IBA BioTAGnology

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A comprehensive manual

MATra
Magnet Assisted Transfection

Last date of revision
April 2011

Version 0015

Now **laminated short protocol** available on request at info@iba-go.com

www.magnet-assisted-transfection.com

Contents

1 Introduction

1.1 General considerations

Magnet Assisted Transfection (MATra) is a new, easy-to-handle and highly efficient method to transfect cells in culture. Using this new technique nucleic acids, such as plasmid DNA, oligonucleotides or siRNA, are in a first step associated with magnetic particles. By applying magnetic force the full nucleic acid dose is then rapidly drawn towards and delivered into the target cells leading to efficient transfection. For MATra, cells must be adherent to the bottom of the culture vessels which requires pretreatment for suspension cells.

Three different procedures according to different initial situations can be followed to apply MATra:

1.1.1 MATra for adherent cells

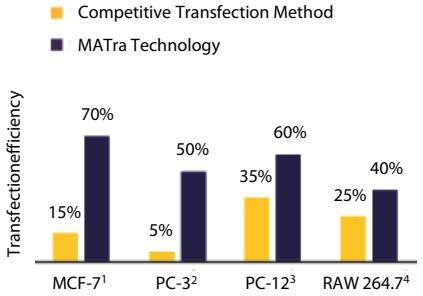
Magnet Assisted Transfection for adherent cells
The nucleic acid has to be combined with MATra-A (plasmid DNA) or MATra-si (siRNA) Reagent and MATra can be performed directly (see 3.2).

1.1.2 MATra for suspension cells

Magnet Assisted Transfection for suspension cells
Suspension cells have to be made adherent first by incubating them with the magnetic reagent MATra-S Immobilizer or by using polylysine plates. Then MATra-A or MATra-si Reagent loaded with the nucleic acid can be applied and MATra can be performed (see 3.2).

1.1.3 MA Lipofection for adherent and suspension cells

Magnet Assisted Lipofection: MATra in combination with lipofection reagents
Transfection with common lipid based (e.g. IBAfect, Lipofectamine, Fugene, Geneporter) or polycationic reagents (e.g. ExGen500 or Superfect) can be enhanced by magnetic assistance. In this case, the nucleic acid is combined with MA Lipofection Enhancer in the presence of the common lipofection reagent (see 3.3). In addition, the MA Lipofection Enhancer can be used for viral transfection.



For indicated cells the following methods were tested. 1-3: Lipofection vs Magnet Assisted Lipofection, 4: Lipofection vs Magnet Assisted Transfection. Data kindly provided by industrial IBA customer.

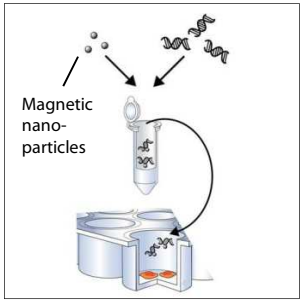
1 Introduction

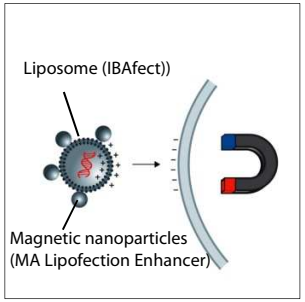
1.2 Methods using MATra reagents

Suspension cells are made adherent with **MATra-S Immobilizer**

Adherent cells

Magnet Assisted Transfection	Magnet Assisted Lipofection
MATra-A Reagent or MATra-si Reagent	MA-Lipofection Enhancer & IBAfect



