Transient plasmid DNA transfection into HeLa and HaCaT cells: a comparison between the efficiency afforded by Biontex Transfection System and Lipofectamine 2000

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Methods

HeLa and HaCaT cells were cultured in D-MEM high glucose medium (Lonza) supplemented with 10% (vol/vol) foetal bovine serum (FBS), 2 mM L-glutamine and 5 mg/ml gentamicin (culture medium, CM). Cells were plated in 35-mm diameter dishes and grown overnight before being processed for transfection of pEGFP-β-Arrestin plasmid DNA. Cells were transfected at 60-70% confluence, which was obtained by plating $6x10^5$ and $5x10^5$ HeLa and HaCaT cells, respectively.

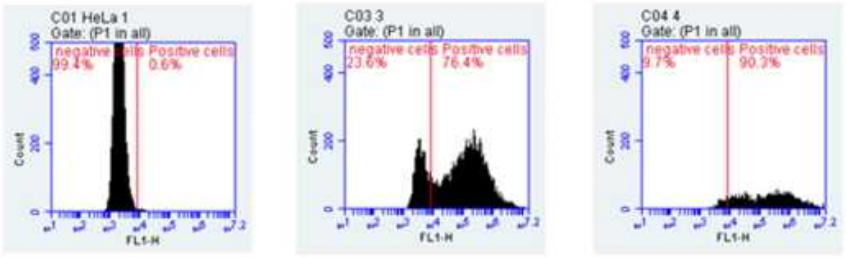
In Lipofectamine 2000 transfection experiments 4 µl of Lipofectamine 2000 (Invitrogen) was mixed with 100 µl of Optimem I (Gibco), incubated for 5 minutes at 25 °C and then added to 100 µl Optimem I containing 2 µg of plasmid DNA. After 20 minute incubation at 25 °C, the liposome/DNA mixture was added dropwise to cell monolayers, which previously had been refed with fresh CM. Cells were incubated with liposomes 18 hours, after which time the transfection mixture was replaced with fresh growth medium. Cells were harvested after 24-36 hours and processed for FACS analysis.

In Biontex transfection experiments, cells were refed with 2 ml of fresh CM containing K2 multiplier (25 µl/35 mm dish for HeLa cells; 50 µl/35 mm dish for HaCaT cells) 2 hours prior to transfection. DNA (2 µg/dish) was diluted in 100 µl Optimem I and mixed with 100 µl Optimem I containing K2 transfection reagent at a 1:4 DNA/K2 (µg:µl) ratio. This mixture was incubated for 20 minutes at 25 °C before being added dropwise to cells. Cells were incubated with transfection mixture for 18 hours, refed with fresh (CM) and harvested for FACS analysis 24-36 hours later.

Results

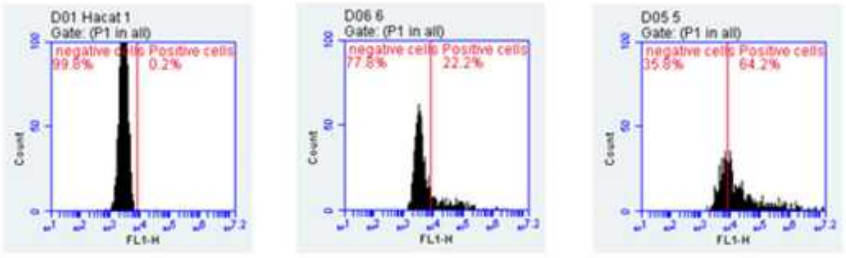
GFP fluorescence was determined by FACS analysis (see Figure 1). In HeLa cells subjected to Lipofectamine 2000 transfection, the expression of GFP was detected in 76,4% of HeLa cells with a mean fluorescence intensity of 437.2. In Biontex experiments GFP positivity increased to 90.3% and the mean fluorescence intensity was 1012.9.

In HaCaT cells, Lipofectamine 2000 achieved a transfection efficiency of 22.2% with a mean fluorescence intensity of 135.9. Biontex K2 reagent increased the transfection efficiency to 64.2 % with a mean fluorescence intensity of 442.5.



A

	Control	Lipofectamine 2000	K2 Reagent
GFP-positive cells (%)	-	76.4	90.3
Mean Fluorescence	-	437.2	1012.92



B

	Control	Lipofectamine 2000	K2 Reagent
GFP-positive cells (%)	-	22.2	64.2
Mean Fluorescence	-	135.9	442,5

Figure 1: Flow cytometry hystograms of HeLa **(A)** and HaCaT **(B)** cells transfected by the Lipofectamine 2000 and K2 transfection protocols. Details are in the text.

Conclusions

In both HeLa and HaCaT cell lines, Biontex K2 reagent afforded a higher transfection efficiency than Lipofectamine 2000. Of note, we observed that Biontex K2-directed gene transfer increased both the percentage of positive cells as well as the expression of ectopic protein (as measured by GFP fluorescence), which is compatible with transduction of a higher number of plasmid copies per cells. This was not at the expenses of cell viability, which was not significantly affected by the transfection procedure in both HeLa and HaCaT cells.

Corroborating the efficacy of K2 transfection system, we obtained satisfying results also by transfecting cells for only 6 hours (instead of the 24 hour-long incubation of cells with the DNA-K2 transfection mixture, as recommended by the manufacturer) (not shown). The shorter transfection procedure may be particularly advantageous when transfected cells need to be split in multiple dishes for further experimental manipulations (e.g. time course or dose-response drug treatment).