IBA

BioTAGnology

www.magnet-assisted-transfection.com

Newsletter

Issue 10

Innovative applications in Neurosciences and Hepatology

pages 5 and 6

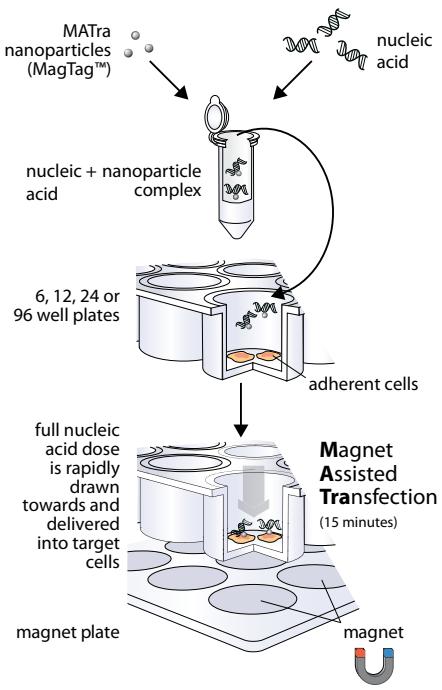
MATra

Magnet Assisted Transfection

MATra - Best practices for best results

- Reduce cost – increase transfection efficiency
- Gentle TAGnology tested to work for almost 200 cell lines and primary cells

MATra principle



Magnet Assisted Transfection (MATra) with MagTag®

Magnet Assisted Transfection (MATra) is a new, easy-to-handle, **very fast** and **highly efficient** technology to transfect cells in culture. All types of nucleic acids from plasmid DNA or siRNA to oligonucleotides can be used with the MATra approach. Data from a variety of species using cell lines or primary tissue culture have accumulated including human, monkey, mouse, rat, xenopus, pig, cat or fish.

Using this new technique, nucleic acids are in a first step associated with specific magnetic nanoparticles (MagTag®). Exploiting magnetic force the full nucleic acid dose is then drawn towards and delivered into the target cells leading to efficient transfection **without disturbing the membrane architecture**, without causing chromosomal damage or leaving a hole in the cell membrane like other transfection technologies. Cellular uptake occurs by either **endocytosis** or pinocytosis. Delivered to the target cells, the DNA is released into the cytoplasm. The magnetic particles are accumulated in endosomes and/or vacuoles. Over time, the nanoparticles are degraded and the iron enters the normal iron metabolism not influencing cellular functions.

Two approaches are possible: for a standard **Magnet Assisted Transfection** "MATra-A Reagent" is used; for more critical cells it is also possible to combine the MATra technology with lipofection ("**Magnet Assisted Lipofection**"). For this purpose, we are offering "MA Lipofection Enhancer" and the high-efficiency lipofection reagent "IBAfect". The MA Lipofection Enhancer can also increase the efficiency of viral transfections.

Both techniques can be used with adherent cells as well as with suspension cells. However, for the latter cells have to be localized at the bottom of the cell culture plate using the MATra-S Immobilizer. MATra can also be adapted to high-throughput transfection assays using robotic stations and adapted protocols.

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				+

Ten good reasons to use MATra technology

1. Greatly improved transfection rates
2. Very gentle method with **almost no toxicity**; membranes remain intact
3. No transfection related side effects on your model system and read out
4. Only **low vector doses** are required
5. Easy protocol; transfections can be performed in original culture plates
6. Functional with serum and serum-free medium
7. Successfully used on many and also critical cell lines (almost 200 cell types tested positively so far)
8. Highly **cost-effective**; no need for expensive instruments (like electroporator or particle gun)
9. Combination with lipofection or viral transfection possible
10. Works excellently for various types of nucleic acids including siRNA or antisense oligo transfections

MATra Specifications

MATra is applicable at different phases of cell confluence, however in general we recommend 50-70% confluence (in some systems a higher visual confluence may result in higher Magnet Assisted Transfection rates).