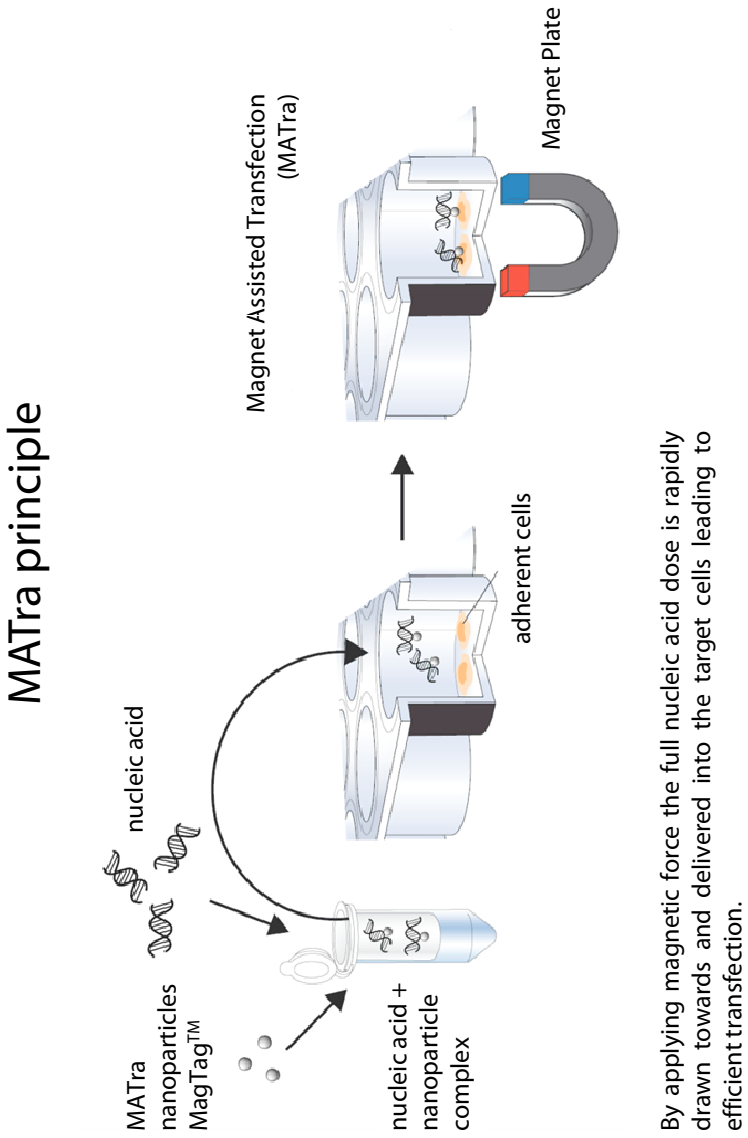


1.3 Suitable type of nucleic acid/ virus

	MATra-A	MATra-si	MA Lipofection Enhancer & IBAfect	MA Lipofection Enhancer only
Plasmid DNA	+	(+)	+	
Antisense Oligo (ODN)	+	(+)	+	
siRNA/miRNA	(+)	+	+	
Adenovirus				+
Retrovirus				+

1 Introduction, continued

1.4 MATra principle



2 Necessary materials and reagents for MATra*

2.1 For adherent cells

- Nucleic acid in **serum-free** and supplement-free medium (e.g. DMEM)
- MATra-A or MATra-si Reagent (MATra-si is a formulation that has been optimized for transfection of siRNA for gene silencing)
- Magnet Plate:
  - Universal Magnet Plate, 8 x 13 cm (for 1 plate or flask up to 75 cm^2) or
  - Universal Magnet Plate, 26 x 26 cm (for 500 cm^2 plates, larger flasks or up to 6 plates at the same time)
  - For quantitative experiments in 96well plates we are offering a special 96 Magnet Bar Plate

2.2 For suspension cells

- Nucleic acid in **serum-free** and supplement-free medium (e.g. DMEM)
- MATra-A or MATra-si Reagent
- MATra-S Immobilizer (can be optionally replaced by the use of polylysine plates)
- Magnet Plate (see above)

2.3 For enhancing lipofection

- Nucleic acid and lipofection reagent like IBAfect or others (e.g. Lipofectamine, Fugene, GenePorter, ExGen500, Superfect)
- MA Lipofection Enhancer
- Magnet Plate (see above)

2.4 For viral transfection

- MA Lipofection Enhancer
- Magnet Plate (see above)

*For order information see page 22

- Do not store MATra Reagents close to magnet! Minimal distance should be 10 cm.
- Do not freeze MATra Reagents!

3 Methods

3.1 General considerations

The instructions given below represent typical protocols that were applied successfully with a large variety of cell types. Optimal conditions may vary slightly from cell type to cell type. Therefore, the amount of nucleic acid and the ratios of the individual components may have to be adjusted to achieve optimal results. Thus, an optimum may be found between toxicity and transfection efficiency. If toxicity is high, reduce the amount of nucleic acid and possibly also the duration of MATra.
In general, for MATra approximately 5 x less DNA compared to lipofection is necessary. Nucleic acids have to be prepared in **serum-free** and supplement-free medium or in physiological saline, because serum may interfere with the transfection complex assembly. Once the transfection complex is formed it can be applied to cells in the presence of serum.

