DNA-transfection of HeLa, SAN and A375 cells using "Biontex K2® Transfection System"

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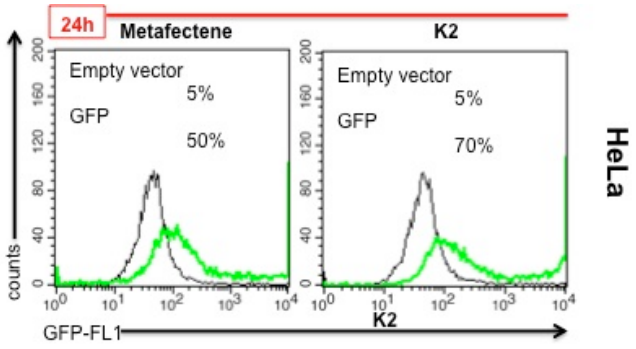
Materials and Methods

Cell culture and transfection.
HeLa cells were cultured in Dulbecco's modified eagle medium (DMEM, Lonza) containing 10% fetal bovine serum (FBS). SAN and A375 (Romano S et al. Cell Death and Diff. 2010) melanoma cells were cultured in 10% FBS RPMI-1640 and 15% FBS DMEM medium (Lonza), respectively. Each cell line was seeded 24h before transfection in 6 well plates, in order to reach 90-100% confluency. Before to start transfection procedure, K2® Multiplier was kept at room temperature (RT) before usage; then it was dripped slowly to the culture medium, mixed by gently swaying the dishes and incubated for 2 hours. After, K2® Transfection Reagent was mixed with free serum medium and left RT during preparation of the DNA/siRNA mix. Plasmid-DNA encoding GFP, or an empty vector, were mixed with medium. For siRNA transfection, two different short-interfering oligoribonucleotide (siRNA) specific for FKBP51, or a non-silencing oligoribonucleotide (NS RNA), were mixed with medium (please find accurate reagent amounts in the table below). Each mix was left 5 min RT and then DNA/siRNA solution was added to the mix containing the K2® Transfection reagent and mixed by inverting the tubes, followed by 20 minutes incubation RT. Transfection solution was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfections were incubated at 37°C and 5% CO_2 for 24 hours, and then medium cell culture was replaced with complete medium. Transfection efficiency was estimated by flow cytometry and QPCR.

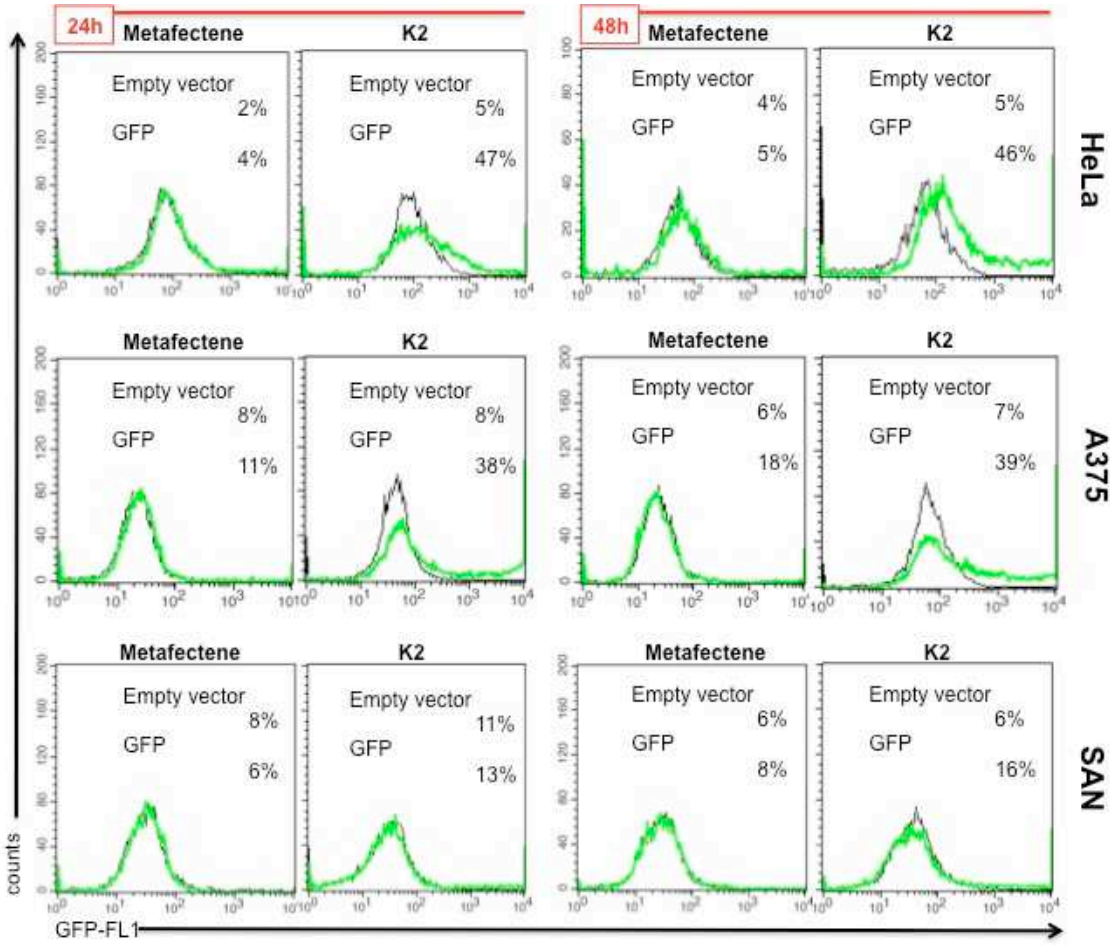
Results

GFP expression in HeLa cells after 24h transfection. Flow cytometryc histograms of HeLa cells transfected with GFP. HeLa cells were transfected, as above described, with 3µg of a plasmid expressing GFP, or with an empty-vector as control. After 24h, K2® Transfection reagent showed increased percentage (70%) of GFP, compared with Metafectene (50%).