MATra is non-toxic at recommended amounts in most cell systems. Still, if higher DNA/MATra or Enhancer amounts are used a medium change is advised within 2 hours.

Reagents for adherent cells

Magnet Assisted Transfection

MATra-A Reagent

contains magnetic nanoparticles (MagTag®) which can be loaded with the nucleic acid of interest. MATra-A Reagent can be used for adherent cells; for suspension cells the MATra-S Immobilizer is required in addition (see below).

MATra-si Reagent

is optimized for siRNA applications. It is suitable for adherent cells; for suspension cells the MATra-S Immobilizer is required in addition (see below). Refer to www.rna-tools.com and page 6 for IBA's RNA custom synthesis.

Magnet Assisted Lipofection and viral transfections

MA Lipofection Enhancer

Transfection with common lipidic or polycationic reagents can be enhanced by magnetic assistance ("Magnet Assisted Lipofection"). In this case, the nucleic acid to be transfected has to be combined with MA Lipofection Enhancer in the presence of a common lipofection reagent. The formulation of MA Lipofection Enhancer, which contains magnetic nanoparticles (MagTag®), has been optimized for use with IBA's lipofection reagent IBAfect, but can also be used with **any other commercially available lipofection reagent**. The MA Lipofection Enhancer can be used for adherent cells; for suspension cells the MATra-S Immobilizer is required in addition (see below).

The MA Lipofection Enhancer is also suitable to enhance the efficiency of **viral** transfections.

Reagent for suspension cells

MATra-S Immobilizer

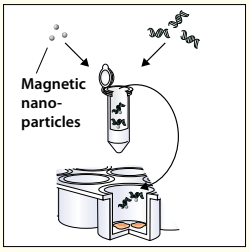
Suspension cells have to be made adherent first by incubating them with the reagent MATra-S Immobilizer which also contains magnetic nanoparticles (MagTag®). Then, Magnet Assisted Transfection or Magnet Assisted Lipofection can be performed (see above).

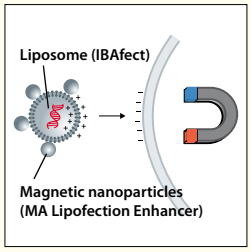
Methods using MATra Reagents

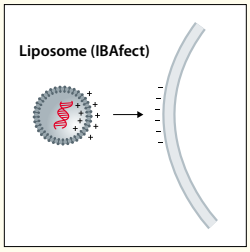
Suspension cells are made adherent with **MATra-S Immobilizer**

Adherent cells

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







We recommend evaluating Magnet Assisted Transfection and Magnet Assisted Lipofection in parallel to determine the optimal method for your particular cells of interest.

Easy protocol
(example with MATra-A Reagent)

1. Dilute nucleic acid in medium
2. Add magnetic nanoparticles (MATra-A Reagent)
3. Incubate 20 - 30 minutes
4. Add medium to adherent cells ($2 - 4 \times 10^5$ cells)
5. Add nucleic acid/nanoparticle solution
6. Place culture plate onto magnet plate
7. Incubate 15 minutes
8. Remove magnet plate

IBAfect

IBAfect is a pentacationic reagent based on DNA/RNA/lipid-complex 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) efficiently entering the cell by endocytosis.

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. IBAfect is recommended to be combined with IBA's MA Lipofection Enhancer (see above).

Magnet plates see page 7.
Order information see page 8.

Examples and Applications

Works for almost 200 cell lines and primary cells !

Including the 5 most popular cell lines

See **supplement page** or **www.magnet-assisted-transfection.com** for a **complete list** of **all cell types** that have been tested positively so far

