METAFECTENE® EASY+

The fast, easy and effective Transfection Reagent for Mammalian Cells

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

Product	Order No.	Size
METAFECTENE® EASY+	T090–1.0	EASY+ Transfection Reagent 1x1.0 ml 10 x EASY+ buffer 1x2.0 ml
METAFECTENE® EASY+	T090–2.0	EASY+ Transfection Reagent 2x1.0 ml 10 x EASY+ buffer 2x2.0 ml
METAFECTENE® EASY+	T090–5.0	EASY+ Transfection Reagent 5x1.0 ml 10 x EASY+ buffer 5x2.0 ml

Shipping: At room temperature

Storage:

EASY+ Reagent	4°C
10 x EASY+ buffer	4°C (**do not freeze**)

Stability: Best before: see label.

Formulations of liposomes like the METAFECTENE® EASY+ 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.

Description

METAFECTENE® EASY+ combines a simple and fast protocol with outstanding transfection efficiency and low toxicity. Therefore transfections can now be performed efficiently using a fixed DNA–lipid ratio, rendering time–consuming optimization obsolete. Because the new protocol of METAFECTENE® EASY+ takes only three days to complete, it also enables two consecutive tests to be performed within one week. METAFECTENE® EASY+ shows no serum inhibition and is the reagent of choice for sensitive cells.

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1. General Information

1.1 Specifications

Application	Transfection of mammalian cells with DNA
Formulation	Cationic lipids with colipids in water
Sterility	tested
Assays	1 ml: up to 750 (24–well); up to 160 (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

Fast, Easy, Efficient – the perfect description of this transfection reagent from Biontex. In intensive in–depth research and development, we succeeded in achieving outstanding transfection results with a fixed DNA–lipid ratio. This elimination of time–consuming optimization processes, combined with a new simple protocol, results in significantly shorter assay times. The transfection efficiency of METAFECTENE® EASY$^+$ achieves outstanding results for a wide range of cells, including sensitive strains.

Storage

METAFECTENE® EASY$^+$ 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 all 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® EASY$^+$ Transfection Reagent.

Cell conditions

Cells to be transfected should be well proliferating and healthy. Cells which have been at full 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 and must 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. Working Instructions

2.1 Remarks concerning the protocol

Tolerance to transfection reagents may vary, sometimes significantly, depending on cell types or cell lines. In the following protocol, cells are transfected in two wells using two different volumes of DNA–lipid complex (lipoplex).

The evaluation of this assay serves as the basis for deciding which of the two lipoplex volumes is ideal for the transfected cells. This volume must be determined empirically for each cell type.

If the lipoplex volume suitable for the cells in question is already known, only this volume need be used. The pipetting charts are given in section 2.8.

The following instructions apply to wells with 1 cm² growth surface (equivalent to a single well of a 48–well plate). A conversion chart for other well formats is given in section 2.7.

2.2 Preparing the reagents

First make up 1× EASY$^+$ buffer from the 10× EASY$^+$ buffer supplied. To do this, mix 1 part 10× EASY$^+$ buffer with 9 parts of sterile water suitable for cell culture under sterile conditions.

Prior to transfection, bring 1x EASY$^+$ buffer and METAFECTENE® EASY$^+$ Transfection Reagent to room temperature and vortex shortly. Bring the DNA solution to room temperature and mix gently.

2.3 Preparing the cells

Prepare 500 µl of cell suspension at a concentration of 4 – 8 x 10^5 cells/ml in complete culture medium.

Fill two wells of an 48-well plate (*Well 1* and *Well 2*) with 250 µl of cell suspension each.

2.4 Preparing the lipoplexes

Place 75 µl 1× EASY$^+$ buffer in a test tube, ideally polypropylene (PP).

Pipette 2.0 µl METAFECTENE® EASY$^+$ Transfection Reagent into the 1× EASY$^+$ buffer and mix the solution by gently pipetting up and down once.

Add 2.0 µg DNA by pipetting and mix as before.

Mix gently!
Shearing forces damage the lipoplex and reduce transfection efficiency.

Then incubate for 15 min at RT.

2.5 Transfection

Now add the lipoplex solution to the wells of cell suspension as follows:

25 µl of lipoplex in *Well 1* and 50 µl in *Well 2*.

Mix the solutions in both wells by gently pipetting up and down once.

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

2.6 Evaluation

Evaluation generally takes place 24 - 72 h later. The best results / highest protein production are generally obtained after 48 h. The optimum time is determined on the basis of the properties of the cells expression product and promoter activity.

2.7 Transfer to other well formats

Format	Area	Cell suspension	1× EASY$^+$ buffer	M. EASY$^+$ Transf. Reagent	DNA	Lipoplex volume	
						Well 1	*Well 2*
96–well	0.3 cm²	2× 100 µl	30 µl	0.6 µl	0.6 µg	10 µl	20 µl
48–well	1.0 cm²	2× 250 µl	75 µl	2.0 µl	2.0 µg	25 µl	50 µl
24–well	1.9 cm²	2× 500 µl	150 µl	3.8 µl	3.8 µg	50 µl	100 µl
12–well	3.6 cm²	2× 900 µl	300 µl	7.2 µl	7.2 µg	100 µl	200 µl
6–well	9.0 cm²	2× 2.2 ml	600 µl	18 µl	18 µg	200 µl	400 µl
60 mm dish	22 cm²	2× 5.5 ml	1.5 ml	44 µl	44 µg	500 µl	1.0 ml
100 mm dish	60 cm²	2× 15 ml	4.5 ml	120 µl	120 µg	1.5 ml	3.0 ml

2.8 Values for known suitable lipoplex volumes

	Lipoplex volume 1			Lipoplex volume 2		
Format	**1× EASY$^+$ buffer**	**M. EASY$^+$ Transf. Reagent**	**DNA**	**1× EASY$^+$ buffer**	**M. EASY$^+$ Transf. Reagent**	**DNA**
96–well	10 µl	0.2 µl	0.2 µg	20 µl	0.4 µl	0.4 µg
48–well	25 µl	0.7 µl	0.7 µg	50 µl	1.3 µl	1.3 µg
24–well	50 µl	1.3 µl	1.3 µg	100 µl	2.5 µl	2.5 µg
12–well	100 µl	2.5 µl	2.5 µg	200 µl	5.0 µl	5.0 µg
6–well	200 µl	6 µl	6 µg	400 µl	12 µl	12 µg
60 mm dish	500 µl	15 µl	15 µg	1.0 ml	29 µl	29 µg
100 mm dish	1.5 ml	42 µl	42 µg	3.0 ml	78 µl	78 µg

2.9 Additional Notes

The culture medium should be replaced when exhausted, particularly when rapidly growing cells are used.

When highly sensitive cells are used, replacement of the medium approx. 6–8 h after seeding may be useful.

If lower cell densities are desired, the volume of cell suspension can be reduced by up to 30%. When growth is under way (6–8 h) culture medium should be added.

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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