DNA-transfection of human cervix epithelial adenocarcinoma HeLa cells using “Biontex K2® Transfection System”

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Materials and Methods

1. HeLa cells (ATCC® CCL-2™) were plated 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 8 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: 500 ng of plasmid-DNA encoding for Green Fluorescent Protein (GFP) was mixed with 50 µl medium without serum.

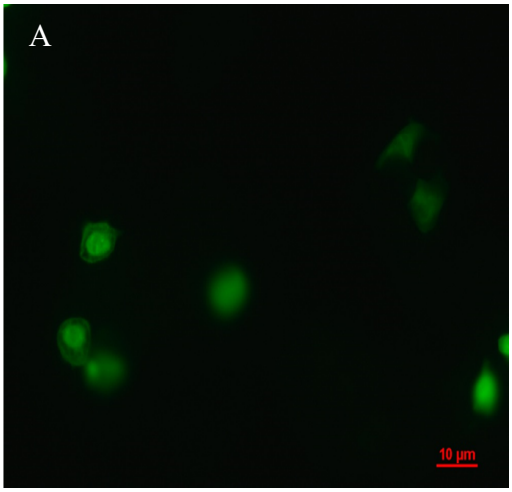
Solution B: 1 µl of K2® Transfection reagent was added to 50 µl medium without serum. Solution A was added to the solution B (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection mix was applied to cells by slow dropwise addition 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 cover glasses.

Evaluation of protein expression and functionality

Transfection efficiency and proper cytoplasmic localization of the fluorescent was evaluated by fluorescence microscopy. Protein expression and functionality was also monitored by confocal fluorescence imaging of GRK2(45-178)GFP when co-expressed with the constitutively active histamine type 2 receptor (H2R).

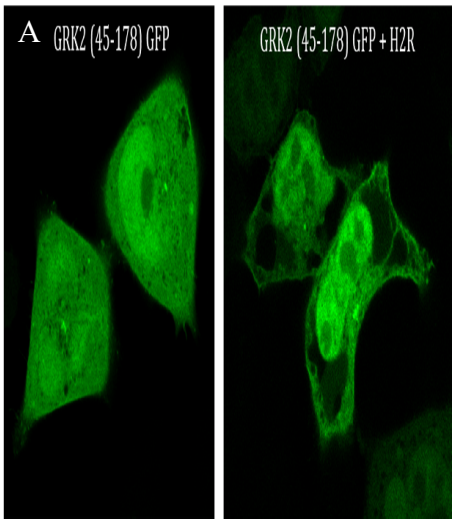
Results

Expression of GFP in HeLa cells



Expression of GFP (A) and Hoechst nuclear stain (B). A 40% of the cells shows fluorescent signal.

Functionality of GRK2-GFP by confocal fluorescence imaging



GRK2 translocation induced by H2R overexpression. HeLa cells were transiently cotransfected with GRK2(45-178)GFP plasmid and histamine type 2 receptor (H2R) (B) or empty vector (A), and fixed after 48hs. Subcellular localization was assessed by confocal microscopy.

Conclusions

Our results show that HeLa cells are efficiently transfected with Biontex K2® Transfection System reagent. Fluorescence microscopy revealed that for this cell line, transfection efficiency is around 40% and that cell physiology was completely preserved. By means of confocal imaging we can conclude that transfected GRK2(45-178)GFP translocate to areas of active signalling as expected.