Adherent cells

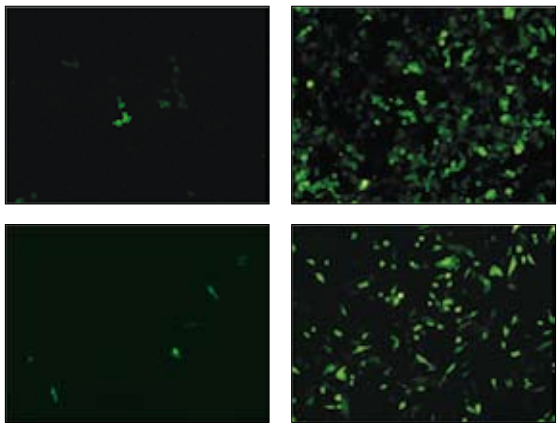
Cell line	Cell type	Organism	MATra-A*	MA Lipofection Enhancer with IBAfect*	IBAfect*
HeLa	cervix epithelial (carcinoma)	human	+	+	+
HEK 293	kidney epithelial	human	+	+	+
BHK -21	kidney epithelial	hamster	n.d.	+	+
CHO	ovary epithelial	hamster	+	+	+
NIH 3T3	fibroblast	mouse	+	+	+

* More information about IBA transfection reagents on page 3

Greatly improved transfection rates !

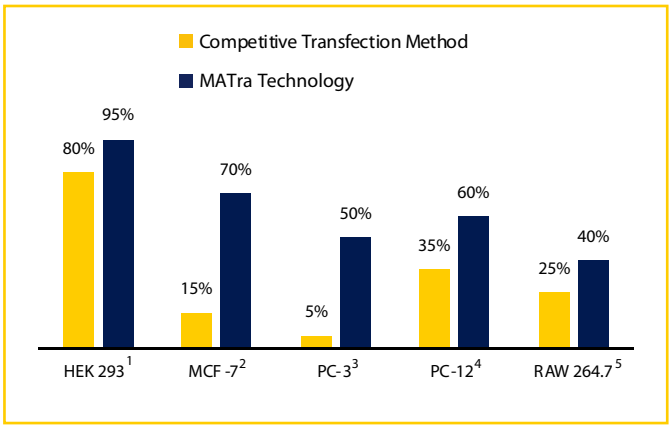
HeLa

CHO-K1



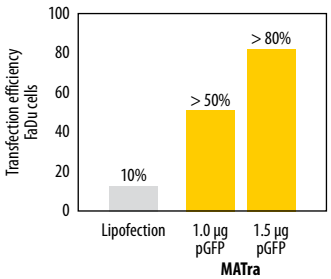
Standard transfection method Magnet Assisted Transfection

Data kindly provided by Dr. Günther Keil, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Island Riems, Germany.



For indicated cells the following methods were tested. 1: Calcium phosphate vs Magnet Assisted Lipofection, 2-4 Lipofection vs Magnet Assisted Lipofection, 5: Lipofection vs Magnet Assisted Transfection. Data kindly provided by industrial IBA customer.

"With MATra we have been able to increase the transfection efficiency to rates as high as 80% at 48 hrs following treatment" stated Olivier Gires from the LMU Munich. *"All cell lines tested showed an increased transfection rate with MATra-A in comparison to lipofection or electroporation protocols."*



