METAFECTENE® PRO

The highly efficient Transfection Reagent for Mammalian Cells

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

Product	Order No.	Size
METAFECTENE® PRO	T040-0.2	0.2 ml
METAFECTENE® PRO	T040-1.0	1.0 ml
METAFECTENE® PRO	T040-2.0	2 × 1.0 ml
METAFECTENE® PRO	T040-5.0	5 × 1.0 ml

Shipping: Room temperature

Storage: 4°C

Stability: Best before: see label.
Formulations of liposomes like the METAFECTENE® PRO 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.

Description

METAFECTENE® PRO is the latest generation transfection reagent setting a new benchmark in mammalian cell transfection. Previous data support the fact that at least equivalent results can be achieved with METAFECTENE® PRO in comparison with METAFECTENE®, but multiple enhancement of efficiency can be obtained eminently with moderately hard or hard-to-transfect cell lines.

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1. General Information

1.1 Specifications

Application	Transfection of nucleic acids into mammalian cells
Formulation	Cationic lipids with colipids in water
Assays	up to 1500 (24-well) or up to 400 (6-well) with 1 ml reagent
Sterility	tested
Cell Culture	tested
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

METAFECTENE® PRO is provided as a ready-to-use solution. It shows no serum inhibition, which makes it a reagent of choice for transfecting sensitive cell lines.

Storage

METAFECTENE® PRO is delivered ***uncooled and 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 reagent is subsequently stored again at 4°C. Freeze-thaw cycles do not affect the reagent. On the contrary, a freeze thaw cycle can reoptimize the gradually changing size distribution of the liposomes in METAFECTENE® PRO.

State of cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results.

Cell confluency

The true confluency of the cells (adherent) to be transfected cannot be estimated visually by using a microscope, but can be optimally determined by means of a growing curve and comparison with counted cells. In many cases a 90-100% covered growing area is correlated with 30-60% true confluency.

The DNA transfection during the exponential growing phase of the cells is essential for optimum results, because of the critical role of cell division in transport of the DNA into the nucleus. The optimal confluency has to be adapted to the cell line used!

Good results are regularly obtained by using a covered growing area of 90–100% (visual confluency, see section 5). Usually transfection of siRNA is independent from cell division and requires a lower cell density at the point of transfection compared to DNA.

Quality of the nucleic acids

Nucleic acid should be of highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Before its use in complex formation with METAFECTENE® PRO, nucleic acid should not be stored diluted in medium for much longer than 5 min. Adsorption of nucleic acid in container materials can result in decrease of transfection efficiency. Polypropylene shows a minimum tendency towards adsorption of transfection reagent and genetic material in comparison to e.g. glass and polyethylene.

Antibiotics

Must be avoided where indicated. In some cases cell death can be caused by use of antibiotics in the transfection medium.

Optimization

Although METAFECTENE® PRO shows a broad peak performance, if optimal results are desired we recommend optimization of the transfection protocol for each combination of plasmid and cell line used. Every cell line has a characteristically optimal nucleic acid-lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ratio and absolute amounts of reagents.[1]

Additionally, a protocol used for other transfection reagents should never be transferred to METAFECTENE® PRO (or any other different transfection reagent). Every transfection reagent possesses its own molecular structure with specific physical properties, which have an important influence on nucleic acid-lipid ratios. Appropriate optimization instructions are given in section 3. As a rule, only little optimization is required if the recommended starting points are used.

More information is available in section 3.

Stable transfection

If you desire stable transfection, follow the usual working instructions for seeding cells with lower density. On the day of transfection, cells should preferably be less than 50% confluent. After the transfection procedure, replace transfection medium with a suitable selected medium containing antibiotics.

1 Probably caused by differing adsorption properties of the tube material based on differing surface dimensions.

2. Working Instructions

2.1 Transfection of adherent cells – Standard protocol for a 12-well plate -

We recommend beginning with the starting points mentioned in the table of section 4 or optimizing by following the optimization protocol.

1. In a 12-well tissue culture plate, seed 1.0 – 4.0 x 10^5 (starting point: 2.0 x 10^5) cells per dish in 1 ml of suitable fresh complete medium.[2]
2. Incubate the cells at 37°C in a CO_2 incubator until growing area is 90–100% covered. The time required will vary among cell types, but will usually take 18–24 hours.
3. The stock solutions of the METAFECTENE® PRO and the DNA or RNA should be at room temperature. Agitate the stock solutions gently before use.

