DNA-transfection of murine neuroblastoma Neuro-2A cells using "Biontex K2® Transfection System".

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Materials and Methods

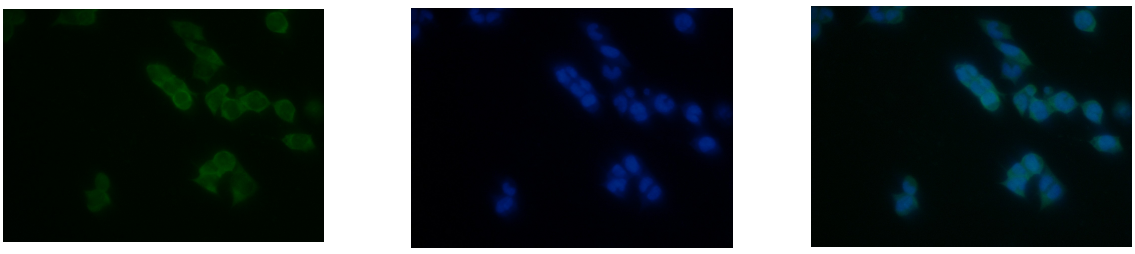
1. Neuro 2A cells (ATCC® CCL-131™) were plate in each well of a 24-well dish in 1 ml of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum and 5 mg/ml gentamicin.
2. Cells were incubated for 24h at 37°C in a CO2 incubator until 80-100% of confluence.
3. Cells in 500 µl medium 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 YFP-hGR (YFP-tagged human glucocorticoid receptor) 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 37C 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 proper cytoplasmic localization of the fluorescent protein after 8 hours cell starving was evaluated by fluorescence microscopy. Protein expression and functionality was also monitored by a reporter assay measuring luciferase activity after 100 nM dexamethasone addition.

Results

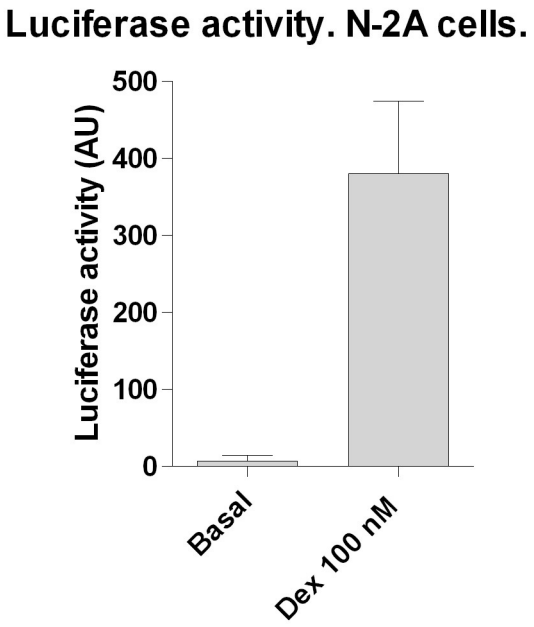
Expression and cellular localization of YFP-hGR.

Neuro-2A cells



Expression of YFP tagged human glucocorticoid receptor (left), Hoescht nuclear stain (center) and merge images (right). Almost all the cells shows fluorescent signal, and the cytoplasmic distribution can be observed.

Functionality of hGR on reporter assays.



Luciferase activity. TAT3-Luc expresion on response to 100 nM dexamethasone. Enzyme activity was determined with SteadyGlo kit (Promega) according to manufacturer's instructions.

Conclusions.
Our results show that Neuro-2A cells are high efficiently transfected with **Biontex K2® Transfection System** reagent.
Fluorescence microscopy revealed that for these cell lines transfection efficiency is almost 100% and that cell physiology was completly preserved. Fluorescence tagged glucocorticoid receptor was located mostly in the cell cytoplasm and by means of a reporter-gene assay we can conclude that transfected glucocorticoid receptor behavior was as expected.