3.1.1 Cell preparation

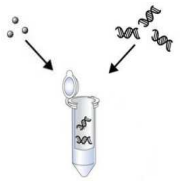
adherent cells

Adherent cells are seeded such that they reach 30-60% maximum density at the time of MATra. 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), confluency reflects maximum density. Cells need to undergo another round of cell division after transfection in order to get plasmid DNA in contact with the transcription machinery in the nucleus but excessive rounds of division lead to adverse dilution of the transfected DNA. Recommended seeding densities depend a) on the cell line, b) on the time available before transfection (i.e. 2 x 10^5 HEK293 cells/cm^2 or 4 x 10^4 CHO-K1 cells/cm^2, respectively, work well when transfections are to be carried out the following day), and c) on the time necessary for evaluation after transfection (e.g. for RNA and protein knockdown analyses we recommend 48 and 72 hours, respectively).
Suspension cell lines are suitably seeded by

a) using MATra-S Immobilizer immediately before MATra (1.5 – 6 x 10^5 cells/cm^2), or
b) culturing them on polylysine-coated plates prior to MATra (at densities as for adherent cells), or
c) briefly centrifuging the cells (2 minutes) to pellet them and use the protocol for adherent cells

In the 96well format the medium is replaced by 150 µl fresh medium immediately prior to transfection (optionally without serum, depending on the cell type).

3.1.2 Nucleic acid preparation



The starting ratio between reagent (µl, MATra-A) and nucleic acid (µg, plasmid DNA) should be 1:1 in each setting. Usually, a nucleic acid amount of 300 ng per cm^2 culture dish yields good results but optimization is worthwhile if the given setting (cell type, culturing conditions, etc.) is intended to be used as a basis for further investigations.

Note: For transfections of siRNA we recommend a starting ratio between reagent (µl, MATra-si) and µg siRNA (1 µg of an average 21mer siRNA double strand corresponds to approximately 77 pmol) of 1:0.2.

Assay format*	Surface*	Surface Factor*	Nucleic acid (plasmid DNA) amount dissolved in **serum-free** and supplement-free medium (e.g. DMEM).	Nucleic acid solution is added to x µl MATra-A Reagent for complex formation	The complex is added to y ml medium supernatant of the cells (may contain serum)
96well plate	0.32 cm^2	1	0.1 µg in 15 µl medium	0.1 µl	0.15 ml
48well plate	0.95 cm^2	3	0.3 µg in 25 µl medium	0.3 µl	0.25 ml
24well plate	1.9 cm^2	6	0.6 µg in 50 µl medium	0.6 µl	0.5 ml
12well plate	3.8 cm^2	12	1.2 µg in 100 µl medium	1.2 µl	1 ml
6well plate	9.5 cm^2	30	3 µg in 200 µl medium	3 µl	2 ml
60 mm dish	21 cm^2	66	6.6 µg in 400 µl medium	6.6 µl	4 ml
100 mm dish	55 cm^2	172	17.2 µg in 1000 µl medium	17.2 µl	10 ml
T-75 flask	75 cm^2	235	23.5 µg in 1500 µl medium	23.5 µl	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 Reagent amount should be adapted according to the difference in surface.

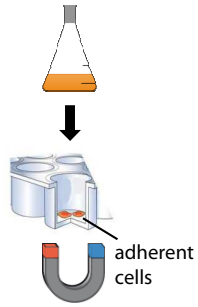
The values given in the table above have to be considered as starting point parameters. In each individual case, optimization may be achieved by performing a titration experiment in the 96well format (see 3.2.3). The surface factor may be used for the determination of the appropriate amount of nucleic acid in the desired format. Besides determining the optimal nucleic acid amount and ratio between nucleic acid and MATra-A/MATra-si Reagent (usually not necessary for plasmid DNA transfections with MATra-A Reagent), cell density and incubation time are further parameters which may be optimized for Magnet Assisted Transfection.

3.2 Standard protocols

Materials and important notes

- Please consider the general remarks under 3.1 prior to starting with the standard protocol, particularly if transfection is intended to be performed in another than the 96well format.
- **Vortex** MATra-A, MATra-si Reagent and MATra-S Immobilizer before use!
- If required, MATra-A or MATra-si Reagent can be pre-diluted with water.
- Incubations on the Magnet Plate may be carried out at 37 °C in the cell incubator.
- Incubation times should be followed exactly.
- Perform a positive control transfection experiment with a well-characterized reporter gene (e.g. GFP, Luciferase).

3.2.1 MATra-S for suspension cells



1. **Dilute the cells to be transfected to 5 x 10^5 - 1 x 10^6 per ml in medium**.
   The medium may contain serum or supplement or may be serum-free; depending on cell type and sensitivity of cells towards serum-free conditions.

