DNA-transfection of adult human dermal fibroblasts using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

Adult human dermal fibroblasts (HDF) cells were cultured in uncoated 96-well or 24-well culture plates (Nunc), in antibiotic-free high glucose Dulbecco's modified eagle medium (Gibco) containing 2% fetal bovine serum. One day prior to transfection, cells were seeded at a density of ~52,000/cm^2 and transfection was performed when cells had reached a confluency of 90-100%, commonly the day after cells were plated out.

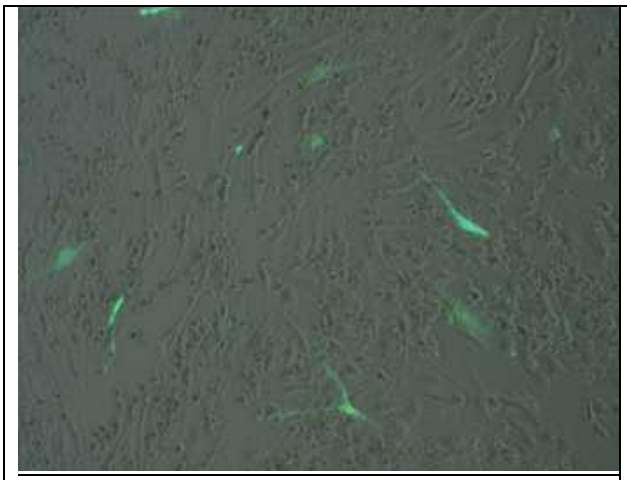
Cell transfection

Cells were switched from DMEM to Opti-MEM® reduced-serum medium (Gibco) for the transfection duration, and were treated with K2® Multiplier for 1 hour prior to DNA transfection, at 37°C with 5% CO2. Reagents were left to reach room temperature during this incubation. K2® Transfection Reagent was mixed with Opti-MEM® Reduced Serum Medium (Gibco), as was plasmid DNA encoding GFP. The DNA solution was added to the solution containing the K2® Transfection reagent and mixed by gentle inversion of the tubes. The final ratio of DNA:K2® reagent was 1:3. The mixture was incubated at room temperature for 20min, to allow complex formation. The transfection complex was applied to the existing medium drop-wise, followed by gentle swirling to mix. Cells were incubated at 37°C and 5% CO^2 for 24 hours, followed by replacement of the medium with DMEM + 2% fetal bovine serum. Transfection efficiency was estimated by fluorescent microscopy 1-3 days post-transfection. An Alamar blue assay (Life Technologies) was used to estimate survival post-transfection, as compared to untransfected cells. Results were compared with those obtained using another transfection reagent (the best of 8 reagents we have tried on adult dermal fibroblasts), used under similar conditions, with the same DNA:reagent ratio and DNA amount.

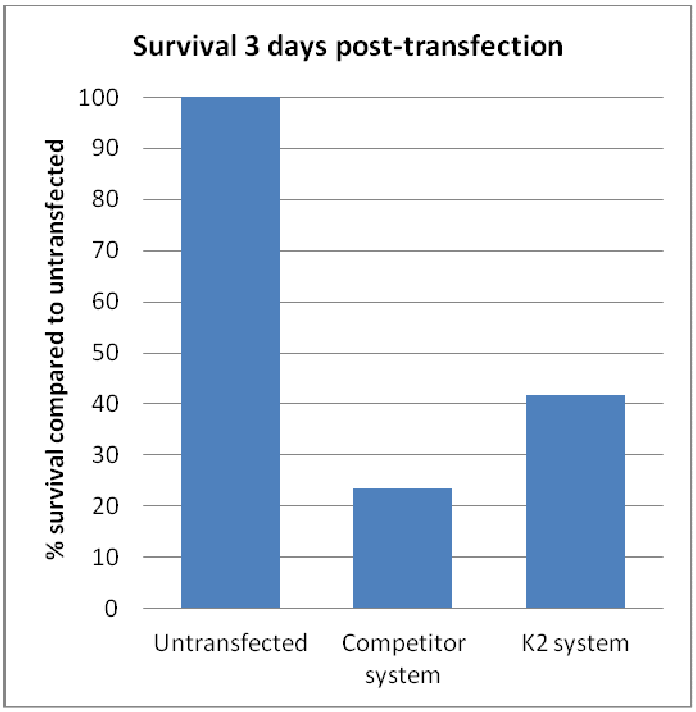
Plate format	Opti-MEM in well	K2® multiplier	K2® Transfection reagent/Opti-MEM® mix	GFP DNA/Opti-MEM® mix
96-well	100µL	2µL	0.489µL/5µL	0.163µg/5µL
24-well	500µL	10µL	3µL/40µL	1µg/40µL

Results

GFP transfection efficiency

	
K2® transfection	Transfection with the best of the tested competitors

Alamar blue assay for estimation of survival



Conclusions

Adult human dermal fibroblasts are notoriously hard to transfect. Their viability drops markedly upon transfection, and efficiency of transfection is not high. Using the K2® system from Biontex was observed to increase the efficiency of transfection for HDF cells, increase the intensity of GFP expressed by the cells, and was found to be less cytotoxic, as compared to the best of the 8 other transfection reagents we tested under these conditions.