METAFECTENE® FluoR

The Transfection Reagent for visualizing Lipofection with DNA

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

Product	Order No.	Size
METAFECTENE® FluoR	T050–0.5	0.5 ml
METAFECTENE® FluoR	T050–1.0	1.0 ml

Shipping: At room temperature

Storage: 4°C

Stability: Best before: see label

Formulations of liposomes like METAFECTENE® FluoR 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® FluoR is a fluorescence-labeled reagent for transfection of eukaryotic cells with DNA. METAFECTENE® FluoR is rhodamine B labeled to enable the transfection process to be tracked and its success evaluated by means of fluorescence microscopy or FACS.

The protocol is designed for optimum cell microscopy, combining cell density suitable for observing cell morphology with a highly efficient transfection process.

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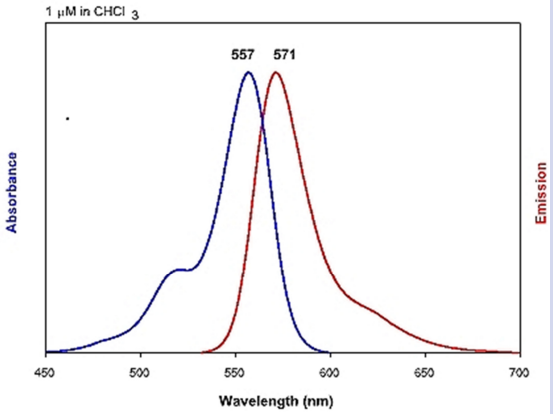
1. General Information

1.1 Specifications

Application	Transfection of eukaryotic cells with DNA; visual tracking of lipoplex during and after transfection process
Formulation	Cationic lipids with colipids in water; covalently bonded rhodamine B label (Ex_{max} = 557 nm, Em_{max} = 571 nm)
Sterility	tested
Assays	1 ml reagent up to 2500 (24–well) or 625 (6–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® FluoR 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® FluoR.

Cell conditions

Cells to be transfected should be well proliferating and healthy. Transfection of cells which have been at full confluency for a while (before seeding) is significantly less efficient than that of cells which are growing rapidly. Therefore it is recommended to use only regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results and have to be excluded.

Quality of genetic material

To achieve optimum transfection results the DNA used should be of the maximum possible purity. Endotoxins and other contaminants can drastically reduce transfection efficiency.

2. Transfection Protocol

2.1 Remarks concerning the protocol

In transfection of eukaryotic cells, the cell nucleus is the intended site of action of the DNA (e.g. of a plasmid). Among many impeding factors, the two principal barriers are the cell membrane and nuclear membrane. The reagent enables the DNA to pass the cell membrane by combining with the DNA to form a complex (lipoplex) which is then actively introduced into the cytoplasm by means of endocytosis. Division of the transfected cells is critical to this process because the lipoplex is not in itself able to pass the nuclear membrane; the DNA can only pass into the cell nucleus during cell division when the nuclear membrane opens.

This means that maximum transfection efficiency rises as the cell division rate increases during treatment of the cells with the lipoplex. The cell division rate of adherent cells is primarily determined by their cell density (cells/cm²). The higher the cell density, the higher the proliferation rate and the higher the maximum transfection efficiency.

However, microscopy often calls for cell densities far lower than those achieved in optimum proliferation of adherent cells, because lower cell densities enable the morphology of individual cells to be observed more closely.

A transfection protocol for low cell densities can therefore only ever aim for a compromise between the parameters of optimum microscopy analysis and transfection efficiency.

Development of the protocol for METAFECTENE® FluoR focused on excellent microscopy analysis. In this protocol, the maximum achievable transfection efficiency is constrained by the relatively low cell proliferation rate, given the need for low cell density.

For this reason, cell density should be optimized for the cell type and the requirements of the assay in question to achieve successful transfection results. More details are given in Section 2.4 "Protocol Optimization".

2.2 Standard Protocol

The following instructions refer to one well with 1 cm² proliferation area (one well of a 48–well plate). For other well sizes see the chart in Section 2.3.

This protocol gives recommended initial values for cell concentration, plasmid amount and DNA:lipid ratio [µg:µl]. All parameters should be optimized for each cell line or cell type as described in section 2.4 "Protocol Optimization.