2. **Mix cell suspension with 30 µl of MATra-S Immobilizer per 1 ml of cell suspension.**

3. **Incubate for 10 - 15 minutes.**

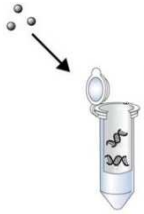
4. **Aliquote 150 µl of the cell suspension per well of a flat-bottom 96well plate placed on a Universal Magnet Plate.**
   Use the surface factor of the table under section 3.1.2 for other formats.

5. **Incubate for 15 minutes.**

6. **During the incubation continue with the standard protocol for MATra-A/MATra-si.**
   Do not perform the medium change of step 3 of the MATra-A/MATra-si protocol. Keep cells on the Magnet Plate until the 15 minute incubation of step 6 of the MATra-A/MATra-si protocol has been terminated.

3.2.2 MATra-A/ MATra-si for adherent cells

MATra nanoparticles

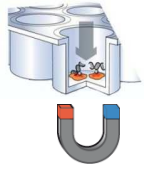


1. **Dilute the nucleic acid amount to be transfected with serum-free and supplement-free medium (e.g. DMEM) as proposed in the table under 3.1.2.**
   For the transfection of cells grown in one well of a 6well plate, for example, 3 µg nucleic acid are diluted with DMEM to an end volume of 200 µl.

2. **Add the diluted nucleic acid to the respective amount of MATra-A Reagent (MATra-si in case of siRNA), e.g. 3 µl for 3 µg nucleic acid of the 6well plate example given above (vortex the MATra Reagent before use), mix thoroughly and incubate at room temperature for 20 minutes.**
   Exceedingly long incubation times lead to inhomogeneous DNA(siRNA):bead precipitation and thus reduced transfection efficiency.

3. **During the incubation of MATra Reagent and nucleic acid, perform a medium change (optional, not for MATra-S protocol).**
   The medium supernatant (which may contain supplements and/or serum) should equal to the volume suggested in the table under 3.1.2 for the different cell culturing formats. E.g. add 2 ml medium to the cells in each cavity of the 6well plate.

4. **Add the DNA(siRNA):bead-mixture from step 2 to the cells and mix immediately.**
   In the case of the 6well plate example, add 200 µl of the DNA(siRNA):bead mixture to the 2 ml supernatant in each well of the 6well plate and mix. Please notice that the supplements and serum are diluted by this step. If the cells used are sensitive to such alterations, a medium change should be performed as proposed under step 7.



Magnet Plate

5. **After mixing, place the plate or flask immediately on the suitable Magnet Plate.**

6. **Incubate for 15 minutes and then remove Magnet Plate.**

7. **(optional) Perform a medium change after 4 - 6 hours of incubation, particularly if transfection has been carried out in serum-free medium.** A medium change is recommended for all fast-growing cell types which are insensitive to medium

Note!

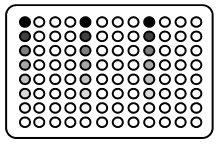
Continued on next page

3.2.2 MATra-A/ MATra-si for adherent cells, continued

change (e.g. HEK 293). For those cell types which are sensitive to medium change, but show toxic effects , it is recommended to titrate MATra-A/MATra-si Reagent or DNA amount (see 3.2.3).

8. **Continue to culture cells as desired until evaluation of transfection efficiency.**
   It is important to wait at least 48 hours before exposing the transfected cells to selection media.

3.2.3 Titration protocol



See page 14 for illustration!

We recommend to optimize transfection conditions in order to get the best results. Several parameters can be optimized:

- Amount of nucleic acid
- Cell density
- Incubation time
- Ratio of MATra-A or MATra-si Reagent/MA Lipofection Enhancer to nucleic acid or virus (usually not necessary for plasmid DNA transfections with MATra-A)

The following titration protocol is designed to find the optimal ratio of MATra-A Reagent to nucleic acid and the optimal amount of nucleic acid.

For adherent cells, seed the cells at the desired density into the columns 2/3/4, 6/7/8, and 10/11/12 of a 96well plate the day prior or at least several hours prior transfection in a total of 150 µl medium per well. For Plasmid DNA it is recommended to titrate cell density instead of ratio between MATra-A Reagent and nucleic acid amount. In all plasmid DNA transfections tested so far an 1:1 (v/w) ratio emerged optimal.

