DNA-transfection of Madin-Darby canine kidney cells (MDCK II cell line) using Biontex K2® Transfection System

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Material and methods

Cell culture

The Madin–Darby canine kidney cell line (MDCK II) was cultured in 75 cm^2 cell culture flasks in the Dulbecco's modified Eagle's medium – high glucose (DMEM) containing 10 % fetal bovine serum in a humidified atmosphere containing 5 % CO_2 at 37 °C. Confluent cells were split twice a week using 0.25% trypsin/EDTA solution.

The MDCK II cells were seeded at a density 2×10^5 cells/well in 24-well plates.
The transfection was performed the following day when the cells had reached a confluency of 90 %.

Cell transfection

The medium in each well was replaced with the fresh DMEM (0.5 ml) right before the transfection (before adding the K2® Multiplier).

2 h before DNA transfection 10 µl K2® Multiplier was added to the each well of cells and gently mixed by slightly moving of the plate.

Adequate amount of DNA and the K2® Transfection Reagent was added to the Opti-MEM® I Reduced Serum Medium (Gibco® by Life Technologies™) and gently mixed by pipetting up and down. Then the both solution was combined (DNA solution was added to the K2® Transfestion Reagent solution), gently mixed and the mixture was incubated at room temperature for 20 min.

After incubation, 60 µl of DNA-lipid complex was added to the each well and gently mixed by shuffling of the plate. The cells were returned back to the incubator (5% CO_2, 37 °C) and incubated for 12 h or 24 h, respectively.

Cell line	**K2® Multiplier [µl]**	**DNA [µg]**	**DNA/ K2®** Transfection Reagent	**Opti-MEM® [µl]** for DNA + K2® Transfection Reagent
MDCK II	10	0.8	1:4	30+30

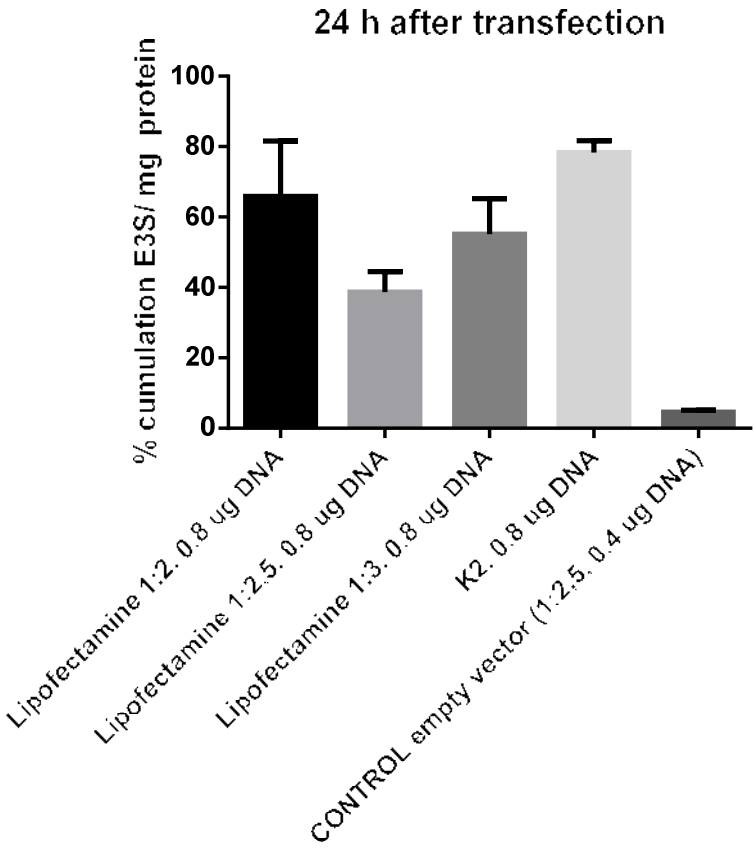
The transfection medium was replaced with the fresh DMEM after 12 h of the incubation and the results were analyzed after 24 h after the transfection. And the transfection medium was replaced with the fresh DMEM after 24 h of the incubation and the results were in this case analyzed after 48 h after the transfection.

