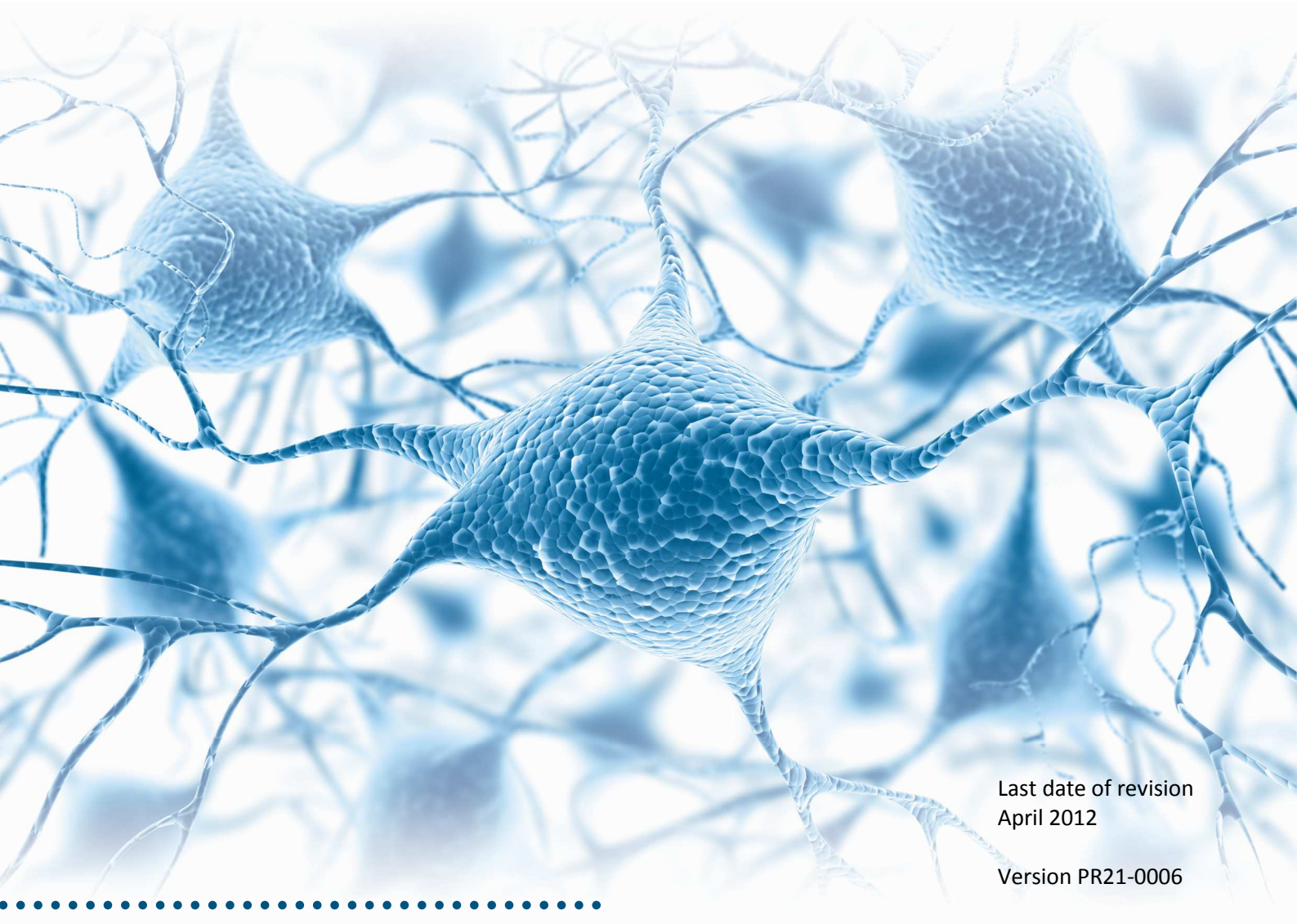
iba
Solutions
For Life Sciences

Cell Transfection with IBAfect

A comprehensive manual



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1 Introduction

1.1 General considerations

IBAfect is a polycationic transfection reagent based on liposome technology. The specifically designed molecular structure of the cationic lipid ensures easy entry of DNA/RNA into cells by condensing DNA/RNA to compact structures (DNA/RNA/lipid-complex) and endosome buffering. IBAfect 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. When results with IBAfect as a lipofection reagent alone are not satisfactory it is recommended to combine it with IBA's MA Lipofection Enhancer (see instructions in the manual for MATra (magnet assisted transfection)).

Starting point methods

1.2 General considerations

Ratio and amount of nucleic acid (plasmid, siRNA or oligonucleotides) and IBAfect

Both nucleic acid amount and IBAfect to nucleic acid ratio can be optimized to obtain the best results. So far, the optimal ratio of (µl) IBAfect to (µg) nucleic acid has been in the range of 2 to 7. As a starting point, we recommend to use the amounts of nucleic acid indicated below and 5 µl IBAfect per µg nucleic.