1. **Dilute 21.6 µg nucleic acid to a final volume of 280.8 µl with serum-free and supplement-free medium (e.g. DMEM).**

2. **Pipet 3.6 µl (1:2 [v/w]), 7.2 µl (1:1 [v/w]), and 14.4 µl (2:1 [v/w]) MATra-A Reagent in well A1, A5 and A9 of the 96well plate and bring the volume in wells A1 and A5 to 14.4 µl by adding 10.8 and 7.2 µl sterile water, respectively. Vortex MATra-A Reagent before use.**

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3.2.3 Titration protocol, continued

3. **Add 93.6 µl nucleic acid solution from step 1 to each well A1, A5, A9 containing MATra-A Reagent and mix well by pipetting up and down.**
   Incubate for 20 minutes at room temperature. The actual duration should be noted for reproduction of results.

3a. (Optional for adherent cells). Perform a medium change prior to transfection. Remove medium supernatant from the seeded cells and replace with 150 µl fresh medium (with or without serum or supplements).

3b. In case of suspension cells (for adherent cells proceed immediately to step 4), dilute the cells to be transfected to 5 x 10^5 - 1 x 10^6 per ml in medium (serum- or supplement-containing or serum-free; depending on cell type and sensitivity of cells towards serum-free conditions).

3c. Mix cell suspension with 30 µl of MATra-S Immobilizer per 1ml of cell suspension. Incubate for 10 - 15 minutes.

3d. Aliquote 150 µl of the cell suspension per well of a flat-bottom 96well plate placed on a Universal Magnet Plate. Incubate for 15 minutes. Use another plate than for performing dilution series of MATra-A Reagent/nucleic acid.

4. **In the meantime add 54 µl serum-free and supplement-free medium (e.g. DMEM) to the residual wells of column 1, 5, 9 of the 96well plate (B1-H1, B5-H5, B9-H9).**

5. **After the incubation from step 3 transfer 54 µl from well A1, A5, A9 to B1, B5, B9 mix by pipetting, transfer 54 µl from B1, B5, B9 to C1,C5, C9 mix by pipetting, transfer 54 µl from C1, C5, C9 to D1, D5, D9 and so on down to H1, H5, H9.**

6. **Transfer 15 µl each in triplicates from column 1, 5, 9 to the columns of the cell culture plate (column 2/3/4, 6/7/8, 10/11/12) where the cells to be transfected have been seeded and mix immediately** (corresponding to nucleic acid amounts: 1000 ng for row A, 500 ng for row B, 250 ng for row C, 125 ng for row D, 62,5 ng for row E, 31 ng for row F, 15 ng for row G and 7.5 ng for row H).

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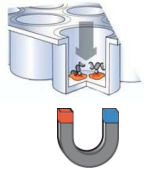
3.2.3 Titration protocol, continued



Magnet Plate

6a. For suspension cells, the plate remains on the Universal Magnet Plate during this step.

6b. For adherent cells, transfer the culture plate on the Universal Magnet Plate after transfer of the MATra-A Reagent/nucleic acid mixture on the cells.

7. **Incubate for 15 minutes on the Universal Magnet Plate.**
   Incubation time may be varied between 5 and 20 minutes for optimization.

8. **Remove Universal Magnet Plate.**

9. **(optional) Perform a medium change after 4 – 6 hours of incubation, particularly if the transfection has been carried out in serum-free medium.** A medium change is recommended for all fast-growing cell types which are insensitive to medium change (e.g. HEK 293).

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

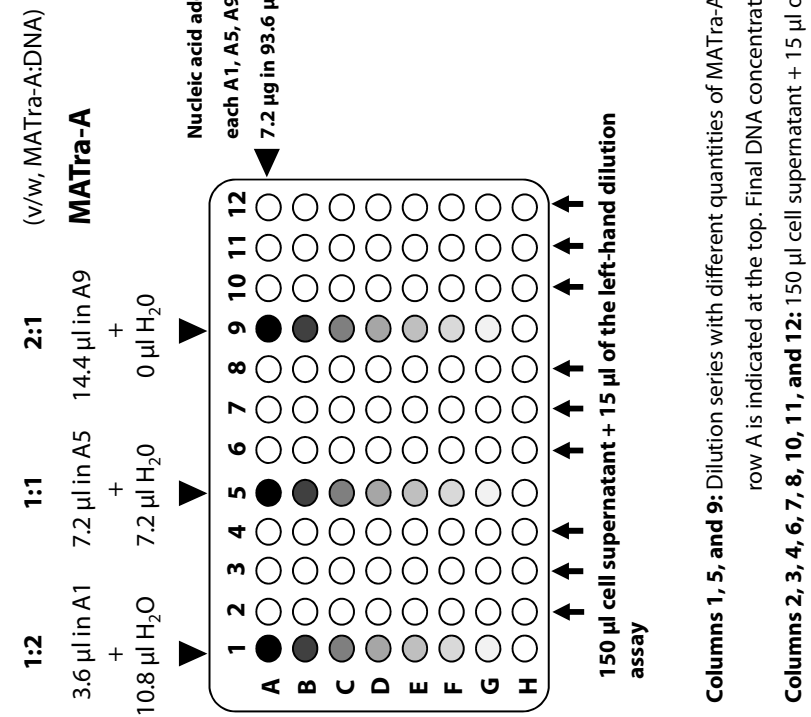
The above mentioned titration protocol can also be used for determination of optimal use of MA Lipofection Enhancer when the DNA solution from step 1 has been prepared with IBAfect or other lipofection reagents and if MATra-A Reagent in step 2 is replaced by the MA Lipofection Enhancer.