Transient transfection of stable carcinoma cells with GFP plasmid

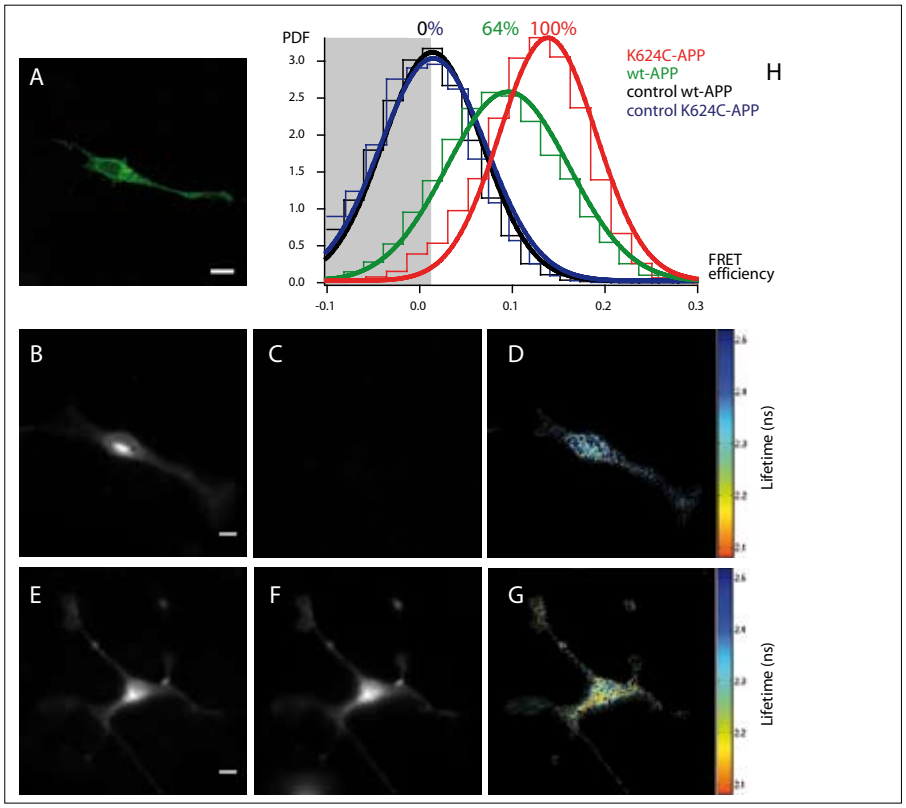
GFP expression in FaDu head and neck cancer cells after transient transfection with pGFP plasmid DNA. FaDu cells (5×10^5 cells per cavity of a 6 well plate) were transfected with 1.0 µg or 1.5 µg pGFP expression plasmid using MATra-A (1 µl/1 µg DNA). GFP fluorescence was detected by flow cytometry after 48 hrs. FaDu cells are typically transfected with standard lipofection reagents with an efficiency of about 10% (1 µg GFP in 5×10^5 cells in 6 wells).
With MATra, expression of GFP was detected in 52.7% (1.0 µg) and 82.55% (1.5 µg) of the cells. Transfection efficiency has been increased 8-fold compared to lipofection.

Data kindly provided by Rauch, Schaffrik, Ahlemann and Gires, LMU Munich and Helmholtz Zentrum Munich, Germany.

MATra – best transfection practices for Neurosciences

Applications in Neurosciences

Neurosciences are a vast and expanding field of research focussing on highly sophisticated and enthralling questions. With Magnet Assisted Transfection IBA offers a very gentle and potent tool for the transfection of many kinds of neuronal cells. Magnet Assisted Transfection is the ideal solution to overcome problems related to the study of complex and easily interrupted systems.



Transfection of APP into neuroblastoma using MATra

Cells maintain their endogenous expression pattern and stay unaffected from transfection related influences:

B103 neuroblastoma cells were plated at 10^5 cells/well in Dulbecco's modified Eagle's medium + 10% fetal calf serum on poly-L-ornithine-coated glass coverslips in 24-well plates (Corning Life Sciences, Lowell, MA) and transfected using 0.2–0.8 µg of plasmid DNA per well and MATra-A beads on a 24 Magnet Bar Plate. The medium was changed 1–2 hrs after transfection, and expression was allowed to proceed for a further 16–24 hrs.

Figure: Investigation of APP dimerization using APP-GFP. A, confocal image of a B103 cell expressing APP-GFP. B–G, wide-field images of B103 cells expressing APP-GFP alone (B–D) or in combination with APP-mCherry (E–G). B and E, GFP channel. C and F, mCherry channel. D and G, GFP lifetime. Scale bars: 10 µm. H, histograms of FRET efficiencies in different experimental conditions. PDF, probability density function.

