METAFECTENE® SI+ Technical Note

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Materials: ON-TARGET plus siRNA targeting human membrane-associated protein (target protein 1)
ON-TARGET plus siRNA targeting human target protein 2
ON-TARGET plus siRNA targeting human GAPDH (positive control)
ON-TARGET plus non-targeting siRNA (negative control)
(all siRNAs from Thermo Scientific/ABgene, Epsom Surrey, UK and at a concentration of 20 pmol/µl)
Two sterile 6-well culture plates
Sterile Eppendorf tubes
0.05 % Trypsin-EDTA solution
Complete medium: RPMI with Glutamax, 10 % fetal calf serum, 0.25 % each of Penicillin/Streptomycin (all from Life Technologies, Darmstadt, DE), 0,4 % bovine insulin (Sigma, St. Louis, MO, USA)
BT549 cells (Breast cancer)

Transfection reagents:
METAFECTENE® SI+
Lipofectamine® 2000 (Life Technologies, Darmstadt, DE)
OptiMEM (Life Technologies, Darmstadt, DE)
Antibiotic-free medium: RPMI with Glutamax, 10 % fetal calf serum (both from Life Technologies, Darmstadt, DE), 0,4 % bovine insulin (Sigma, St. Louis, MO, USA)

All steps were conducted under sterile conditions.

Protocol for the transient transfection of BT549 cells
A total of 0.5 x 10^6 cells/well was seeded and grown to 80 % confluence in complete medium at 37°C in a humidified incubator under 5 % CO_2 conditions. By the day of transfection, the complete medium was replaced by antibiotic-free medium and the cells were incubated for a minimum of 1 h.
In the meantime, the respective transfection reagents were pipetted into an 1,5 ml Eppendorf tube (Table 1 and 2).

For transfections using METAFECTENE® SI+:
All steps were performed according to manufacturer´s instructions. 150 µl 1 x SI+ Buffer was mixed with METAFECTENE® SI+ and 200 pmol siRNA were added and carefully mixed (Table 1). After 15 min incubation at room temperature, the METAFECTENE® SI+ - siRNA complexes were pipetted onto the cells and mixed by swinging the plates for a few times.

	SI⁺ Buffer	METAFECTENE® SI⁺	siRNA
1. mock:	150 µl	7,2 µl	-
2. + siRNA.NK:	150 µl	7,2 µl	10 µl
3. + siRNA.GAPDH:	150 µl	7,2 µl	10 µl
4. + siRNA.TP1:	150 µl	7,2 µl	10 µl
5. + siRNA.TP2:	150 µl	7,2 µl	10 µl
6. + siRNA.TP1 + siRNA.TP2:	140 µl	14,4 µl	10 µl + 10 µl

Table 1: The amounts of respective reagents for the transfection using METAFECTENE® SI⁺. TP: target protein, NK: negative control.

For transfections using Lipofectamine® 2000:
All steps were performed according to manufacturer´s instructions. The respective siRNAs were added to OptiMEM and in a separate tube, 10 µl of Lipofectamine® 2000 were mixed with OptiMEM and incubated for 5 min at room temperature (Table 2). Both approaches were combined, mixed carefully and incubated for 20 min at room temperature. Then, the Lipofectamine® 2000-siRNA complexes were pipetted onto the cells and mixed by swinging the plates for a few times.

	I.		II.	
	siRNA	OptiMEM	Lipofectamine® 2000	OptiMEM
1. mock:	-	250 µl	10 µl	240 µl
2. +siRNA.NK:	10 µl	240 µl	10 µl	240 µl
3. +siRNA.GAPDH:	10 µl	240 µl	10 µl	240 µl
4. +siRNA.TP1:	10 µl	240 µl	10 µl	240 µl
5. +siRNA.TP2:	10 µl	240 µl	10 µl	240 µl
6. +siRNA.TP1 +siRNA.TP2:	10 µl + 10 µl	230 µl	10 µl + 10 µl	230 µl

Table 2: The amounts of respective reagents for the transfection using Lipofectamine® 2000. TP: target protein, NK: negative control.

