DNA-transfection of human lung carcinoma cells H1299 using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

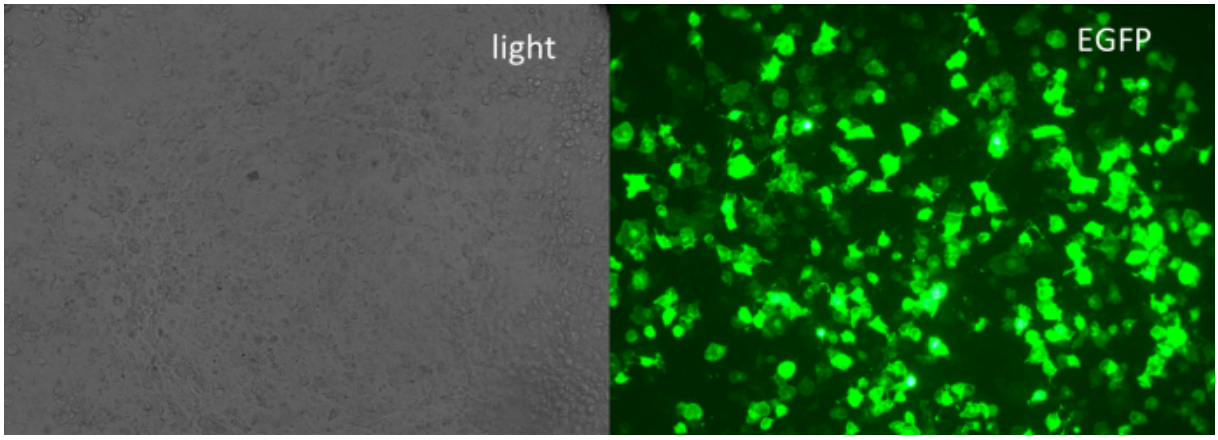
2.5 x 10^4 H1299 cells resuspended in 250 µl DMEM/10% FCS were seeded in chamber slides (8 chambers, IBIDI) and transfection was performed 48 h after cultivation at 100% confluence.

Cell transfection

For transfection, 5 µl K2® Multiplier was added to the cell culture medium and cells were incubated for 2 h at 37°C. During this time 15 µl OptiMEM and 0.6 µg DNA (pEGFP-ITPKA) were mixed (solution A). In addition, 15 µl OptiMEM was mixed with 1.2 µl K2® Transfection Reagent. Thereafter, solution A was mixed with solution B and after incubation for 20 min at room temperature this solution was added to the cell culture medium. 24 h after incubation, transfected cells were washed with PBS and fixed with paraformaldehyde solution (4% in PBS).

Results

H1299 cells transfected with the vector pEGFP-ITPKA



Conclusions

Fluorescence microscopy shows successful transfection of DNA encoding for EGFP-ITPKA with high transfection rates. ITPKA binds to and thus labels F-actin. Our result shows healthy cells with normal actin cytoskeleton and morphology. Taken together, the data show high transfection rates (80-90%) 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 90%.

2. Physiology.

Even at high transfection rates, H1299 cells appear perfectly healthy. Morphology and cytoskeleton remain fully intact after DNA transfection.