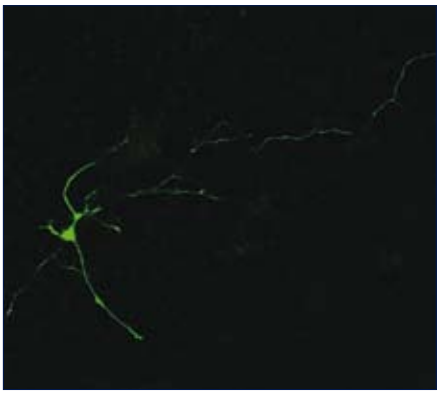
Expression levels were high enough to aquire fluorescence lifetime images (Fig. B-G), which permitted calculating the levels of interaction between APP-GFP molecules in the cell (Fig. H)

Data kindly provided by Dr. Matthias Gralle, Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany.

"Several liposomal methods were tried out, but the transfection efficiency was low, and the transfected cells were rounded and visibly unhealthy. With MATra, the expression pattern of APP-GFP was indistinguishable from the known expression pattern of endogenous APP, and the cells maintained the typical elongated morphology with protrusions", said Dr. Gralle at MPI, Leipzig. Published in JBC 2009, see page 7.

Neurons transfected with eGFP plasmid

Primary hippocampal neurons (E14) were grown on 15 mm glass coverslips on a 12 well plate at a density of 150.000/cm^2. The neurons were transfected 4 d.i.v. with pSyn-eGFP using 25 µl MATra complex per well (prepared by adding a MATra-A Reagent-DNA complex mixture (2.8 µg cDNA; 2.8 µl beads) into 175 µl neuronal medium without serum). The cells were fixed 6 d.i.v. with 4% PFA and imaged.



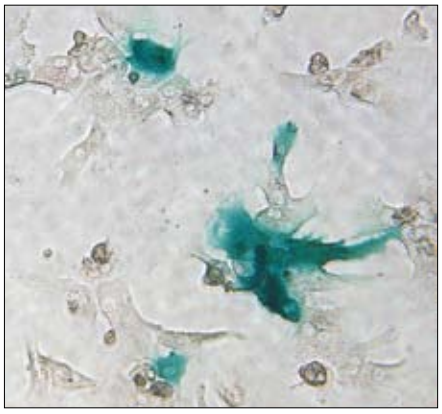
"With MATra we can transfect and modulate the expression levels of exogenous proteins in highly sensitive primary neurons without any toxicity. Once optimized, double and even triple transfections with different DNA ratios are easily achieved" said Dr. Mika Ruonala, Center for Membrane Proteomics, Frankfurt.

Data kindly provided by Dr. Mika Ruonala, Center for Membrane Proteomics, University of Frankfurt, Germany; ruonala@em.uni-frankfurt.de.

Applications in Hepatology

Magnet Assisted Transfection (MATra) of primary rat hepatocytes

Primary hepatocytes are of great scientific interest and are commonly used as a model system to study mechanisms of cell growth and differentiation, biotransformation, drug pharmacokinetics and toxicity of drugs. However, most methods fail to efficiently transfect primary hepatocytes.

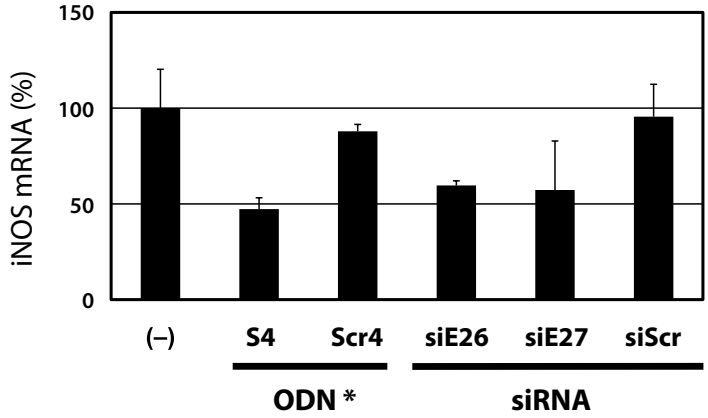


Hepatocytes were transfected with pCMV-LacZ, a CMV enhancer/promoter-driven β-galactosidase plasmid.

Data kindly provided by Prof. Dr. Mikio Nishizawa, Department of Biomedical Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan.

