DNA-transfection of human colon adenocarcinoma cells (DLD-1) using "Biotex K2® Transfection System"

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Materials and Methods

Cell culture and transfection
DLD-1 cells were cultured in Dulbecco's modified eagle medium (Sigma Aldrich, Lot RNBD2282) containing 10% fetal calf serum (FCS), 1% Pyruvat and 1% Penicillin/Streptomycin. 24 hours before transfection, cells were seeded in 24 well plates with $0{,}8x10^5$ cells and 500 µl medium per well to reach a confluency of 80%. Prior transfection, cells were treated with K2® Multiplier that was dripped slowly into the medium and incubated for 2 hours at 37°C and 5% CO_2. During incubation plasmid-DNA encoding luciferase was mixed with medium without serum (Sigma Aldrich, Lot RNBC9342) (solution A). The indicated amounts of K2® Transfection reagent was added to serum-free medium and left at room temperature (solution B). Solution A was added to solution B and gently mixed by pipetting. After incubation for 20 minutes at room temperature, transfection solution was applied to cells by dropwise addition into the medium, followed by gently swaying the dishes. Transfected cells were incubated for 24 hours at 37°C and 5% CO_2.

Results

To optimize the K2® transfection system, DLD-1 cells were transfected with different ratios of pGl3control plasmid-DNA encoding luciferase to K2® Transfection reagent, using different amounts of multiplier. The following table shows the amounts of DNA, K2® Transfection reagent and multiplier:

	5 µl Multiplier				10 µl Multiplier				15 µl Multiplier			
0,4 µg DNA	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2
0,6 µg DNA	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2
0,8 µg DNA	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2
1 µg DNA	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2	1:2 DNA/K2	1:3 DNA/K2	1:4 DNA/K2	1:5 DNA/K2

Luciferase expression was measured 24 hours after transfection, using a Centro LB 960 luminometer (Berthold). As shown in Figure 1 luciferase expression was increased with a DNA:K2® Transfection reagent ratio of 1:4 or 1:5. The analysis indicated optimal levels of luciferase expression using 0,8 µg DNA, a DNA:K2® Transfection reagent ratio of 1:4, and 5 µl multiplier produced.

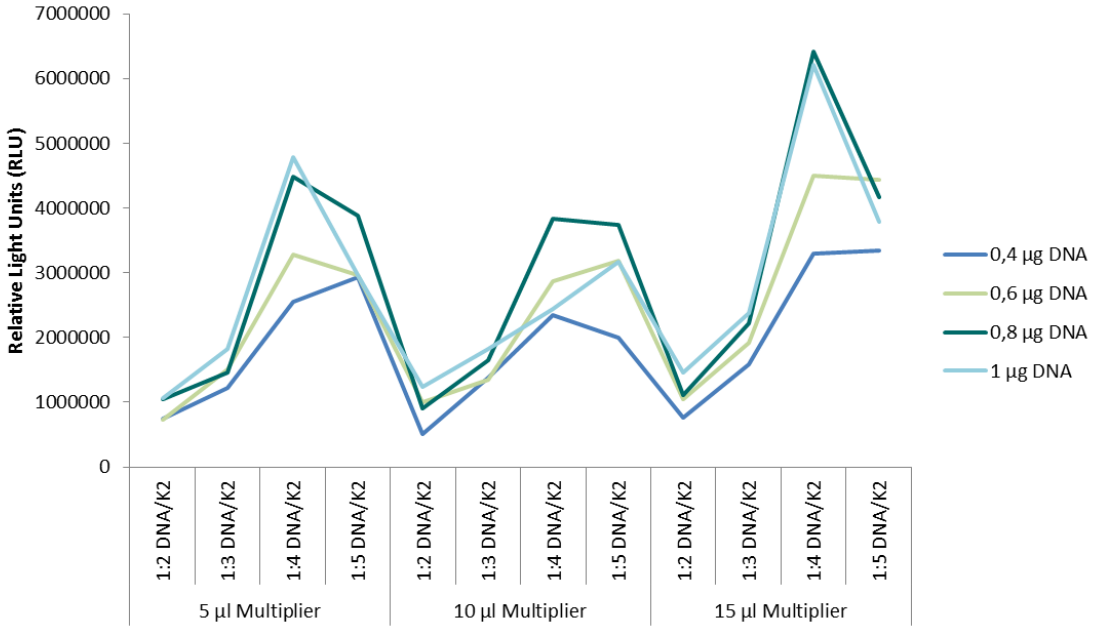

Figure 1: Analysis of luciferase expression with different transfection conditions using the K2® Transfection system. The expression was measured in relative light units for different amounts of transfected DNA, DNA:K2® Transfection reagent ratio, and pre-incubated multiplier.

Conclusion

In order to optimize the K2® transfection system, pGl3control plasmid-DNA encoding luciferase was transfected into DLD-1 cells using different amounts of DNA, DNA:K2® Transfection reagent ratio, and multiplier. The results showed a high transfection efficiency with no indication of cytotoxic effects after an incubation of 24 hours. The comparison of the different transfection conditions indicated an optimal transfection efficiency of DLD-1 cells using 5 µl K2® Multiplier, 0,8 µg plasmid-DNA and a DNA:K2® Transfection reagent ratio of 1:4.