protocol for the transfection of HaCat cells with Biontex K2®Transfection Reagent. Blue bars represent the cell viability evaluated under the light microscope. With increasing amount of DNA and K2®Transfection Reagent, cell viability was reduced. Green bars represent the transfection efficiency assed by fluorescent microscopy of a GFP transfected plasmid. The amount of positively transfected cells was increasing with increasing amount of DNA and K2®Transfection reagent.

The medium was changed after 24 hours to remove dead and apoptotic cells and cells were incubated for another 24 hours for an evaluation after 48 hours. Two days after transfection, there was no massive increase in GFP positive cells, however it seemed that the cells in lane E and F showed a smaller increase in GFP fluorescence. The absent increase in GFP positive cells could be due to a cell confluence of nearly 100% already after 24 hours post-transfection. Therefore, I would recommend seeding HaCat cells in a 48-well plate at a density of 0.5×10^5 cells/well, to allow more space for cell division. Another

recommendation is to replace the medium 6 hours post-transfection with fresh medium, to reduce the toxicity. In my opinion the combination in well E4 (0.5 µg DNA, with a ratio of 1:2 (DNA:K2®Transfection Reagent) and D1 (0.2 µg DNA, with a ratio of 1:4 (DNA:K2®Transfection Reagent) gave the best results for transfecting HaCat cells, with an acceptable cell viability in relation to transfection efficiency. Figure 2 shows an example of the transfection result of HaCat cells.

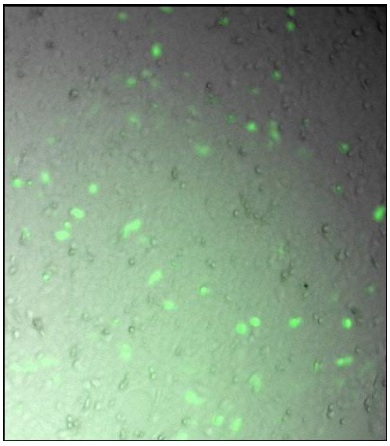

Figure 2. Example image of HaCat transfection with CMV-GFP using the Biontex K2®Transfection System.

Conclusion

It is well known that transfection of HaCat cells is not easy and only yields low transfection efficiencies between 20-30%, maximal 50%. In this assay the transfection efficiency of a CMV-GFP-plasmid in HaCat cells with the Biontex K2®Transfection Reagent was investigated in a 48-well format. The results show that the optimal amount of DNA transfected in a 48-well plate was 0.5 µg with a ratio of 1:2 DNA to K2®Transfection Reagent and a 2 hour pre-transfection application of 5 µl K2®Multiplier per well. In a transfection experiment of the same plasmid with Lipofectamine®2000 only 0-3% of HaCat cells could be transfected successfully, whereas the Biontex K2®Transfection System definitely achieved the expected transfection efficiency of 20-30%. It is recommended that the medium is refreshed 6 hours post-transfection to reduce cytotoxic effects to a minimum.