Assay format*	Surface*	Surface Factor*	Nucleic acid (plasmid or siRNA) amount dissolved in serum free and supplement free medium (e.g. DMEM).	IBAfect solution in serum and supplement free medium to be combined with nucleic acid solution for complex formation	The complex is added to and mixed with y ml medium supernatant of the cells (may contain serum)
96 well plate	0.32 cm^2	1	0.08 µg in 10 µl medium	0.4 µl in 10 µl medium	0.15 ml
48 well plate	0.95 cm^2	3	0.2 µg in 12.5 µl medium	1 µl in 12.5 µl medium	0.25 ml
24 well plate	1.9 cm^2	6	0.5 µg in 25 µl medium	2.5 µl in 25 µl medium	0.5 ml
12 well plate	3.8 cm^2	12	1 µg in 50 µl medium	5 µl in 50 µl medium	1 ml
6 well plate	9.5 cm^2	30	2.4 µg in 100 µl medium	12 µl in 100 µl medium	2 ml
60 mm dish	21 cm^2	66	5.3 µg in 200 µl medium	26.5 µl in 200 µl medium	4 ml
100 mm dish	55 cm^2	172	13.8 µg in 500 µl medium	69 µl in 500 µl medium	10 ml
T-75 flask	75 cm^2	235	18.8 µg in 750 µl medium	94 µl in 750 µl medium	15 ml

* The basis for the above mentioned suggestions were cell culture materials from Corning/Costar. Surfaces may differ for materials from other manufacturers. If other cell culture materials are used, nucleic acid and MATra A amount should be adapted according to the difference in surface. Adsorption processes with the vessel material cause necessity of optimization of complex amount and DNA/RNA:lipid ratio for each change to a considerably different format.

Cell preparation
The DNA transfection during the exponential growing phase of the cells is essential for optimal results, because of the very important role of cell division regarding the transport of the DNA/RNA into the nucleus. Generally, good results are obtained with growing, healthy cells having reached 30-60% of their maximum density.

Antibiotics
Antibiotics should be avoided where indicated although until now no problems arose when complex formation and transfection were performed in the presence of antibiotics.

Optimization
Generally, only little optimization is required if the recommended starting point protocols are used. The instructions represent typical protocols that were applied successfully with a large variety of cells. However, as transfection is a procedure depending on many parameters we recommend optimization of the transfection protocol for each combination of plasmid and cell line used, if optimal results are desired. Please consult Chapter 3 to get information on critical parameters and general tips and tricks.

1.3 Protocols

Please consider the general remarks under 2.1 prior to starting with the protocol.

Protocol for adherent cells, example protocol for 12 well plate

1. Incubate the cells at 37°C in a CO_2 incubator until they are at 30-60% maximum density. For a cell type that continues to grow after having reached an apparently confluent monolayer (e.g. HEK293) it is recommended to determine the maximal density it finally reaches. For all other cell types (e.g. COS-7), this value reflects 30-60% confluent cell layer.

2. The solutions of DNA/RNA and IBAfect should have an ambient temperature and should be gently vortexed prior to use.

3. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of glass, polypropylene or polystyrene:
   Solution A: 1.2 µg of DNA/RNA in 50 µl medium free of serum and antibiotics;
   Solution B: 3.6 µl of IBAfect in 50 µl medium free of serum and antibiotics.
   Each solution is prepared by gentle pipetting up and down.

4. Combine the two solutions, mix gently by carefully pipetting several times (do not vortex or centrifuge!), and incubate at room temperature for 15-20 min to form the DNA/RNA:lipid complex.

5. Add the solution containing the DNA/RNA:lipid complexes to the wells with the cells and mix gently. (If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3-6 hours and replace it with medium). Incubate at 37°C in a CO_2 incubator.

6. Continue to culture cells as desired until evaluation of transfection efficiency.

Protocol for suspension cells

1. In a 12 well tissue culture plate seed 0.6 x 10^6 cells in 2 ml fresh suitable complete medium.

2. The solutions of DNA/RNA and IBAfect transfection reagent should have ambient temperature and should be gently vortexed prior to use.

3. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of glass, polypropylene or polystyrene for each transfection:
   Solution A: 1.2 μg of DNA/RNA in 50 μl medium free of serum and antibiotics;
   Solution B: 3.6 μl of IBAfect in 50 μl medium free of serum and antibiotics.
   Each solution is prepared by gentle pipetting up and down.

4. Combine the two solutions, mix gently by carefully pipetting several times (do not vortex or centrifuge!), and incubate at room temperature for 15-20 min to form the DNA/RNA-lipid complex.

5. Add the DNA/RNA-lipid complexes to the cell suspension and mix gently. (If toxicity is a problem by use of very sensitive cells, remove the transfection mixture after 3-6 hours and replace it with medium). Incubate at 37°C in a CO_2 incubator.

