Transfection of Neuroblastoma cells using "Biontex K2® Transfection Reagent"

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Cell culture

SH-SY5Y, Kelly and IMR-32 cells were cultured on 6-well plates. 2 ml of DMEM/Ham's F12 (SH-SY5Y) or RPMI 1640 (Kelly, IMR-32) containing 10% FCS and 1% Penicillin/Streptomycin were used per well. The cells were seeded the day before transfection at a density leading to 90-100% of confluence and then incubated at 37°C and 5% CO_2 for 24 hours.

Cell transfection

Cell transfection was performed following the manufacturer's manual:
20 µl of the "K2® Multiplier" were mixed with 2 ml of fresh medium. 2 hours before transfection the medium in the well was removed and this mixture was added to the cells. For each well 4 µg of DNA (eGFP containing plasmid) and serum free medium (DMEM/Hams F12 or RPMI 1640) were mixed to reach a volume of 50 µl (solution A). Furthermore, 16 µl of "K2® Reagent" were mixed with 34 µl of serum free medium (solution B). In the end, solution A was added to solution B and incubated for 15 minutes. Then, the whole 100 µl of this mixture were added drop by drop.
Cells were incubated 5, 12 and 24 hours at 37°C and 5% CO_2 before changing the medium.

Analysis

The transfection efficiency was determined using FACS analysis after a total incubation of 48 hours.

Conclusion

Neuroblastoma cells are well known to be difficult to transfect. Here, the K2® transfection system was compared with other common transfection reagents (Figure 1). Although still not ideal, using the K2® reagent clearly resulted in the highest transfection efficiencies.

Furthermore, the cells were incubated for different times with the K2® Reagent. Longer incubation times didn´t lead to a significant improvement of transfection efficiency (Figure 2).

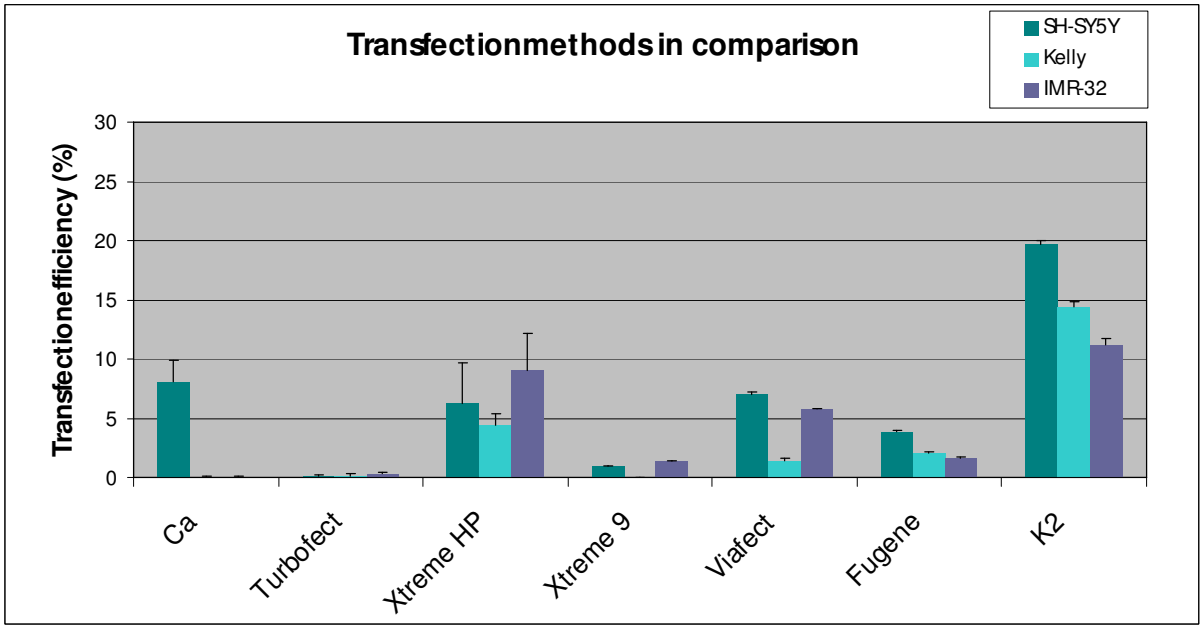

Figure 1: Transfection methods in comparison. The K2® reagent clearly resulted in the highest transfection efficiencies.

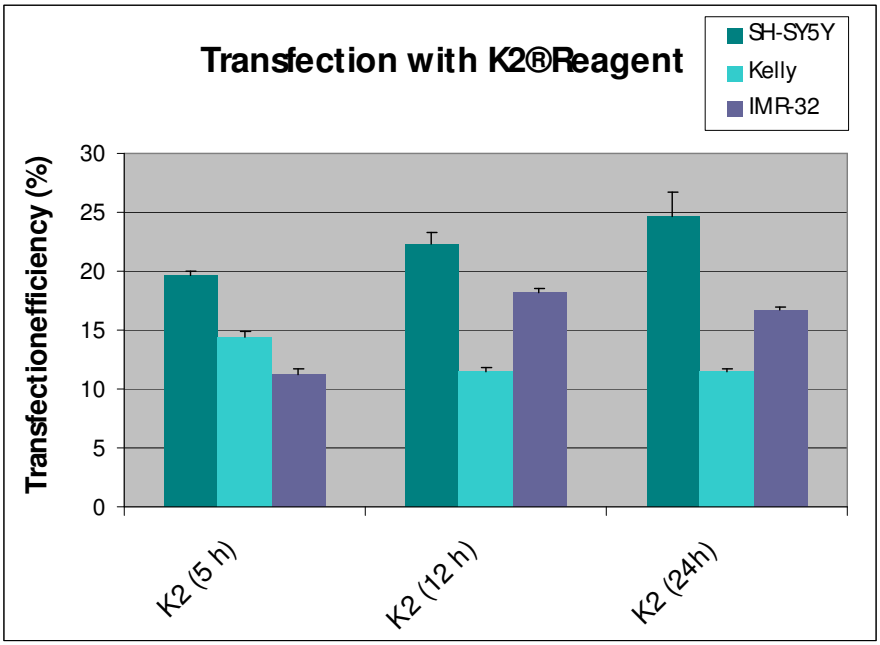

Figure 2: The three cell lines were incubated for different time periods with K2® system. This did not lead to a significant change of transfection efficiency.