Biontex

METAFECTENE® SI+

The siRNA and miRNA transfection reagent for eukaryotic cells

Highlights

- Suitable for siRNA and miRNA transfection
- Optional "Fast Forward" protocol
- Specific optimization of lipid composition for gene silencing
- Predefined RNA/lipid ratio results with less optimization effort
- Efficient knockdown and low siRNA volume
- Rapid protocol: Two independent assays within one week

Technology

A broadly based screening process enabled a highly active lipid composition precisely aligned to siRNA and miRNA transfection to be identified from a comprehensive library of lipids. In this context, a specifically optimized buffer composition for lipoplex formation – the SI+ buffer – and a significantly simplified protocol were developed. These individual features combine to form a unique composition which enables outstanding siRNA and miRNA transfection rates to be achieved.

Product Specifications

Application	Transfection of eukariotic cells with siRNA or miRNA
Content	Lipid formulation and sufficient quantity of SI+ buffer
Assays	Up to 4000 (96-well) or 650 (24-well) per 1ml reagent

Order Numbers

Product	Order No.	Size	CHF
Test sample Metafectene®SI+	T100-0.2	200 µl	
SI+ buffer		400 µl	0.-
Metafectene®SI+	T100-1.0	1.0 ml	
SI+ buffer		2.0ml	320.-

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Transfection of miRNA into MCF-7 breast cancer cells with Metafectene SI+

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Materials

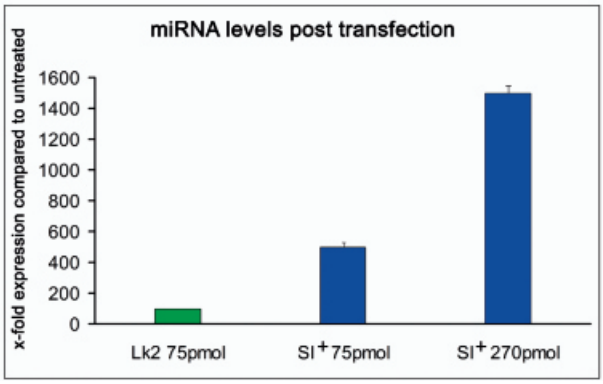
miRIDIAN human hsa-miR-27a-3p Mimic
(Thermo Fisher C-3005-03-0005)

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 $2x10^5$ 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 75pmol 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 270pmol 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, total RNA 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