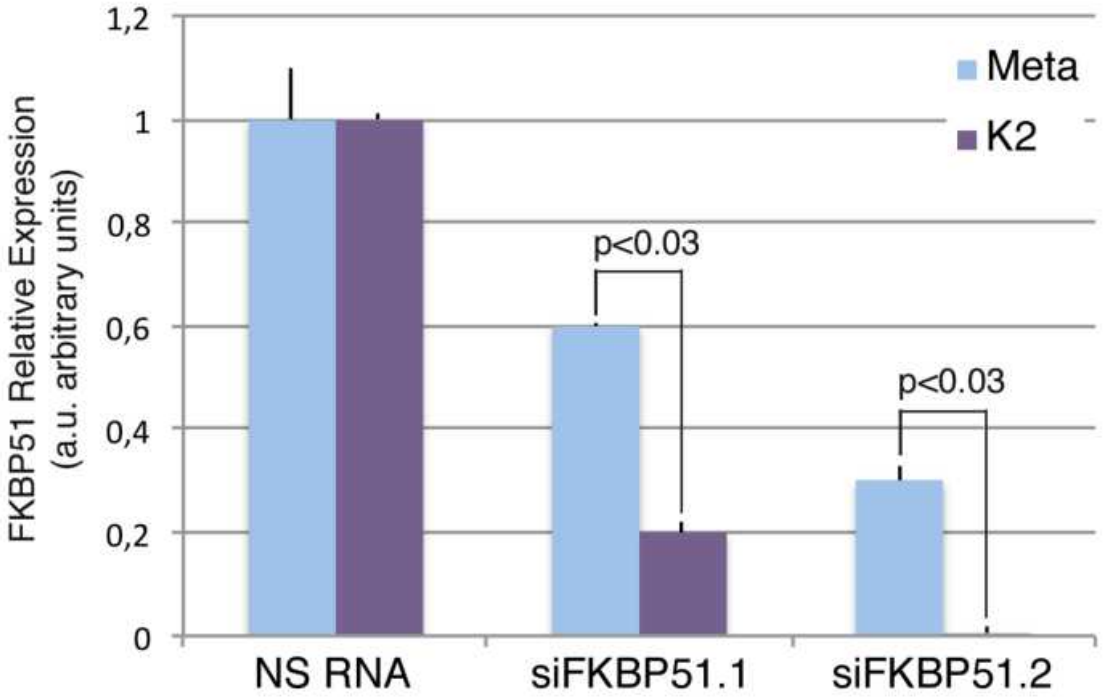
Dish size	Medium	K2® Multiplier	K2® Transfection reagent	Medium for K2® Transfection reagent/DNA	DNA
35mm	2ml	20µl	6µl	120µl	3µg

GFP expression in HeLa, SAN and A375 cells after 24 and 48h transfection, with low DNA amount. Flow cytometryc histograms of HeLa, SAN and A375 cells transfected with GFP. Cells were transfected, as above described, with 0.6µg of a plasmid expressing GFP, or with an empty-vector as control. K2® Transfection reagent works better than Metafectene also with lower DNA amounts, particularly in HeLa and A375. Moreover, K2 reaches it maximal efficacy already at 24h, and does not show significant increase after further 24h.



Dish size	Medium	K2® Multiplier	K2® Transfection reagent	Medium for K2® Transfection reagent/DNA	DNA
35mm	2ml	20µl	6µl	120µl	0.6µg

FKBP51 silencing in SAN melanoma cells, after 24h transfection. FKBP51 transcript average levels obtained from three different experiments. Cells were transfected, as above described, with 50nM of two FKBP51 specific siRNAs and a NS RNA as control. Knock down of FKBP51 resulted significantly increased in cells transfected with K2® Transfection reagent. FKBP51 expression was evaluated in QPCR with specific primers for FKBP51.



Dish size	Medium	K2® Multiplier	K2® Transfection reagent	Medium for K2® Transfection reagent/DNA	siRNA
35mm	2ml	20µl	6µl	120µl	50nM

Conclusions

Results from flow cytometry and QPCR show successful transfection of GFP-DNA in three different cell lines, also at a lower amounts of DNA. Cells showed a perfectly healthy morphology and a healthy phenotype at microscope observation; cells vitality was confirmed in flow cytometry by FSC/SSC analysis. Moreover, QPCR results showed high efficacy of K2 reagent also in siRNAs tranfection, in a cell line system hard to transfect, as well as SAN melanoma cells. Taken together, these data show high transfection rates without any cytotoxic effects of the transfection system.

Compared with other transfection reagents, and with the associate Metafectene reagent, which usually show a good transfection efficacy, the K2® Transfection system is particularly advantageous because of its higher efficacy, vitality of treated cells and it is also convenient in terms of reagent amounts to be used in the experiment, given the high efficacy also with very little amounts of exogenous material.