Following characterization of the transfected cells, the optimal nucleic acid amount and nucleic acid:MATra-A Reagent ratio for efficient MATra can be determined and extrapolated to other formats by using the surface factor mentioned in the table under section 3.1.2.

Note! **The ratio of MATra-si Reagent to siRNA should be optimized in a range of 1:0.5 to 1:0.005.**

If required, MATra-A or MATra-si Reagent can be pre-diluted with water.

3.2.3 Titration protocol, continued



Columns 1, 5, and 9: Dilution series with different quantities of MATra-A Reagent. Amount of MATra-A Reagent added in row A is indicated at the top. Final DNA concentrations are given to the right. Final volumes: 54 µl.

Columns 2, 3, 4, 6, 7, 8, 10, 11, and 12: 150 µl cell supernatant + 15 µl of the left-hand dilution assay.

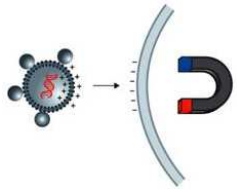
3.2.4 Large plate formats

26 x 26 cm

The 26 x 26 cm Universal Magnet Plate can be used for transfections of cells grown on larger plates (up to 500 cm^2). While the same general principle applies, a few points should be considered.

- Optimizing conditions can be easily carried out in a small scale by calculating cell number, DNA (siRNA), and MATra-A (MATra-si) amounts for the respective growth surface area.
- For larger plates an uniform liquid level over the cells is even more important. Make sure plates are not inclined during the transfection.
- Equal distribution of the nucleic acid:bead complex on large plates can be challenging. Alternatively add the entire medium to the nucleic acid:bead complex, aspirate the supernatant off the cells and add the premixed solutions to the plate.

3.3 MA Lipofection



<u>MA Lipofection Enhancer in combination with common lipofection reagents, e.g. IBAfect</u>

IBA provides an efficient polycationic transfection reagent based on liposome technology (IBAfect). The efficiency of IBAfect and also that of similar reagents of other suppliers can be enhanced through magnetic assistance simply by mixing them with MA Lipofection Enhancer, usually leading to improved transfection efficiencies. There are two strategies of using MA Lipofection Enhancer: One is to prepare a standard nucleic acid complex with IBAfect, followed by mixing with MA Lipofection Enhancer. The second strategy is to first mix nucleic acid with MA Lipofection Enhancer followed by mixing this complex with IBAfect. In this case, the instructions for IBAfect are used with the only exception that instead of nucleic acid alone, a mixture of nucleic acid and MA Lipofection Enhancer is added to the lipofection reagent. Depending on the lipofection reagent, the mixing order of the components may influence the final transfection efficiency. It is recommended to use 1 µl of MA Lipofection Enhancer per microgram of nucleic acid in initial experiments. However, depending on the cell to be transfected and the commercial lipofection reagent used, the optimal composition may be found above or below this ratio. A similar titration experiment as described under 3.2.3 may be performed for determining optimal conditions.

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3.3 MA Lipofection, continued

If required, MA Lipofection Enhancer can be pre-diluted with water.

Vortex MA Lipofection Enhancer before use!

Example protocol for IBAfect for a titration experiment in the 96well format:

1. Add 3.6 µl IBAfect to 146.4 µl **serum-free** and supplement-free medium (e.g. DMEM) and mix by pipetting up and down.
2. Dilute 1.2 µg of nucleic acid in 150 µl **serum-free** and supplement-free medium (e.g. DMEM).
3. Mix the nucleic acid solution with the IBAfect dilution by pipetting up and down (do not vortex).
4. Incubate for 15-20 minutes.
5. Add the resulting 300 µl of nucleic acid complex to 1.2 µl of MA Lipofection Enhancer (**Vortex** MA Lipofection Enhancer before use!) and mix immediately by pipetting up and down several times.
6. After further 15 minutes of incubation add 12.5 µl or 25 µl or 50 µl of the resulting mixture, each in triplicates, to the cells (corresponding to 0.05 µg or 0.1 µg or 0.2 µg of nucleic acid per well, respectively) and mix immediately and thoroughly with the medium (final volume 150 µl).
7. Immediately after mixing, place the cell culture plate for 15 minutes on the Universal Magnet Plate. This parameter may be varied between 5 and 20 minutes for optimization.
8. Remove the Universal Magnet Plate and cultivate cells under standard conditions until evaluation of transfection efficiency.
9. Optionally perform a medium change after 4 - 6 hours of incubation. A medium change is recommended for all fast-growing cell types which are insensitive to medium change (e.g. HEK293).