RNA means single stranded RNA (ssRNA), not siRNA!

4. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces.

Solution **A:**	0.5 – 1.5 µg of DNA or RNA to 50 µl serum- and antibiotic-free medium or 1xPBS
Solution **B**:	1.0 – 7.0 µl of METAFECTENE® PRO to 50 µl serum- and antibiotic-free medium or 1xPBS

5. Mix the solutions gently by carefully pipetting one time.

Ratios will require optimizing based on various factors (see sections 3 and 4).
The DNA or RNA lipid ratio has to be kept between 1:2 and 1:7 [µg DNA or RNA : µl METAFECTENE® PRO]!

Please note the order of addition in the following step: Add the DNA or RNA solution into the transfection reagent solution and not in reverse!

6. Combine the two solutions, **mix gently by pipetting up and down once** and incubate at room temperature for 15–20 min.

Shear stress may destroy the DNA or RNA lipid complex!

7. After incubation time add as soon as possible the DNA or RNA-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.[3]
8. Depending on cell type and promoter activity, assay cells for gene activity 24–72 h following the start of transfection.

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2 Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

3 If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3–6 hours and replace it with medium.

2.2 Transfection of suspension cells – Standard protocol for a 12-well-plate -

1. In a 12-well tissue culture plate, seed 0.4 – 1.6 x 10^5 cells per dish in 1 ml suitable fresh complete medium.
2. The stock solutions of METAFECTENE® PRO and the DNA or RNA should be at room temperature. Agitate the stock solutions gently before use.
3. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces.

   Solution **A:** 0.5 – 1.5 µg of DNA or RNA to 50 µl serum- and antibiotic-free medium or 1xPBS
   Solution **B**: 1.0 – 7.0 µl of METAFECTENE® PRO to 50 µl serum- and antibiotic-free medium or 1xPBS

4. Mix the solutions gently by carefully pipetting one time.

Ratios will require optimizing based on various factors (see sections 3 and 4).
The DNA or RNA lipid ratio has to be kept between 1:2 and 1:7
[µg DNA or RNA : µl MEATAFECTENE® PRO]!

Please note the order of addition in the following step: Add the DNA or RNA solution into the transfection reagent solution and not in reverse!

5. Combine the two solutions, **mix gently by pipetting up and down once** and incubate at room temperature for 15–20 min.

Shear stress may destroy the DNA or RNA lipid complex!

6. After incubation time add as soon as possible the DNA or RNA-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.[4]
7. Depending on cell type and promoter activity, collect cells by centrifugation and assay cells for gene activity 24–72 hours following the start of transfection.

4 If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3–6 hours and replace it with complete medium.

2.3 Transfection of siRNA – Protocol for initial optimization in a 24-well plate -

1. In a 24-well tissue culture plate, seed 0.1 – 1.0 x 10^5 cells per dish in 0.5 ml suitable fresh complete medium. For most cell types this range of cell amount will achieve the desired density of 30–50% visual confluency.[5]
2. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
3. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene) for each transfection. **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces:

Tube	R1	R2	R3	R4
Serum-free medium or 1xPBS	30 µl	30 µl	30 µl	30 µl
siRNA	0.1 µg (ca. 7.5 pmol)	0.2 µg (ca. 15 pmol)	0.5 µg (ca. 40 pmol)	2 µg (ca. 150 pmol)

Tube	M1	M2	M3	M4
Serum-free medium or 1xPBS	40 µl	40 µl	40 µl	40 µl
METAFECTENE® PRO	0.5 µl	1 µl	2.5 µl	10 µl

4. Mix the solutions gently by carefully pipetting one time.

Please note the order of addition in the following step: Add the siRNA solution into the transfection reagent solution and not in reverse!

