METAFECTENE® SI+

Especially developed for the transfection of mammalian cells with siRNA or miRNA

For ordering information, MSDS, publications and application notes see www.biontex.com

Description	Cat. No.	Size
METAFECTENE® SI+	T100–0.2	0.2 ml
METAFECTENE® SI+	T100–1.0	1.0 ml
METAFECTENE® SI+	T100–2.0	2 × 1.0 ml

Shipping: At room temperature

Constituents: METAFECTENE® SI+ Transfection Reagent
10× SI+ buffer

Storage: 4°C

Stability: Best before: see label
Formulations of liposomes like the METAFECTENE® SI+ Transfection Reagent change their size distribution after long storage at 4°C, which can have slightly adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. We recommend performing a freeze-thaw cycle before first use and subsequently monthly to yield optimal results.

Use: Only for research purposes *in vitro*, not intended for human or animal diagnostic, therapeutic or other clinical uses.

METAFECTENE® SI+ is a composition of lipids especially developed for the transfection of mammalian cells with siRNA or miRNA. It generates excellent knockdown even with small amounts of RNA, due to its new siCOM technology.

METAFECTENE® SI+ is designed specifically for transfection with siRNA and miRNA. It enables transfection to be performed with extremely small amounts of reagent, resulting in excellent cell viability and minimizing off-target effects. In addition, METAFECTENE® SI+ can be used with a fast forward protocol enabling two consecutive experiments to be performed per week (standard protocol with 48 h incubation time) and is ideal for automated laboratory testing.

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1. General Information

1.1 Specifications

Application	Transfection of mammalian cells with siRNA or miRNA
Formulation	Composition of lipids in water
Sterility	Tested
Assays	About 4000 (96-well) or about 650 (24-well)
Storage	4°C

1.2 Quality Control

Standard transfection assay. Absence of bacterial and fungal contamination is verified using thioglycolate medium.

1.3 Explanatory Remarks

Storage

METAFECTENE® SI+ is delivered uncooled and all individual constituents should be stored in a refrigerator at 4°C after receipt. Storage for several days at room temperature is not a problem provided that the constituents are subsequently stored again at 4°C. Freeze-thaw cycles do not affect the constituents. On the contrary, a freeze thaw cycle can reoptimize the gradually changing size distribution of the liposomes in the METAFECTENE® SI+ Transfection Reagent.

State of the cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at total confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing well (70 – 90% coverage of the growth area). It is therefore recommended to use only regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically diminish transfection efficiencies. Biontex offers MycoSPY® und MycoRAZOR® for fast, reliable detection and efficient removal of mycoplasmal contaminations.

Quality of the genetic material

The RNA should be of the maximum purity if optimal knockdown results are desired.

Note: RNA is sensitive to ubiquitous RNases. Appropriate precautions should therefore be taken!

2. Working Instructions

2.1 Description

Sections 2.2 – 2.6 describe a transfection protocol in which cells in two wells of a 48–well plate (each with 1 square centimeter growth area) are treated with two different quantities of an RNA/lipid complex (lipoplex). Section 2.7 shows a chart of quantities for other well formats.

In this fast-forward protocol cells are seeded shortly before the addition of the lipoplex.

This experiment is then evaluated to give the optimum amount of lipoplex for the cells. All further experiments involving the same cells and the same RNA use this optimum amount of lipoplex in transfection. Amounts for these explicit transfection protocols are given in Section 2.8.

In rare cases, e.g. cells that are hard to transfect or highly sensitive, optimization of the RNA:lipid ratio may be useful (Section 2.9).

2.2 Prepare Reagents

First of all dilute 1 aliquot of 10× SI^+ buffer with 9 aliquots sterile water (suitable for cell culture) under sterile conditions to prepare 1× SI^+ buffer.

Before transfection bring the 1× SI^+ buffer, the METAFECTENE® SI^+ Transfection Reagent and the RNA stock solution (with a concentration of at least 20 µM = 20 pmol/µl) to room temperature. Agitate all reagents gently before use.

2.3 Prepare Cells

Prepare 250 µl cell suspension in complete cell culture medium with a concentration of $2.0 \cdot 10^5$ cells/ml.

Fill each of two wells (*well 1* and *well 2*) with 250 µl of cell suspension.

Incubate the cells under normal culture conditions (e.g. 37°C in CO_2–containing atmosphere) until the lipoplex is added.

2.4 Prepare Lipoplexes

Place 45 µl 1× SI^+ buffer in a reaction vessel (preferably polypropylene).

Pipet 2.4 µl METAFECTENE® SI^+ Transfection Reagent into the 1× SI^+ buffer and mix by gently pipeting up and down once.

Add 90 pmol RNA by pipeting and mix gently once.

Mix gently!
Shear forces damage the lipoplex and impair transfection efficiency.

Incubate for 15 min at room temperature.

2.5 Transfection

Now divide the lipoplex solution between the two wells filled with cell suspension:

15 µl of lipoplex in *well 1* and 30 µl in *well 2*. *Well 1* now contains approx. 30 pmol RNA and *well 2* approx. 60 pmol.

Incubate the cells under the usual conditions.

2.6 Evaluation

Evaluation of the experiment (e.g. real–time PCR or reporter gene assay) is done 24 – 72 h after the addition of the lipoplex. The highest knockdown is often achieved after 48 h. The optimal point in time is determined by the properties of the cell type and the expression rate of the protein as well as by the half-life of the expressed protein, if applicable.

Use the results to select the optimum amount of lipoplex (1 or 2) for the cells used.

