

FAQ Magnet Assisted Transfection (MATra)

Frequently asked questions concerning Magnet Assisted Transfection (MATra)

General Considerations

[1] What is the principle of MATra?

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 nanoparticles. Exploiting 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 adhere to the bottom of the culture vessels which makes special precautions necessary for suspension cells.

[2] What is the difference between MATra-A Reagent, MATra-si Reagent, MATra-S Immobilizer and MA Lipofection Enhancer?

The four different reagents are used for different procedures:

1. MATra-A Reagent

For adherent cells the nucleic acid has to be combined to the MATra-A Reagent and MATra can be performed. Suspension cells have to be made adherent first before MATra can be performed (see MATra-S Immobilizer).

2. MATra-si Reagent

The MATra-si Reagent was developed specifically for MATra with siRNA and miRNA and can be used for adherent and suspension cells as described for the MATra-A Reagent.

3. MATra-S Immobilizer for suspension cells

In this case, cells have to be made adherent first by incubating them with the magnetic reagent MATra-S Immobilizer. Then MATra-A/MATra-si Reagent loaded with the nucleic acid is applied and MATra can be performed.

4. MA-Lipofection Enhancer used in combination with standard transfection reagents

Transfection with common lipidic (Lipofectamine, Fugene, Geneporter) or polycationic (e.g. ExGen500 or Superfect) reagents can be enhanced by magnetic assistance. In this case, the nucleic acid to be transfected has to be combined with MA Lipofection Enhancer in the presence of the common transfection reagent. Furthermore, MA Lipofection Enhancer can be associated with viruses (adenovirus, retrovirus) to enhance entry of viruses into the cells.

[3] Which equipment is required?

In addition to the reagents described above you require a magnet plate. We offer a small Universal Magnet Plate (6 x 13 cm) which is suitable for 6, 12, 24, 48 or 96 well plates as well as for T75 flasks and 60 mm dishes; the large Universal Magnet Plate (26 x 26 cm) is suitable also for those formats and in addition for 100 mm dishes. The 96 Magnet Bar Plate (8 x 12 cm) is optimized for transfection in 96 individual wells.

[4] Is the magnet plate efficient for all types of cell culture supports?

The Universal Magnet Plate (two sizes: 6 x 13 cm or 26 x 26 cm) works with different types of cell culture supports (6, 12, 24, 48 or 96 well plates as well as T75 flasks, 60 mm dishes (6 x 13 cm plate) and 100 mm dishes (26 x 26 cm plate) by creating a homogeneous magnetic field. The 96 Magnet Bar Plate (8 x 12 cm) is designed like a 96-well microplate and contains 96 little magnets optimized for sensitive read-out assays in a 96-well format to minimize intra-assay variances. In comparison to different 96-well plates the "Cellstar Greiner bio-one No. 655180 Tissue Culture Plate with Lid, Flat Bottom" fits well to the 96 Magnet Bar Plate (8 x 12 cm).

[5] What is the size of the magnetic nanoparticles?

Magnetic particles range between 100 and 200 nm in hydrodynamic diameter.

[6] What happens to the magnetic nanoparticles complexed with nucleic acids? Are they incorporated into the cells? Do they have cytotoxic effects?

The magnetic nanoparticles enter the cells essentially by endocytosis and pinocytosis. Basically, MATra concentrates the genetic material on the cell surface and promotes its entry into the cells. The nucleic acids are then released into the cytoplasm. Magnetic nanoparticles are not toxic for the cells at the recommended or even at higher doses. After 24 - 72 hours, the nanoparticles are localized in "vacuoles" (membrane surrounded compartments). The magnetic nanoparticles are made of iron oxide, which is biodegradable, and of a polymer coating. The magnetic nanoparticles do not influence cellular functions, as far as we know. Observed toxic effects have been entirely related to transfection reagents which have been combined with MATra and have been dose related. MATra allows reducing doses below toxicity thresholds while maintaining satisfactory efficacy.

[7] What is the efficiency of MATra?