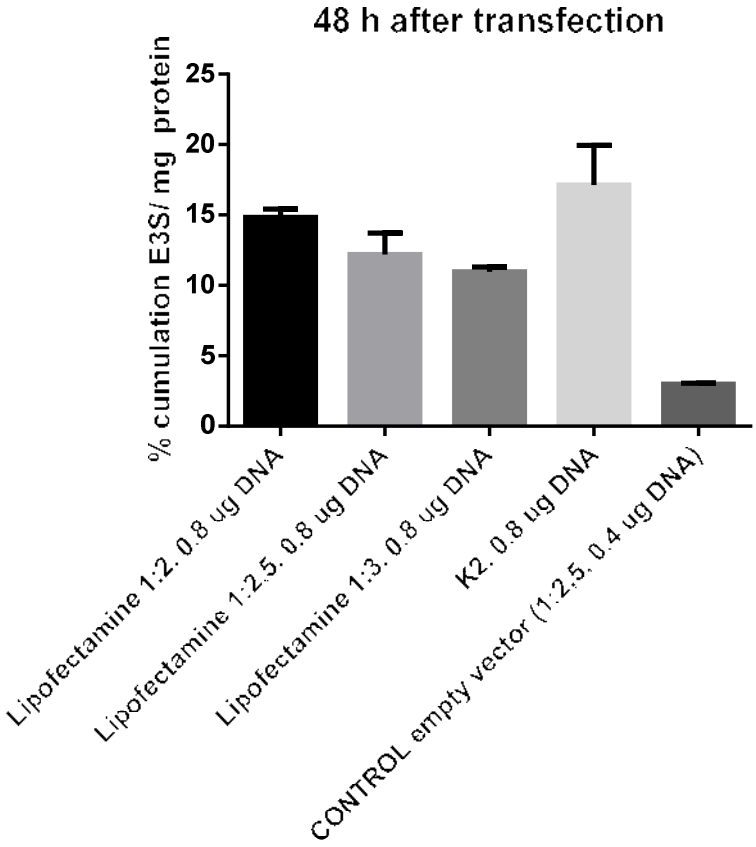
Evaluation of the transfection

The success rate of the transfection after 24 h and 48 h was observed and compared with the cells transfected by the Lipofectamine® 2000 Transfection Reagent (Invitrogen™) using 0.8 µg DNA/well in the ratio DNA:Lipofectamine® 2000 1:2, 1:2.5 and 1:3. As a control served the MDCK cells transiently transfected with the empty vector (using the Lipofectamine® 2000 Transfection Reagent).

The MDCK cells were transfected with the OATP2B1 transporter and the accumulation study (24 h and 48 h after the transfection) was performed with the standard substrate of OATP2B1: radioactively labeled [^{3}H]estrone 3-sulfate (ammonium salt) (E3S) (American Radiolabeled Chemicals).

Results





Conclusions

Transient transfection of MDCK II cells with the OATP2B1 expression construct was successfully performed using the K2® Transfection System. We observed **16.8** fold higher accumulation of [^{3}H]estrone 3-sulfate in the cells transfected using K2® Transfection System versus the control after 24 h and **5.7** fold increase after 48 h. For the transient transfection of the MDCK II cells we obtained better results with the K2® Transfection System than the Lipofectanine® 2000 Transfection Reagent in the both cases. We achieved **1.2** fold increase of accumulation of [^{3}H]estrone 3-sulfate using the K2® Transfection System versus using the most successful ratio of the Lipofectamine® 2000 Transfection Reagent (1:2, 0.8 µg DNA) after 24 h as well as after 48 h.These findings also confirm proper functionality of the MDCK II cell model transfected using the K2® Transfection System.