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3.3 MA Lipofection, continued

For other lipofection reagents like Fugene, Lipofectamine, GenePorter, ExGen500 or Superfect the same steps as described above are carried out. The primary nucleic acid complex is prepared according to the instructions of the manufacturer. For example, in step 1, 3.6 µl Fugene + 146.4 µl medium, 3.6 µl Lipofectamine™ 2000 + 146.4 µl medium, or 6.0 µl GenePorter + 144 µl medium are used (please follow the manual of the respective manufacturer for the exact ratio of reagent to nucleic acid). The residual protocol remains the same. However, it should be kept in mind that the alternative mixing order (first mixing nucleic acid and MA Lipofection Enhancer followed by mixing with the common reagent) may be advantageous.

3.3.1 MA Lipofection Enhancer in combination with **viruses**

Viral infection depends highly on the presence of the respective cell surface receptors (CAR for adenovirus; CD4 for HIV etc.). Unfortunately, many target cells for basic research and gene therapy are non-permissive to viral gene delivery as the respective receptors are down regulated or even missing (e.g. tumor tissues and apical surface of lung epithelium may express variable, little or none of the required receptors).
A solution for this problem is the association of viral vectors with MA Lipofection Enhancer to permit efficient infection of non-permissive cells as could already be shown for adenovirus or retroviruses.

Protocol:

1. Cells should be plated in the same manner as required for standard viral gene delivery. For example, the confluency can be high for adenoviral vectors but must be low for retroviral vectors because retroviruses require cell division for infection. Cells must be plated the day prior transfection.
2. Pipet the required amount (see examples in the table on page 19) of MA Lipofection Enhancer in a reaction vessel appropriate to harbor the volume of virus preparation aimed to be added in step 3.

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3.3.1 MA Lipofection Enhancer in combination with **viruses**, continued

3. Add virus preparation (e.g. retroviral supernatant or purified adenovirus diluted in HBS, PBS or cell culture medium) to the reaction vessel containing MA Lipofection Enhancer and mix immediately by pipetting or gentle vortexing. Incubate 20 minutes at room temperature.

4. The ratio virus:MA Lipofection Enhancer should be adjusted according to the virus titer and cell types used. For optimization, as a starting point the use of 1.5 µL, 3 µL, 6 µL and 12 µL of MA Lipofection Enhancer with a fixed quantity of virus preparation:supernatant is recommended.

5. Add the mixture prepared in step 3 in duplicate or triplicate to the cells.

6. Place the cell culture plate on the Magnet Plate for 30 minutes.

7. Remove the Magnet Plate. Optionally perform a medium change.

8. Cultivate cells under standard conditions until evaluation of transgene expression.

9. This protocol should be adjusted depending on the viral vector, the quantity of virus and the cell types used.

Cell type	Virus	MA Lipofection Enhancer
K562	adenovirus (200 MOI)	6 µl
human PBL	adenovirus (500 MOI)	3-6 µl
NIH-3T3	adenovirus (200 MOI)	3-6 µl
NIH-3T3	Retrovirus (1-5x10^3 X gal CFU/ml)	3-6 µl

4 Troubleshooting

Problem	Suggestion
Cell culturing plate does not fit perfectly onto magnet plate.	• A small spacing between the bottom of the culturing plate and the magnets is not critical. The culturing plate should, however, not be placed inclined on the magnet plate which would lead to inhomogeneous transfection.
Transfection is not homogeneous over the whole culturing area	• Reduce time of complex formation between the magnetic beads and nucleic acids • Mix the nucleic acid:MATra bead complex and the cell culturing medium supernatant over the cells thoroughly **prior** to placing the culturing plate onto the magnet plate. Do not mix by circular motion. It may be recommendable, particularly in case of large surfaces, to add the culture supernatant to the formed bead:nucleic acid complex in a suitable vessel, to mix thoroughly and then to apply the homogeneous mixture back to the cells.
Low efficiency	• Avoid serum or other charged macromolecules when incubating magnetic particles and nucleic acids, otherwise proteins from serum will bind to the MATra-A Reagent; once the MATra-A/DNA complex is formed it can be applied to cells in the presence of serum. • Optimize cell density • Optimize/titrate amount of nucleic acid:bead complex to be applied to the cells by using the titration protocol in the 96well format (see 3.2.3) • Extend readout time (e.g. 24 h longer than usual) • Incubate cells for prolonged time in the magnetic field • Use freshly thawed cells that have been passaged at least once. Cells that have been in culture for a long time may become resistant to transfection. • Use other cell line if possible • Perform a positive control transfection experiment with a well-characterized reporter gene (e.g. GFP, Luciferase) • Mycoplasma contamination alters transfection efficiency

