DNA-transfection of human embryonic kidney cells 293 (HEK293) using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

Human embryonic kidney 293 (HEK293) cells were cultured in 100mm (Sarstedt) or 29mm glass bottom dishes (in vitro scientific), in antibiotic-free high glucose Dulbecco's modified eagle medium (Sigma-Aldrich) containing 10% fetal calf serum. The amount of medium for 100mm and 29mm dishes were 10mL and 1mL, respectively. Before cells were seeded, the glass bottom of the 29mm dishes was surface coated with poly-D-lysine for 30 minutes and subsequently washed in PBS. This coating was omitted for 100mm dishes. Transfection was performed when cells had reached a confluency of 90-100%.

Cell transfection

(Please find accurate reagent amounts in the table below)

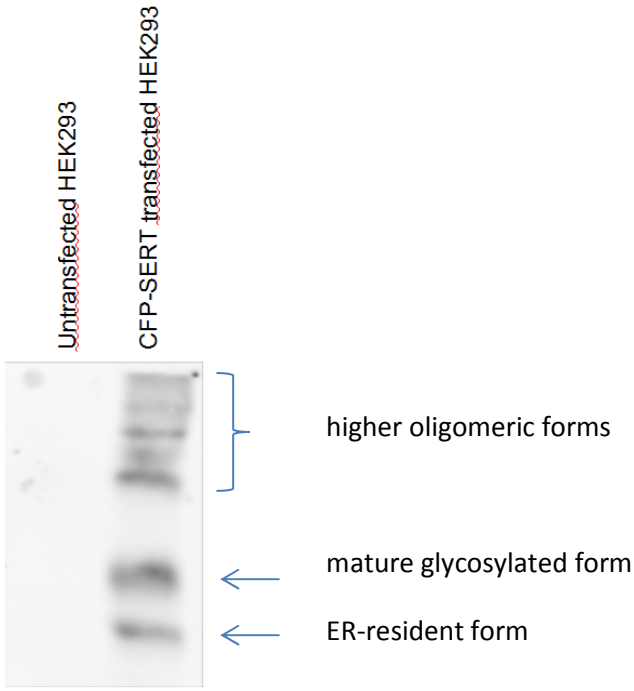
Cells were treated with K2® Multiplier, 2 hours before DNA transfection. For this **K2®** Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes. K2® Transfection Reagent was mixed with Opti-MEM® Reduced Serum Medium (life technologies) and left on room temperature during preparation of the DNA. Plasmid-DNA encoding either CFP-SERT (CFP-tagged serotonin transporter) or YFP-HSP70 was mixed with Opti-MEM. DNA solution was added to the solution containing the K2® Transfection reagent (not the other way around) and mixed by inverting the tubes, followed by 15 minutes incubation at room temperature. Transfection solution was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfections were incubated at 37C and 5% CO2 for 24 hours. Transfection efficiency was estimated by fluorescence microscopy. Protein expression was also monitored by immunoblotting for proteins encoded by the transfected plasmids.

Dish size	DMEM	K2® Multiplier	K2® Transfection reagent	Opti-MEM® for K2® Transfection reagent/DNA	DNA CFP-SERT	DNA YFP-HSP70
29mm	1mL	6,25 uL	1,5 uL	50uL/50uL	1ug	0.1ug
100mm	10mL	125 uL	30uL	500uL/500 uL	20ug	-

(The 29mm dishes were used for a FRET-experiment, therefore I was careful not to overexpress the fluorescent proteins, the 100mm dishes were used for a immunoprecipitation, for this I sought for a high protein yield, therefore the protocols are not directly convertible regarding the surface area)

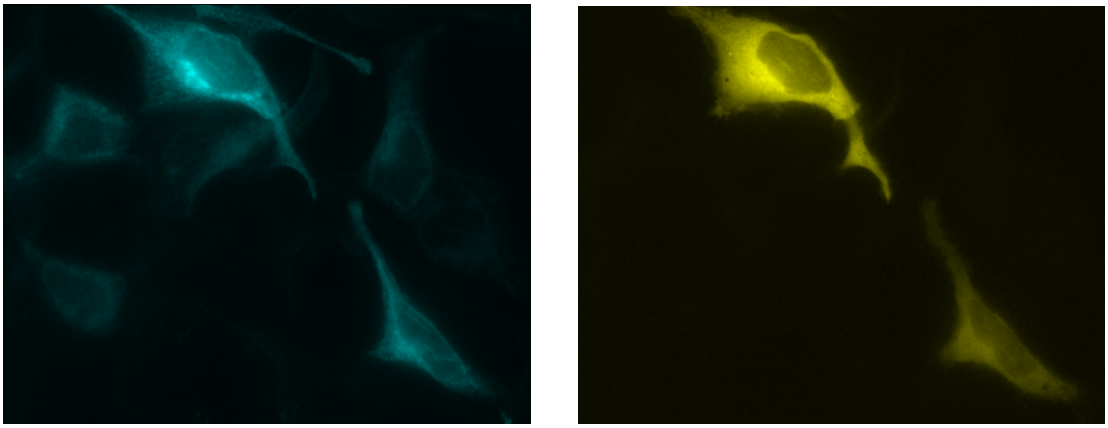
Results

Immunoprecipitated CFP-SERT



Cotransfection of CFP-SERT (ER retained mutant) and YFP-HSP70

CFP-SERT^{PG-AA} YFP-HSP70



Conclusions

For the immunoblot DNA encoding CFP-SERT was transfected into HEK293 cells using the K2® Transfection system. The blot shows a band of the expected size (~100kDa). Aside from this, it shows a slightly higher band representing maturely glycosylated protein and higher bands representing oligomeric forms of the transporter. Therefore it can be assumed that major physiological cellular mechanisms are not negatively affected by the transfection system. Fluorescence microscopy shows successful transfection of two different DNAs with high and moderate transfection rates, whereas the moderate transfection rate was desired for the sake of this specific experiment. The cells show a perfectly healthy morphology and a healthy phenotype of the endoplasmic reticulum, where this mutant of the transporter is retained. Taken together, my data show high transfection rates (70–80%) without any cytotoxic effects of the transfection system.

In contrast to other transfection reagents 2 properties of the K2® Transfection system are particularly advantageous.

1. Efficiency.

   Cells can be transfected at higher confluence. This increases the protein yield or decreases the amount of transfection reagent required for a certain result achieved with other transfection systems. The transfection rate was approximately 80%.

2. Physiology.

   Even at high transfection rates, HEK293 cells appear perfectly healthy. Both, protein maturation and the phenotype of the endoplasmic reticulum remain fully intact after DNA transfection.