5. Combine the solutions R1 + M1, R2 + M2, R3 + M3, R4 + M4, **mix gently by pipetting up and down once** and incubate at room temperature for 15–20 min.
6. After incubation time add as soon as possible the siRNA-lipid complexes dropwise to the cells and **swirl the wells with extreme care** to ensure distribution overall. Incubate at 37°C in a CO_2 incubator.
7. Depending on cell type, siRNA, stability of mRNA and the protein being targeted, assay for gene knockdown 24–72 hours following the start of transfection. Once the siRNA-lipid complex has been added to the cells, there is no need to replace with fresh medium.[6]

To obtain the highest efficiency and low non-specific effects, optimize transfection conditions by varying siRNA to lipid ratio.

5 Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

6 If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3–6 hours and replace it with medium. When using serum-free medium during siRNA transfection procedure, replace the complex-containing medium with serum-containing medium 3–6 hours after start of transfection.

2.4 Co-Transfection Experiments (siRNA-DNA)

METAFECTENE® PRO works well in co-transfection of short inhibitory RNA and plasmid DNA. Usually transfection of plasmids requires a higher cell density at the point of transfection compared to siRNA. Note following recommendations:

1. Plate cells are as those described for transfection of plasmid DNA.
2. Maintain the same *total* nucleic acid to lipid ratio as that used for siRNA alone. If you need to increase the total amount of nucleic acid, increase the amount of METAFECTENE® PRO in proportion to the total amount [µg] of nucleic acid.
3. Always use a volume of METAFECTENE® PRO [µl] that is at least double the total final mass of nucleic acid [µg].

3. Optimization

3.1 Critical optimization parameters

Ratio of nucleic acid to METAFECTENE® PRO

The most important optimization parameter is the ratio of nucleic acid to METAFECTENE® PRO. For successful transfection a slightly net positive charge of the nucleic acid–METAFECTENE® PRO–complex is required.

The optimal nucleic acid–METAFECTENE® PRO–ratio depends on the cell line.

Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the absolute amount of nucleic acid-lipid-complex may be required.

Optimal ratio of nucleic acid-METAFECTENE® PRO and concentration of nucleic acid-lipid-complex may vary with the number of cells. An excessive amount of the complex can lead to over expression and/or lysis of cells (lipids are also lysis reagents!). It is necessary to keep the number of seeded cells and incubation period constant until the transfection procedure for a reproducible optimization of these parameters.

Numbers of cells to seed

For details see section 5.

Effect of serum

As yet, nearly all cell lines transfected with METAFECTENE® PRO have shown superior results if transfection is performed in the presence of serum. Nevertheless, special cell lines may show different behaviour. Accordingly transfection can be performed without serum, under serum-reduced (e.g. 5% serum) or full-serum (e.g. 10% serum) condition.

Presence of serum during **complex formation** between METAFECTENE® PRO and nucleic acid is strictly forbidden!

Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of nucleic acid to METAFECTENE® PRO and concentration of nucleic acid-lipid-complex may vary with different serum concentrations.

Optimization of these critical parameters by following the optimization instructions (see chapter 3.3) should give satisfactory results.

3.2 Further optimization parameters

These parameters can be further optimized by a step-by-step procedure:

Incubation time with transfection complex

Cells can be exposed to the transfection complex within a variety of time ranges (i.e. 3–72 hours). Depending on the sensitivity of the transfected cell line, short or long exposure is possible.

Time range between transfection and evaluation

Assay for gene activity should be performed 24–72 hours after the start of transfection. The optimal time is dependent on cell type, promoter activity and expression product (e.g. toxicity).

Using PBS in DNA-lipid complex formation instead of serum-free medium

Numerous experiments showed that the use of 1x PBS in nucleic acid-lipid complex formation instead of serum- and antibiotic-free medium delivers transfection rates with improved reproducibility and in some cases higher transfection rates, particularly with lower volumes of lipids. (Preparation of PBS see chapter 6.1)

3.3 Optimization instructions

For optimizing purposes, use reporter gene plasmids such as pCMVßGal, pND2Lux, pEGFP etc.