MATra is a universally efficient method, convenient for a lot of cell types (cell lines and primary cells). The transfection efficiency depends on many parameters such as cell type,

cell density, plasmid-DNA construct, cell culture conditions etc. For example, we have tested the MA Lipofection Enhancer reagent (associated with several commercial transfection reagents) on several primary cells and we achieved at least a 30% increase of transfection efficiency (compared to the transfection reagent used without MA Lipofection Enhancer). In several experiments transfection efficiency could be increased by up to 100 fold. In the same way, oligonucleotides could be delivered in 90% of human umbilical vein endothelial cells (HUVEC). For MATra-A Reagent the efficiency is for instance 40% in porcine aortic endothelial cells (PAEC) or 75% in NIH-3T3. Please refer to our [list](#) “Successfully Transfected Cells”.

[8] Is MATra designed for stable or transient transfections?

MATra is suitable for transient and stable transfections.

[9] Which specifications are required for the nucleic acids, such as purity, concentration, size, conformation (linear or circular)?

MATra is suitable for any kind of nucleic acids. Nucleic acids should not contain impurities such as toxins (endotoxins), nucleases, agarose residues that can affect vector:bead assembly or cells to be transfected. The purity of nucleic acids always influences the transfection efficiency.

[10] Are MATra-A Reagent and MA Lipofection Enhancer suitable for co-transfection?

Yes, co-transfection with MATra reagents is possible and reproducible if the total amount of nucleic acid is considered for the calculation of the ratio between nucleic acid and MATra reagents.

[11] Is MATra compatible with serum-containing culture media?

Yes, MATra is compatible with serum-containing and serum-free culture media. Thus, you can use your regular complete cell culture media during transfection but - *important note* - do not use serum and other culture media supplements during complexation of the nucleic acid with the magnetic nanoparticles. In the case, serum-free media is not available you can use regular buffer for complexation of the nucleic acid with the magnetic nanoparticles, since the small amounts used will not dilute the medium to a significant amount.

Cells

[12] What is the difference in protocol for suspension cells and adherent cells?

We have tailored and optimized a protocol for suspension cells that is detailed in the instruction manual. Briefly, during the incubation step when MATra-A Reagent/nucleic acid complexes or MA Lipofection Enhancer/nucleic acid/transfection reagent (or virus) complexes will form, dilute the cells to be transfected to 5×10^5 - 1×10^6 per mL in medium

(with or without serum or supplement) and perform one of the following options to sediment the cells at the bottom of the culture dish thus making them susceptible to MATra.

- **Option 1:** Mix cell suspension with 30 μ L of MATra-S Immobilizer per 1mL of cell suspension.
 - a. Incubate for 10-15 minutes.
 - b. Distribute cells to the culture dish placed upon the magnet plate (volume of culture medium containing cells depends on the culture dish size; see transfection volume as suggested in the protocol).
 - c. Incubate for 15 minutes.
 - d. Add the mixture containing the nucleic acid/MATra-A Reagent complexes to the cells while keeping the culture plate on the magnet plate, continue to incubate for 15 minutes and then proceed as explained in the Instruction Manual.
- **Option 2:** Seed the cells on polylysine-, gelatin-, or collagen-coated plates (e.g. use polylysine-coated 15 mm glass coverslips placed in a 12 well plate) and continue with the protocol for adherent cells (see Instruction Manual).
- **Option 3:** Briefly centrifuge the cells (2 minutes) in order to pellet them and continue with the protocol for adherent cells (see Instruction Manual).
- **Option 4:** Use antibody against cell surface antigen immobilized on microplate and continue with the protocol for adherent cells (see Instruction Manual).

[13] Does MATra work for primary cells?

MATra is an ideal method for transfecting primary cells. Numerous primary cells have been successfully transfected with this technology; see our list “Successfully Transfected Cells”.

If your particular cell type is not mentioned, this does not imply that MATra does not work. Since the MATra technology is quite new only some out of the entire range of cell types and cell lines of interest could be studied up to date. Nevertheless, our customers' feedback is exceptionally enthusiastic about primary cell transfection.