6. Continue to culture cells as desired until evaluation of transfection efficiency.

Optimization

1.4 Important parameters

IBAfect:nucleic acid ratio

The most important optimization parameter is the ratio of IBAfect to DNA/RNA.
For successful transfection a slightly net positive charge of the IBAfect:DNA/RNA-complex is required. Every cell line has an optimal DNA/RNA:lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ratio and absolute amounts of reagents (probably the different adsorption of the tube`s material based on different surface dimensions is responsible for this fact). The transfer of a protocol used for other transfection reagents to IBAfect should never be attempted as every transfection reagent possesses its own molecular structure with specific physical properties, which have an important influence on DNA/RNA:lipid ratios.

Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the absolute amount of DNA/RNA-lipid-complex may be required.
Note: Optimal ratio of IBAfect:DNA/RNA and concentration of DNA/RNA-lipid-complex may vary with the number of cells. An amount of the complex that is too high can lead to overexpression or/and lysis of cells (lipids are also lysis reagents!). Therefore, it is necessary for a reproducible optimization of these parameters to keep the number of seeded cells and incubation period constant until the transfection procedure.

Effect of serum

A further important parameter is the serum. Up to now, nearly all cell lines transfected with IBAfect showed superior results if transfection is performed in the presence of serum. Nevertheless, special cell lines may exhibit another behavior. Adaptation is possible and transfection can be performed without serum, under serum reduced (e.g. 5%, use of OptiMEM from Invitrogen) or full serum (e.g. 10%) conditions. Presence of serum during complex formation between IBAfect and DNA/RNA should be strictly avoided as serum may inhibit complex formation. Once the complex is formed, the contact with serum is possible.
Note: Optimal ratio of IBAfect:DNA/RNA and concentration of DNA/RNA-lipid-complex may vary with different serum concentrations.

Cells

Cells to be transfected should be well proliferating and healthy. Cells which have been sitting 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. Cells should also be regularly checked for contaminations. Microbial contaminations for example, with mycoplasma or fungi, can drastically alter transfection results. The DNA transfection during the exponential growing phase of the cells is essential for optimal results, because of the very important role of cell division regarding the transport of the DNA/RNA into the nucleus. Regularly, good results are obtained with 30-60% of the maximum density. However, the optimal confluency has to be adapted to the used cell line.

Nucleic acid to be transfected

DNA/RNA should be of highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Before its use in complex formation, DNA/RNA should not be stored longer than 5min diluted in medium. Adsorption of DNA/RNA in container materials can result in decrease of transfection efficiency.

1.5 Further optimization parameters

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

Addition of the transfection complex to fresh seeded cells

Addition of the transfection complex to adherent cells immediately (within 1 hour) after their seeding into adequate culture plates can result in a considerable increase of transfection efficiencies. It is not necessary to change the incubation time.
With this procedure the time of a transfection experiment can be shortened by 24 hours!

Incubation time with transfection complex

Cells can be exposed to the transfection complex in a large time range between 3 and 72 hours.

Duration between transfection and evaluation

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

1.6 Tips and tricks

1. Important: Avoid the contact of pure IBAfect and of pure DNA/RNA solutions with tube materials (e.g. 96 well plates) for longer than one minute.
Conclusion: Add as soon as possible serum- and antibiotics free medium to prepare the solutions!

2. DNA/RNA- and IBAfect solutions diluted in medium should be combined within 5min.

3. Decreased cell growth or toxicity is often associated with very high transfection activity (overexpression). This effect can be avoided by transfection of cultures with higher confluency or with a lower amount of the IBAfect/DNAorRNA complexes.

4. Do not add antibiotics to media during transfection as this may decrease transfection efficiency.

Related Products

Cat. no.	Description	Amount
7-2001-020	MATra-A Reagent	for 200 µg nucleic acid
7-2001-100	MATra-A Reagent	for 1000 µg nucleic acid
7-2002-020	MATra-S Immobilizer	up to 7 Mio. cells
7-2002-100	MATra-S Immobilizer	up to 35 Mio. cells
7-2003-020	MA Lipofection Enhancer	for 200 µg nucleic acid
7-2003-100	MA Lipofection Enhancer	for 1000 µg nucleic acid
7-2004-000	96 Magnet Plate	1 plate
7-2005-020	IBAfect	for 30-100 µg nucleic acid
7-2005-050	IBAfect	for 75-250 µg nucleic acid
7-2005-100	IBAfect	for 150-500 µg nucleic acid
7-2005-500	IBAfect	for 750-2500 µg nucleic acid
7-2006-000	24 Magnet Plate	1 plate
7-2011-000	Universal Magnet Plate, 8 x 13 cm	1 plate
7-2012-000	Universal Magnet Plate, 26 x 26 cm	1 plate

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