DNA-transfection of murine hippocampal nueronal HT22 cells using "Biontex K2® Transfection System".

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Materials and Methods

1. HT22 cells were plate in each well of a 24-well dish in 1 ml of Dulbecco's modified eagle medium (DMEM) supplemented with 5% fetal calf serum and 5 mg/ml gentamicin.

2. Cells were incubated for 24h at 37°C in a CO2 incubator until 80-100% of confluence.

3. Cells in 500 µl medium were treated with 7.5 µl of K2® Multiplier 2 hours before adding the lipoplex. For this, K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes.

4. For each well of a 24-well dish there were prepared:

Solution A: 750 ng of plasmid-DNA encoding either for Green Fluorescent Protein (GFP) or for firefly luciferase under the control of a GR responsive promoter (TAT3-Luc) was mixed with 50 µl medium without serum.

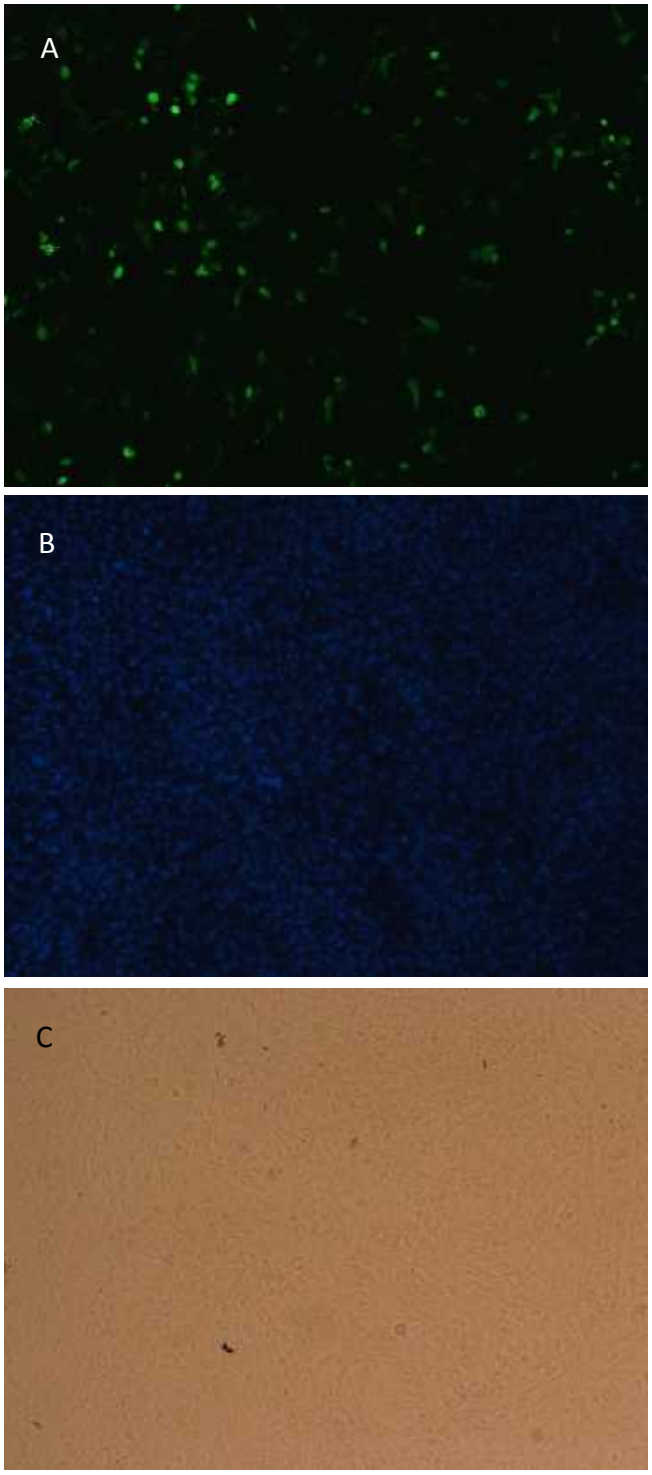
Solution B: 2.25 µl of K2® Transfection reagent was added to 50 µl medium without serum. Solution A was added to the solution B (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection mix was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfected cells were incubated at 37ºC and 5% CO2 for 24 hours and then the cells were reseeded on 96 wells plates or cover glasses.

Evaluation of protein expression and functionality.

Transfection efficiency and localization of the fluorescent protein was evaluated by fluorescence microscopy. Protein expression and functionality was also monitored by a reporter assay measuring luciferase activity after dexamethasone addition.

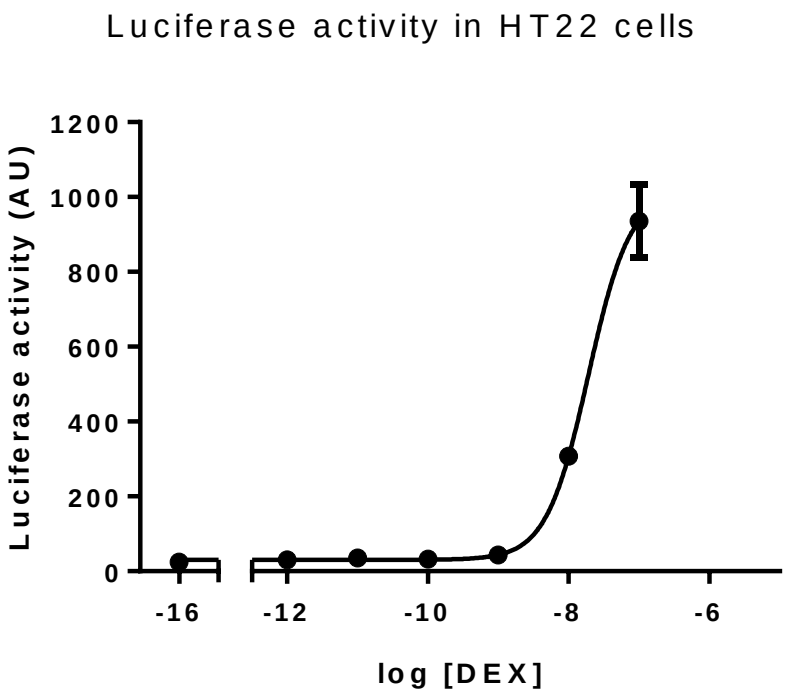
Results

Expression of GFP in HT22 cells.



Expression of GFP (A), Hescht nuclear stain (B) and visible light (C). A 40% of the cells shows fluorescent signal.

Functionality of hGR on reporter assays.



TAT3-Luc expresion on response to dexamethasone. Enzyme activity was determined with SteadyGlo kit (Promega) according to manufacturer's instructions.

Conclusions

Our results show that HT22 cells are efficiently transfected with Biontex K2® Transfection System reagent. Fluorescence microscopy revealed that for these cell lines transfection efficiency is arround 40% and that cell physiology was completly preserved. By means of a reporter-gene assay we can conclude that transfected GR responsive promoter (TAT3-Luc) behavior was as expected.