[14] Does MATra work with neurons?

Both MATra-A Reagent & MA Lipofection Enhancer are working with primary cortical and hippocampal primary neurons. Transfection efficiency was 15-20% with MATra-A Reagent and even higher with MA Lipofection Enhancer combined with other transfection reagents.

[15] Has MATra been tested with non-mammalian cells?

MATra has been developed and tested for mammalian cells, but we have also examples for other species like fish or Xenopus; see our list “Successfully Transfected Cells”.

[16] Does MATra work with all types of nucleic acids? What about siRNA?

MATra reagents are universal: they can be used with all types of nucleic acids (naked DNA, plasmid, siRNA, mRNA) and without DNA size limits (successfully used with 150 kB plasmids (genomic context vectors GCV's)).

[17] With which type of virus can MA Lipofection Enhancer be associated?

MA Lipofection Enhancer can be potentially associated with any virus. It has been successfully associated with adenovirus, lentivirus, baculovirus, and retrovirus.

[18] When MA Lipofection Enhancer is used does the mixing order of components influence the final transfection efficiency?

All commercial transfection reagents can be associated with MA Lipofection Enhancer reagent by simple mixing in order to generate magnetic delivery systems. Depending on the transfection reagent used, the mixing order of components may influence the final transfection efficiency of MATra. We recommend to use 1 µL of MA Lipofection Enhancer per µg of DNA in initial experiments (for antisense oligos or siRNA ratios need to be optimized) and to prepare the DNA / transfection reagent complexes according to the reagent's manufacturer instructions first and then add the nanoparticles. The usual final incubation step after mixing DNA & the commercial transfection reagent can be omitted by adding directly the magnetic particles into the mixture. However, depending on the cell line to be transfected and the commercial transfection reagent used, the optimal composition may require the optimization of the MA Lipofection Enhancer/DNA ratio.

Protocol

[19] Which quantity of DNA has to be used?

The protocols for MATra-A Reagent and MA Lipofection Enhancer are as simple as follows:

MATra-A Reagent: use 1 µL of MATra-A Reagent per µg of DNA.

MA Lipofection Enhancer: use 1 µL MA Lipofection Enhancer per µg of DNA.

For doses less than 1 µg DNA, predilute MATra-A Reagent or MA Lipofection Enhancer with deionized water.

The quantity of DNA to be used depends on cell types, size of the culture dishes etc. (please refer to the Instruction Manual).

[20] Why should MATra-A Reagent or MA Lipofection Enhancer be prediluted in deionized water?

IBA recommends a ratio of 1 μL of reagent per μg of DNA. For DNA doses less than 1 μg , less than 1 μL of reagent has to be used which is difficult to be transferred accurately. Thus, dilution ensures more accurate experimentation.

[21] How many transfections can be performed with 100 μL reagent?

It depends on the quantity of DNA used per transfection. If 1 μg of DNA is necessary per transfection, 100 assays can be achieved with 100 μL of reagent.

[22] Should MATra be processed in serum free medium?

It is one of the great benefits of the method that the presence of serum does not interfere with MATra. Only the incubation with nucleic acid and MATra-A Reagent or MA Lipofection Enhancer should be done in a medium free of serum or supplements. Serum and supplements could interfere with the assembly of the nucleic acid and the magnetic nanoparticles. Subsequent transfection via MATra is, however, not affected by serum or supplements and can thus be performed in their presence.

[23] Are protocols available for transfection in cell culture dishes other than 96-well plates?

The protocol is essentially the same for all formats; only the reagent volume has to be adapted to the quantity of nucleic acid used according to the table in the instruction manual.

[24] Where do the cells have to be incubated during the 15' of incubation on the magnet plate?

In principal, the plates can be left at any place, like under the clean bench or in the incubator, depending on the sensitivity of the cells.

[25] Can other tests be performed after the transfection?

MATra should not influence the experimental tests following transfection such as flow cytometry, ELISA assays, luminescence and colorimetric assays.