Biontex

Biontex K2-Transfection Technical Note

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Materials:

Plasmid pDsRedN1 (Clontech) encodes a fusion protein carrying a fusion of Drosophila CathepsinD N-terminally of Red
Sterile 12-well tissue culture plates
75 ml cell culture flasks
Sterile Eppendorf tubes
Glass coverslips
Trypsin solution
DMEM (Gibco) + 10 % fetal calf serum
MPR-minus MEF cells (SV40-immortalised)
Fluoromount G (SouthernBiotech)

Transfection reagents:
K2-Transfection Multiplier
K2-Transfection Reagent

Stable transfections of MPR-minus MEF cells (SV40 immortalised) were obtained using the K2-Transfection-Kit . Efficiencies were up to 30 and even 50%, however these cells lost the expression after several passages.

Optimization of the transient K2-Transfection for MPR-minus MEF cells (SV40 immortalised) previously stably transfected with the sorting LERP-receptor:

Cells were grown in a 75 ml cell culture flask in DMEM with 10% serum to near confluency, thereafter trypsinated and seeded onto glass coverslips in sterile cell culture 24-well plates ($1x10^5$/0.5ml medium/well). By the time of transfection (typically next day), the cells were covering about 90% of the plate surface (90% optical confluency, corresponds to about 60% true confluency; checked by growth curve).

Optimization of the transfection with K2-Transfection Kit was carried out as follows (all reagents at room temperature):

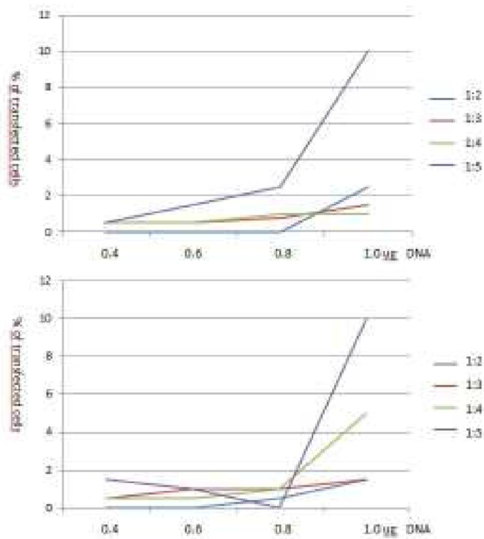
The indicated amount of K2-Multiplier (See Table I) was added to each well 2h before transfection.
DNA in serum-free cell culture medium(SFM) was mixed with various ratios of K2-Transfection Reagent in SFM by pipetting as indicated in Table I. The tubes were allowed to stand at room temperature for 15 – 20 min for complexes to form. At the end of the incubation time, the Lipoplex -mixtures were pipetted onto the cells, mixed by gently agitating the plates and thereafter incubated overnight (37°C /5% CO_2 incubator).
The transfection efficiency with K2-Transfection Reagent was compared to the calcium phosphate transfection method since no commercial reagent was successfull for the transfection of these cells until now.

24 h post transfection starting time, the cells were washed with PBS and fixed with paraformaldehyde. After repeated washing with PBS, the cells were mounted in FluoroMount (SouthernBiotech). The transfection rate was determined by counting the per cent of fluorescent cells expressing the Red-tagged protein under a Zeiss 510 confocal laser scanning microscope. At least 300 cells per sample were scored for expression.

Tabel I. Transient Transfection efficiencies of MPR-minus MEF cells previously stably transfected with the sorting LERP-receptor using the K2 reagent

DNA: K2-Reagent Ratio	Transfected cells (%) 2.5 ul K2-Multiplier			
	0.4 ug DNA	0.6 ug DNA	0.8 ug DNA	1.0 ug DNA
1:2	0	0	0	2.5
1:3	0.5	0.5	0.8	1.5
1:4	0.5	0.5	1	1
1:5	0.5	1.5	2.5	10

DNA: K2-Reagent Ratio	Transfected cells (%) 5 ul K2-Multiplier			
	0.4 ug DNA	0.6 ug DNA	0.8 ug DNA	1.0 ug DNA
1:2	0	0	0.5	1.5
1:3	0.5	1	1	1.5
1:4	0.5	0.5	1	5
1:5	1.5	1	n.d.	10



Conclusions:

Using the optimized transfection conditions for MPR-minus MEF previously stably transfected with the LERP-receptor cells with the K2-Reagent, transfection efficiencies of about 10% were reached compared to 0.2% for calcium phosphate.

A very important variant to be considered is the confluency of the cells by the time of transfection. The cells should be in an active proliferative phase and show an optical confluency of about 90% by the time of transfection. Though some variation of confluency is tolerated without significantly affecting the transfection efficiencies, we observed highly decreased transfection efficiences when the cells were seeded either too dense or too sparse for transfection. In contrast, the incubation time of the transfection mixture with the cells seems to be of minor

importance, since approximately same transfection rates were observed when the cells were incubated either for 4 h or overnight with the transfection mixture, no toxicity was detected.

Figure 1 shows an example of the transfection efficiency using the K2-Transfection Reagent. Already 24 h posttransfection, the expression levels of individual cells are relatively high.

Figure 1 Legend: MPR-minus MEF cells previously stably transfected with the LERP-receptor were transiently transfected with a plasmid encoding for a Red-fusion protein of Drosophila cathepsin D using the K2-Transfection Reagent according to the optimized conditions described in this study.