Metafectene SI Technical Note

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Materials:
miRIDIAN human hsa-miR-27a-3p Mimic (Thermo Fisher C-3005-03-0005)
Sterile 6-well tissue culture plates
Sterile Eppendorf tubes
Trypsin solution (Biochrome)
DMEM + 20 % fetal calf serum
MCF-7 breast cancer cell line cultivated according to ATCC
Transfection reagents:
METAFECTENE SI+
Lipid Lk2

Transfection of miRNA (miR-27a) into adherent MCF-7 breast cancer cells
MCF-7 cells were grown in a 75 ml cell culture flask in DMEM with 20 % serum, without antibiotics, to 80 % confluency, thereafter trypsinated, resuspended and seeded onto 6-Well plates in the number of $2*10^5$ cells and a final volume of 2 ml media per well.

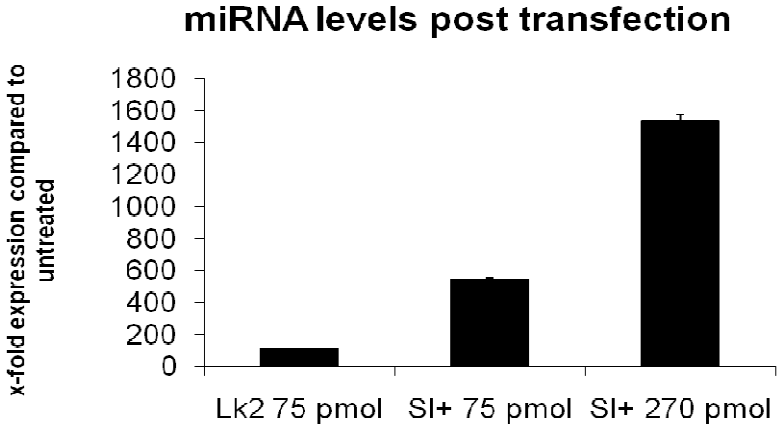
On the next day, the transfection was performed with two different reagents.
One commercially available transfection reagent, Lipid Lk2 was conducted per the reagent's protocol with a miRNA amount of 75 pmol per well.

The transfection with METAFECTENE SI^+ was carried out in two different amounts of miRNA Mimics. Once, with 75 pmol per well (as comparison to Lipid Lk2) and once with 270 pmol as stated in the protocol for Metafectene SI.

All reagents were thawed at room temperature. One Eppendorf 1,5 ml tube was prepared for each concentration.
Per tube 150 µl 1x SI+ Buffer were provided first, 7,2 µl Metafectene SI^+ were added and mixed by gentle pipetting.
The according amounts of miRNA were added from 50 µM stock solution.
After 15 min of incubation time, the agent was applied to the cells by distributing single drops over the area of the media and mixed by tilting the plate cautiously.

The plate was incubated at 37°C and 5% CO_2 atmosphere, followed by exchanging the medium after 4h to minimize toxic effects.

24h post medium change, the cells were lysed , totalRNA were prepared. Analysis of miRNA levels was performed via qPCR as described in Kopp et al. [1]



Conclusions:
Using untreated MCF7 for normalization, transfection of miRNA utilizing all reagents was successful.

While METAFECTENE SI$^+$ was superior to Lipid Lk2 almost 5 fold when using 75 pmol miRNA. The transfection efficiency was additionally improved by increasing the amount of transfected miRNA Mimic, showing proportionality between used amount of miRNA and transfection efficiency.
Summarized, Metafectene SI$^+$ is a very useful reagent for the transfection of miRNA mimics with great efficiency and limited toxic effects.

[1] Kopp F, Oak PS, Wagner E, Roidl A (2012) miR-200c Sensitizes Breast Cancer Cells to Doxorubicin Treatment by Decreasing TrkB and Bmi1 Expression. PLoS ONE 7(11): e50469. doi:10.1371/journal.pone.0050469