> ⓘ **For siRNA applications see section 2.3.**

1. Follow the working instructions, varying the amount of METAFECTENE® PRO within the interval proposed in the table in section 4 (e.g. 12-well plate: 2 µl, 4 µl, 6 µl, 8 µl, 10 µl, 12 µl etc.). Keep the number of cells at the beginning of the transfection procedures and the DNA or RNA amounts constant at the recommended starting-points. The serum concentration during incubation with the DNA or RNA-lipid-complexes should be the same as the concentration with which the cells are cultured.
2. Follow the working instructions, varying the quantity of the DNA or RNA (e.g. 1 µg, 1.5 µg, 2 µg, 2.5 µg, 3 µg etc.) and keeping the proposed interval of METAFECTENE® PRO proportional (see step 1). Also, keep the number of cells at the beginning of the transfection procedures constant at the recommended starting-point. The serum concentration during incubation with the DNA or RNA-lipid-complexes should be the same as the concentration with which the cells are cultured. Determine the optimal DNA or RNA and lipid amounts.
3. Repeat steps 1 and 2 with serum-reduced and serum-free conditions.
4. Repeat steps 1 and 2 with other starting-points for the number of cells at the beginning of the transfection procedures.

Example for a 12-well format

1. In a 12-well tissue culture plate, seed 1.0 – 4.0 x 10^5 (starting point: 2.0 x 10^5) cells per dish in 1 ml of suitable fresh complete medium.[7]
2. Incubate the cells at 37°C in a CO_2 incubator until growing area is 90–100% covered. The time required will vary among cell types, but will usually take 18–24 hours.
3. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
4. Pipet 50 µl serum- and antibiotic-free medium or 1xPBS to each below stated well using a cell culture grade 96-well plate. Following add:

0.5 µg	DNA or RNA in A1 – A4
1.0 µg	DNA or RNA in B1 – B4
1.5 µg	DNA or RNA in C1 – C4

   and mix the solutions gently by carefully pipetting one time.
5. Pipet 50 µl serum- and antibiotic-free medium or 1xPBS to each below stated well using a cell culture grade 96-well plate. Following add:

1 µl, 2 µl, 4 µl, 6 µl	METAFECTENE® PRO in D1 – D4
2 µl, 4 µl, 8 µl, 12 µl	METAFECTENE® PRO in E1 – E4
4 µl, 8 µl, 12 µl, 16 µl	METAFECTENE® PRO in F1 – F4

   and mix the solutions gently by carefully pipetting one time.

Please note the order of addition in the following step: Add the DNA or RNA solution into the transfection reagent solution and not in reverse!

6. Combine the corresponding wells (A1 + D1, A2 + D2 etc., B1 + E1, B2 + E2 etc., C1 + F1, C2 + F2 etc.), **mix gently by pipetting up and down once** (shear stress can destroy the developing complex!) and incubate at room temperature for 15–20 min.
7. After incubation time add as soon as possible the DNA or RNA-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.[8]
8. Depending on cell type and promoter activity, assay cells for gene activity 24–72 h following the start of transfection.

If results are satisfactory, scale up or down to other desired vessel sizes. In this case see table on following section 4.

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7 Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

8 If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3–6 hours and replace it with complete medium.

4. Up- and Downscale

1. In the majority of cases, the optimum ratio range of nucleic acid [µg] to METAFECTENE® PRO [µl] is between 1:2 and 1:7. For siRNA applications use special siRNA optimization protocol.
2. Adapt the ratio of nucleic acid:lipid to your size of wells by the appropriate proportional factor.
3. Adsorption processes of the vessel material with the agents used necessitate optimization of the amount of lipoplex and the nucleic acid-lipid ratio for each change to a significantly different format.

Reagent quantities for different sizes of culture vessels

(Proposed starting points for optimization in brackets)

Culture plate	96-well plate	24-well plate	12-well plate	6-well plate	60mm	100mm
Growth areas [cm^2]	0.31	1.9	3.7	9	22	60
Proportional factor	0.03	0.2	0.4	1.0	2.5	6.7
Adherent cells to seed* (day before transfection) [$\times 10^5$]	0.10 – 0.60 (0.30)	0.4 – 2.0 (1.0)	1.0 – 4.0 (2.0)	2.5 – 10.0 (5.0)	6.0 – 24.0 (12.0)	15.0 – 60.0 (25.0)
Suspension cells to seed* (day of transfection) [$\times 10^5$]	0.04 – 0.24 (0.12)	0.16 – 0.8 (0.40)	0.4 – 1.6 (0.8)	1.0 – 4.0 (2.0)	2.4 – 9.6 (4.8)	6.0 – 24.0 (10.0)
Cell suspension volume [ml]	0.15	0.5	1.0	2.0	4.5	12.0
DNA or RNA amount [µg]	0.04 – 0.3 (0.1)	0.08 – 1.0 (0.5)	0.2 – 2.0 (1.0)	0.4 – 5.0 (2.0)	0.8 – 12.0 (6.0)	1.6 – 34.0 (14.0)
METAFECTENE® PRO amount [µl]	0.2 – 4.0 (0.6)	0.4 – 7.0 (2.0)	0.8 – 15.0 (3.0)	1.6 – 35.0 (6.0)	3.2 – 90.0 (18.0)	6.4 - 250 (42.0)
Dilution volume of DNA or RNA [µl]	15 - 30	30	50	100	300	700
Dilution volume of METAFECTENE® PRO [µl]	10 - 50	10 - 50	50	100	300	700
Total volume [ml]	0.175 – 0.23	0.54 – 0.58	1.1	2.2	5.1	13.4