2.7 Transfer to other formats

Format	Growth area	Cell suspension	1× SI$^+$ buffer	M. SI$^+$ Transf. Reagent	RNA	Lipoplex amount	
						well 1	*well 2*
96 well	0.3 cm²	2× 100 µl	22.5 µl	0.72 µl	27 pmol	7.5 µl	15 µl
48 well	1.0 cm²	2× 250 µl	45 µl	2.4 µl	90 pmol	15 µl	30 µl
24 well	1.9 cm²	2× 500 µl	90 µl	4.6 µl	170 mol	30 µl	60 µl
12 well	3.6 cm²	2× 900 µl	180 µl	8.6 µl	320 pmol	60 µl	120 µl
6 well	9.0 cm²	2× 2.2 ml	450 µl	21.6 µl	810 pmol	150 µl	300 µl
60 mm dish	22 cm²	2× 5.5 ml	1350 µl	53 µl	2.0 nmol	450 µl	900 µl
100 mm dish	60 cm²	2× 15 ml	4.0 ml	144 µl	5.4 nmol	1.35 ml	2.7 ml

2.8 Explicit transfection protocols

If the optimum amount of lipoplex (1 or 2) for the cells to be transfected is already known, the following chart can be used as a basis for further procedure.

Amounts given refer to transfection of a single well with the given format. The process of lipoplex preparation is the same as described in Section 2.4.

	Lipoplex amount 1 (*well 1*)			Lipoplex amount 2 (*well 2*)		
Format	**1× SI$^+$ buffer**	**M. SI$^+$ Transf. Reagent**	**RNA**	**1× SI$^+$ buffer**	**M. SI$^+$ Transf. Reagent**	**RNA**
96 well	7.5 µl	0.24 µl	9 pmol	15 µl	0.48 µl	18 pmol
48 well	15 µl	0.8 µl	30 pmol	30 µl	1.6 µl	60 pmol
24 well	30 µl	1.5 µl	57 pmol	60 µl	3.0 µl	114 pmol
12 well	60 µl	2.9 µl	108 pmol	120 µl	5.8 µl	216 pmol
6 well	150 µl	7.2 µl	270 pmol	300 µl	14.4 µl	540 pmol
60 mm dish	450 µl	17.6 µl	660 pmol	900 µl	35.2 µl	1.3 nmol
100 mm dish	1.35 ml	48 µl	1.8 nmol	2.7 ml	96 µl	3.6 nmol

2.9 Advanced optimization

Adjustment of the RNA:lipid ratio is generally unnecessary in transfection with METAFECTENE® SI+. However, changes to transfection parameters may be useful in the case of hard-to-transfect or highly sensitive cells.

The following chart shows all useful combinations of reagent amount and RNA amount for the various well formats. Standard amounts are underlined.

	Lipoplex amount 1					Lipoplex amount 2				
Format	**1× SI buffer**	**M. SI+ [µl]**			**RNA**	**1× SI buffer**	**M. SI+ [µl]**			**RNA**
96 well	7.5 µl	0.12	0.24	0.36	9 pmol	15 µl	0.24	0.48	0.72	18 pmol
48 well	15 µl	0.40	0.80	1.2	30 pmol	30 µl	0.8	1.6	2.4	60 pmol
24 well	30 µl	0.76	1.5	2.3	57 pmol	60 µl	1.5	3.0	4.6	114 pmol
12 well	60 µl	1.45	2.9	4.3	108 pmol	120 µl	2.9	5.8	8.6	216 pmol
6 well	150 µl	3.6	7.2	10.8	270 pmol	300 µl	7.2	14.4	21.6	540 pmol
60 mm dish	450 µl	8.8	17.6	26.4	660 pmol	900 µl	17.6	35.2	52.8	1.3 nmol
100 mm dish	1.35 ml	24	48	72	1.8 nmol	2.7 ml	48	96	144	3.6 nmol

3. Miscellaneous

3.1 Important Information

This reagent is developed and sold for research purposes and *in* vitro use only. It is not intended for human therapeutic or diagnostic purposes.

3.2 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex or one of its authorized distributors.

4. Related Products

Product	Cat. No.	Product	Cat. No.	Product	Cat. No.
Transfection					
METAFECTENE® EASY+	T090	METAFECTENE® PRO	T040	METAFECTENE®	T021
METAFECTENE® SI+	T100	METAFECTENE® FluoR	T050	INSECTOGENE	T030
DOTAP	T010	METAFECTENE® 3D-SC	T110	METAFECTENE® 3D-HG	T120
Protein Purification					
Ni-NTA Agarose	R040	Ni-IDA Agarose	R010	Highflow Ni-IDA Agarose	R011
GST Agarose	R030	Co-IDA Agarose	R020	Highflow Co-IDA Agarose	R021
Empty S-Columns	P-50S	Empty L-Columns	P-50L	Empty XL-Columns	P-50XL
Empty Spin-Columns	SP-50	Empty Cartridges	C010		
Proteofection					
PROTEOfectene®	E010	PROTEOfectene® AB	E020		
Cell Culture					
MycoSPY®	M030	MycoRAZOR®	M040		
Microfection Kits					
µ-Transfection Kit VI	K010	µ-Proteofection Kit VI	K030		
µ-Transfection Kit VI FluoR	K020	µ-Proteofection Kit VI AB	K040		
Buffer and Positive Controls					
1x PBS	S011	EASY^{+} buffer (10x)	EA010		
SI^{+} buffer (10x)	SO010	R-Phycoerythrin	S020		

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