4 Troubleshooting

Problem	Suggestion
Toxic effects	• Optimize cell density at the time of transfection. Adherent cells are seeded such that they reach 30-60% density at the time of MATra. If the cell density is too low, increased toxicity may be observed. For suspension cells it is necessary that the cells are immobilized on the well bottom. • Reduce the incubation time of complexes with the cell. Medium can be changed 4 – 6 hours after performing MATra • Reduce amount of nucleic acid:bead complex to be applied to the cells. Higher amounts of MATra-A/MATra-si Reagent or MA Lipofection Enhancer may be toxic for cells and can reduce transfection efficiency. For MATra approximately 5 x less DNA compared to lipofection is necessary. • Check the purity of the molecule of interest to be delivered, e.g. lipopolysaccharides which are endotoxins will cause cell death. • Use other cell line if possible

5 Related products

5.1 Order information MATra reagents

Product	Amount	Cat. no.
MATra-A Reagent	200 µl (for 200 µg nucleic acid)	7-2001-020
MATra-A Reagent	1 ml (for 1000 µg nucleic acid)	7-2001-100
MATra-si Reagent	200 µl (for 200 µg siRNA)	7-2021-020
MATra-si Reagent	1 ml (for 1000 µg siRNA)	7-2021-100
MATra-S Immobilizer	200 µl (for up to 7 Mio. cells	7-2002-020
MATra-S Immobilizer	1 ml (for up to 35 Mio. cells	7-2002-100
MA Lipofection Enhancer	200 µl (for up to 200 µg nucleic acids)	7-2003-020
MA Lipofection Enhancer	1ml (for up to 1000 µg nucleic acids)	7-2003-100
IBAfect	500 µl (for 75-250 µg nucleic acid)	7-2005-050
IBAfect	1 ml (for 150-500 µg nucleic acid)	7-2005-100
IBAfect	5 ml (for 750-2500 µg nucleic acid)	7-2005-500

5.2 Order information Magnet Plates

Product	Amount	Cat. no.
Universal Magnet Plate, 8 x 13 cm	1 plate	7-2011-000
Universal Magnet Plate, 26 x 26 cm	1 plate	7-2012-000
96 Magnet Bar Plate, 8 x 12 cm	1 plate	7-2004-000
On special request:		
24 Magnet Bar Plate, 8 x 12 cm	1 plate	7-2006-000
Set of 4 Magnet Bar Plates	4 x 24 Magnet Bar Plates	7-2008-000
Set of 4 Magnet Bar Plates	4 x 96 Magnet Bar Plates	7-2009-000
Set of 4 Magnet Bar Plates	2 x 24/2 x 96 Magnet Bar Plates	7-2010-000

5. 3 MATra assay formats

Formats	Volume of MATra-A Reagent recommended per well [µl]	Transfections per 200 µl vial
96well plate	0.1	2000
48well plate	0.3	667
24well plate	0.6	333
12well plate	1.2	167
6well plate	3	67
60 mm dish	6.6	30
100 mm dish	17.2	12
T-75 flask	23.5	9
25 x 25 cm plate	156	1*

*156 µl MATra-A are required for 500 cm^2; please titrate to optimize for your application.

6 Appendix

Contact us!

Visit our Web site at **www.magnet-assisted-transfection.com** for:

- References
- Application Notes
- FAQs
- Overview of cells successfully transfected with MATra

For more information or technical assistance contact us:
at **info@iba-go.com** or tel. (Germany): **+49 (0) 551-50672-0** and tel. (USA): **1-877-IBA-GmbH** (1-877-422-4624) .

Note!

Being a specialist for RNA synthesis IBA is offering **siRNA** for your transfection experiments with **MATra-si**. For more information and ordering visit our Web site **www.rna-tools.com** or contact us at **oligo@iba-go.com**.

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