Preparing Cells

On the **1st day** of the assay prepare 250 µl of cell suspension with a concentration of 0.5-1.0 x 10^5 cells/ml in complete culture medium and add the suspension to one well of a 48-well plate.

Incubate the suspension for 24 hours under standard culture conditions for the cells used (e.g. 37°C in atmosphere containing CO_2).

Preparing and Adding the Lipoplex

Preparing the solutions

On the **2nd day,** bring the METAFECTENE® FluoR and the DNA solution to room temperature and vortex briefly prior to lipoplex formation.

Prepare the following solutions in sterile vessels appropriate for cell culture (tubes, reaction vessels, well plates; preferably of polypropylene). **Always introduce the medium first** to prevent the reagent and DNA solutions from coming into direct contact with the vessel surface:

DNA solution: 0.05 – 0.2 µg DNA (initial value: 0.1 µg) to 15 µl serum– and antibiotic–free medium

Lipid solution: 0.2 – 1.3 µl METAFECTENE® FluoR (initial value: 0.5 µl) to 15 µl serum– and antibiotic–free medium

The volumes of DNA and lipid given are sufficient for a DNA:lipid ratio [µg/µl] of 1:4 – 1:6,5. The recommended initial value is 1:5 µg:µl.

Lipoplex formation

Now add the DNA solution to the lipid solution and mix gently by pipetting up and down once.

Please note the sequence of adding the solutions:
The DNA solution is added to the METAFECTENE® FluoR solution!

Incubate the solution for 15–20 min at room temperature.

Adding the lipoplex

Now add 30 µl of lipoplex solution to the incubated cells in the culture medium and agitate gently to mix.

High shearing forces can damage the DNA–lipid complex (lipoplex)!

Then incubate under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Changing medium 5 h after adding the lipoplex

Replace the medium with fresh complete culture medium 5 h after adding the lipoplex.

Evaluation

The rhodamine label enables the transfection process to be visualized and lipoplex localization and uptake to be tracked. In addition, METAFECTENE® FluoR can be used in combination with a suitably fluorescence-labelled DNA to deliver information on the localization of the genetic material introduced, the genetic product and the lipoplex.

The time of evaluation should be selected with respect to the object of evaluation. As a basic principle, microscopic observation (e.g. live imaging) is possible from the point where the lipoplex is added; however, care should be taken to replace the medium – as stated in the protocol – 5 hours after lipoplex addition and not before, otherwise significantly reduced transfection rates will result.

Expressed protein amounts frequently peak after 48 hours. The optimum time is determined by the characteristics of the cell type and expression product and by promotor activity. To optimize image quality for fluorescence or transmitted–light microscopy, the medium can be replaced by 1× PBS in the final evaluation.

2.3 Transfer to other formats

Format	Growth area [cm²]	Cell suspension [µl]	Medium f. lipoplex formation [µl]	METAFECTENE® FluoR [µl]	DNA amount [µg]	Lipoplex volume [µl]
96–well	0.3	100	2× 10	0.1 – 0.65 (0.25)	0.025–0.1 (0.05)	20
48–well	1.0	250	2× 15	0.2 – 1.3 (0.5)	0.05 – 0.2 (0.1)	30
24–well	1.9	500	2× 30	0.4 – 2.6 (1.0)	0.1 – 0.4 (0.2)	60
12–well	3.6	1000	2× 50	0.8 – 5.2 (2.1)	0.2 – 0.8 (0.4)	100
6–well	9.0	2000	2× 100	1.6 – 10.4 (4.2)	0.4 – 1.6 (0.8)	200
60 mm dish	22	5000	2× 250	3.9 – 26.0 (10.4)	1.0 – 4.0 (2.0)	500
100 mm dish	60	15000	2× 750	10 – 65 (26)	2.5 – 10 (5.0)	1500

The chart gives the recommended initial reagent volume and DNA amount for each format in brackets.

2.4 Protocol Optimization

Optimum cell density

The potential level of transfection efficiency is strongly affected by cell density at the time of adding the lipoplex (see Section 2.1). For this reason, the first parameter to be optimized should be the cell concentration of the cell suspension seeded on the first day.