In both approaches, the cells were incubated for 6 h at 37°C in a humidified incubator under 5 % CO_2 conditions, then the supernatant was removed, the cells were washed with PBS, transferred into cell culture flasks and incubated in complete medium. 72 h post-transfection, the supernatant was removed, the cells were trypsinized, pelleted (1500 rpm, 5 min) and lysed in T-PER® tissue protein extraction reagent buffer (Thermo Scientific, Rockford, IL, USA) supplemented with Complete Mini Protease inhibitor (Roche, Mannheim, DE) and Phosphatase Inhibitor cocktail 2 and 3 (Sigma, St. Louis, MO, USA).

SDS-PAGE and Western blots:
The protein concentrations were determined using the BCA Protein Assay Reagent (Thermo Scientific, Rockford, IL, USA). For Western blot analysis, 50 µg of protein lysates were separated by SDS-PAGE and transferred onto a 45 µm nitrocellulose membrane (GE Healthcare/Amersham, Buckinghamshire, UK). The membrane was blocked in a 5 % skim milk T-BST solution for 1 h at 37°C. The antibodies against the target proteins and the controls were incubated overnight in 5% BSA/T-BST at 4°C and were detected using HRP-linked secondary antibodies incubating for 1 h in 5% skim milk solution at room temperature.

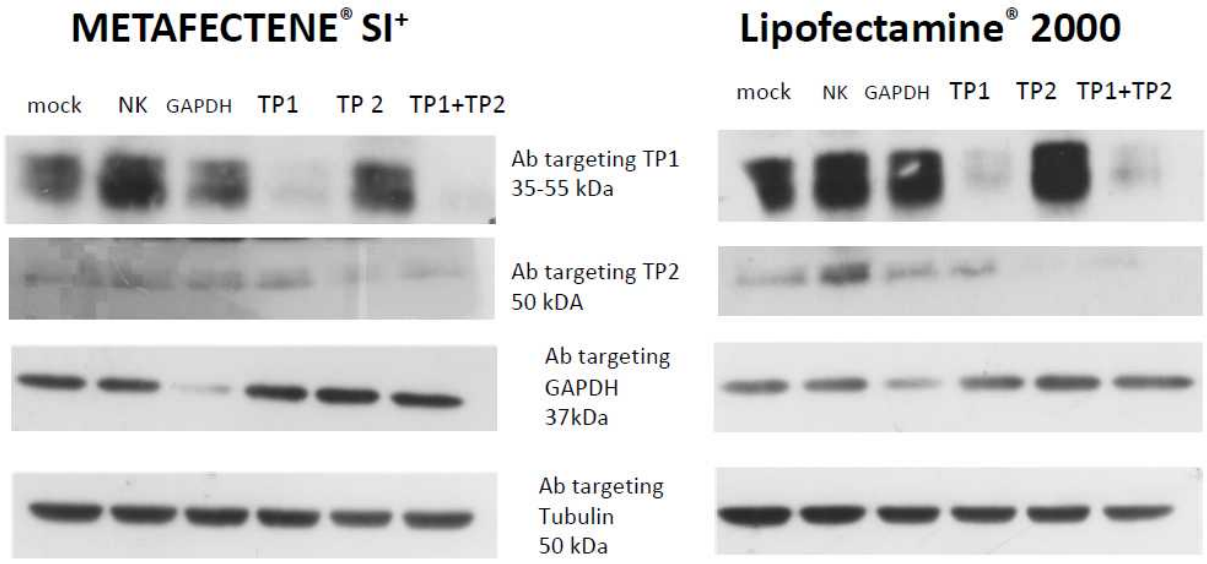

Figure 1: Western blot analysis of BT549 cell lysates derived from transient (72h) transfections using Lipofectamine® 2000 and METAFECTENE® SI+. TP: target protein, NK: negative control, GAPDH: positive control.

Conclusions

The Western blot analysis (Figure 1) demonstrates a very efficient downregulation of the target proteins using either METAFECTENE® SI+ or Lipofectamine® 2000. Although both approaches were conducted under the same conditions and at the same time, GAPDH (positive control) seems to be stronger downregulated when METAFECTENE® SI+ was used. Furthermore, in comparison with the Lipofectamine® 2000 protocol, the transfection protocol using METAFECTENE® SI+ is time-saving due to less pipetting and intermediate steps. This is very advantageous, in particular when a lot of approaches should be conducted. Moreover, no toxic effects on BT549 cells were observed using the transfection reagents under the previously described conditions.