Plasmid-transfection of human HPAF-II (ATCC® CRL-1997™) cells using "Biontex K2® Transfection System".

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Materials and Methods

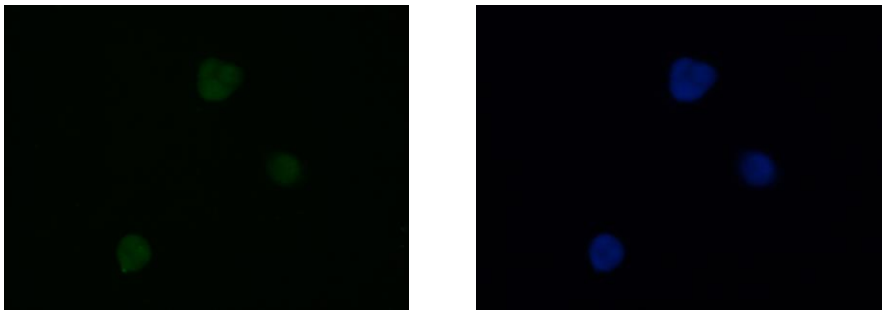
1. 100,000 HPAF-II cells (ATCC® CRL-1997™) were plate in each well of a 24-well dish in 1 ml of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum and 5 mg/ml gentamicin.
2. Cells were incubated for 24h at 37°C in a CO_2 incubator until 80-100% of confluence.
3. Cells in 500 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 GFP (Green Fluorescent Protein).
Solution B: 1.5 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 37°C and 5% CO_2 for 24 hours and then the cells were reseeded on cover glasses.

Evaluation of protein expression
Transfection efficiency and proper cytoplasmic localization of the fluorescent protein after 8 hours cell starving was evaluated by fluorescence microscopy.

Results

Expression and cellular localization of GFP

HPAF-II cells



Expression of GFP (left) and Hoescht nuclear stain (right). Almost all the cells shows fluorescent signal and the cytoplasmic distribution can be observed.

Conclusions

Our results show that HPAF-II cells are efficiently transfected with **Biontex K2® Transfection System** reagent.
Fluorescence microscopy revealed that for this cell line, transfection efficiency is almost 70% and that cell physiology was completely preserved (data not shown).