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments. Usually transfection of plasmids requires a higher cell density at the point of transfection compared to siRNA.

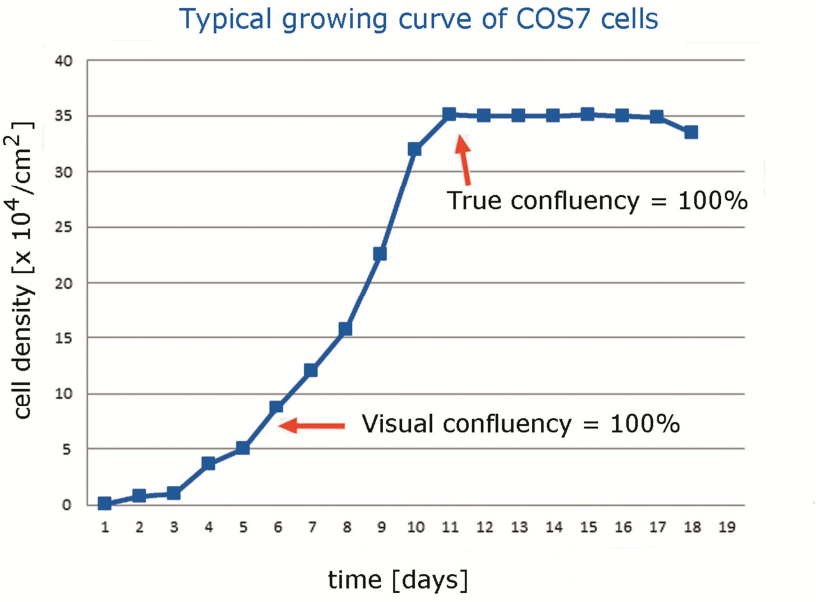
5. Troubleshooting

1. Avoid any contact of pure METAFECTENE® PRO and of pure nucleic acid solution with tube materials (e.g. 96-well plates).

   Conclusion: Serum- and antibiotics-free medium must be pipetted first.

2. Nucleic acid- and METAFECTENE® PRO-solutions diluted in medium should be combined within 5 min.

3. Confluency determined visually ("visual" confluency = percentage of growth surface covered with cells) is **not identical** with confluency determined by growing curve (= true confluency). Best results are obtained if transfection is performed at the highest possible proliferation state (= 30–60% true confluency). This often corresponds with visual confluency of 90–100%.



4. Decreased cell growth or toxicity is often associated with very high transfection activity (over expression). This effect can be avoided by transfection of cultures with higher confluency or with a lower amount of METAFECTENE® PRO–nucleic acid complex.

5. Do not add antibiotics to media during transfection as this may cause cell death and decrease transfection efficiency.

6. In case of very sensitive cells, remove the transfection mixture after 3–6 h and replace it with complete medium.

6. Miscellaneous

6.1 Buffer

10x PBS			
40 g	NaCl	1 g	KH_2PO_4
1 g	KCl	5.75 g	$Na_2HPO_4 \cdot 2\ H_2O$
The salts are weighed, mixed, topped up to 500 ml with water and dissolved, then autoclaved.			

1x PBS
100 ml 10x PBS is diluted 1:10 with water in a 1l volumetric flask and autoclaved.

6.2 Important Information

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

METAFECTENE® is a registered trademark of Biontex Laboratories GmbH.

6.3 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when stored and used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex Laboratories GmbH.

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