The aim is to achieve the best possible compromise between observability (low cell density is an advantage) and transfection rate (high cell density is an advantage). The prepared cell concentration should be as high as possible while still allowing evaluation to be carried out at the desired time.

In many cell types, preparation of a cell suspension at a concentration of 0.5–1.0 x 10^5 cells/ml leads to full confluency after 2–3 days. This recommended initial concentration should be adjusted depending on the requirements of the experiment.

Optimization protocol

In the following optimization protocol cells are transfected in nine vessels (wells or dishes) with three DNA amounts (I, II and III) and three different DNA:lipid ratios (1:4 / 1:5 / 1:6.5 µg:µl). To achieve this, three different lipoplex solutions are prepared at the given DNA:lipid ratios and three wells are filled with three different volumes of solution.

The following chart shows the nine resulting lipoplex compositions, taking optimization for a 48–well format as an example:

	DNA:Lipid Ratio in µg:µl ➡		
DNA amount ⬇	1:4, 0.05 µg	1:5, 0.05 µg	1:6.5, 0.05 µg
	1:4, 0.1 µg	1:5, 0.1 µg	1:6.5, 0.1 µg
	1:4, 0.2 µg	1:5, 0.2 µg	1:6.5, 0.2 µg

The optimum parameters for DNA amount and DNA:lipid ratio apply only to the specific combination of the selected cell type and cell density.

Seeding the cells

On the first day of the assay, prepare a cell suspension using the concentration determined for the cell type in question and distribute among nine culture vessels. The volume of cell suspension is given in the chart in section 2.3.

Incubate the suspension for 24 hours under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Preparing the solutions

Label four reaction vessels (ideally of PP) "DNA solution", "Lipid 1:4", "Lipid 1:5" and "Lipid 1:6.5".

Then place the DNA solution and the three lipid solutions in the corresponding vessels. The following chart shows the volumes of medium and reagent and the DNA amounts for each culture format.

The following example applies to wells of a 48–well plate each with 1 cm² growth area:

DNA solution: 90 µl serum**–/**antibiotic–free medium and 1.2 µg DNA

Lipid solutions:

1:4 30 µl serum**–** and antibiotic–free medium and 4.8 µl METAFECTENE® FluoR

1:5 30 µl serum**–** and antibiotic–free medium and 6.0 µl METAFECTENE® FluoR

1:6.5 30 µl serum**–** and antibiotic–free medium and 7.8 µl METAFECTENE® FluoR

Format	Growth area [cm²]	Medium for DNA solution [µl]	DNA amount [µg]	Medium for lipid solutions [µl]	Lipid volume [µl]		
					1:4	1:5	1:6,5
96–well	0.3	60	0.6	3× 20	2.4	3.0	3.9
48–well	1.0	90	1.2	3× 30	4.8	6.0	7.8
24–well	1.9	180	2.4	3× 60	9.6	12.0	15.6
12–welll	3.6	300	4.8	3× 100	19.2	24.0	31.2
6–well	9.0	600	9.6	3× 200	38.4	48.0	62.4
60 mm dish	22	1500	24	3× 500	96	120	156
100 mm dish	60	4500	60	3× 1500	240	300	390

Lipoplex formation

Add 30 µl of DNA solution to each of the three lipid solutions, 1:4, 1:5 and 1:6.5 and mix gently by pipetting up and down once.

Please note the sequence of adding the solutions:
The DNA solution is added to the METAFECTENE® FluoR solution!

Incubate the solution for 15-20 min at room temperature.

Adding the lipoplex

Now pipette the lipoplex solution into the nine culture vessels of proliferating cells. Add three different volumes of each of the three lipoplex solutions 1:4, 1:5 und 1:6.5, as follows:

	Lipoplex volume [µl]		
Format	V_1	V_2	V_3
96–well	5	10	20
48–well	7.5	15	30
24–well	15	30	60
12–well	25	50	100
6–well	50	100	200
60 mm dish	125	250	500
100 mm dish	375	750	1500

Then incubate under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

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 or animal therapeutic or diagnostic purposes.

METAFECTENE® is a registered trademark of Biontex Laboratories GmbH.

3.2 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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