Magnet Assisted Transfection technology enables:

- **Transfection of slowly dividing cells such as hepatocytes**
- **A non-viral transfection method for cells, that are difficult to transfect**
- **High transfection efficiencies using DNA, ODN* and siRNA**



Both sense ODN and siRNAs effectively degrade iNOS mRNA. Rat hepatocytes (1.2 x 10^6 cells/well) were transfected with 1 µg ODNs (S4 and Scr4) or siRNAs (siE26, siE27, and siScr) using 1 µL MATra-A reagent. Total RNA was extracted from IL-1β-treated hepatocytes and subjected to real-time PCR of iNOS and EF-1α mRNA (n=3). Expression levels of iNOS mRNA were normalized by those of EF-1α mRNA.

As shown in the Figure, the sense ODN S4 corresponding to iNOS gene exon 27 **reduced the level of iNOS mRNA to 63.9%** to that of mock transfection. We designed siRNAs (siE26 and siE27) corresponding to iNOS gene exon 26 and 27, respectively. When they were introduced, iNOS mRNA was reduced to the levels which were comparable to that when S4 was introduced. For details see www.magnet-assisted-transfection.com/siRNA.html.

* oligodeoxyribonucleotide

„MATra is a powerful tool to transfect difficult cells, such as primary cultured rat hepatocytes, with ALL the types of DNA/RNA including plasmid DNA, ODN, and siRNA." Prof. Dr. Nishizawa, Ritsumeikan University, Kusatsu, Japan

References see page 7 and www.magnet-assisted-transfection.com.

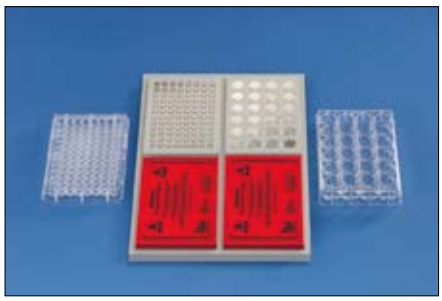
IBA: Your provider of dsRNA

High quality RNA and attractive pricing! See also www.rna-tools.com.

Ready-to-use: RNA duplexes, purified, deprotected and annealed, plus negative control (sense and antisense); **unlabeled**	
scale: 0.2 µmol **amount:** 20 – 40 nmol (140 – 280 µg) dsRNA; 5-10 nmol ssRNA as control cat.no. 5-0515-113	1.0 µmol 50 – 100 nmol dsRNA; 10 – 20 nmol ssRNA as control cat.no. 5-0515-114
Ready-to-use: RNA duplexes **with fluorescent label or biotin**, purified, deprotected and annealed, plus negative control (sense and antisense)	
scale: 1.0 µmol **amount:** 50 – 100 nmol dsRNA; 10 – 20 nmol ssRNA as control Cat.no. 5-0516-124	
Ready-to-use: control siRNA (ß-Actin; lamin A/C) 5, 10 or 25 nmol, unlabeled	
Cat.no. 5-0515-142/3 or 4 (lamin A/C) Cat.no. 5-0515-152/3 or 4 (ß-Actin)	

Magnet Plates

- Universal magnet plate (8 x 13 cm) suitable for Magnet Assisted Transfection of cells grown in culture plates, e.g. 6well, 12well, 24well, 48well or 96well plates, T75 flasks, 60 mm dishes etc.
- Universal magnet plate (26 x 26 cm) suitable for Magnet Assisted Transfection of cells grown in culture plates, e.g. 6well, 12well, 24well, 48well or 96well plates, T75 flasks, T175 flasks, 60 mm dishes, 100 mm dishes etc.
- For 96well culture plates we are offering a special 96 Magnet Bar Plate (8 x 12 cm) optimized for transfection in 96 individual wells
- Compatible with culture plates from the most common suppliers
- Can easily be decontaminated with 70% ethanol
- Can be used within incubators and robots
- Several generations of magnet plate development (type of magnet and magnetic field) resulted in a unique plate for which a utility patent application is issued



Set of 4 magnet plates on a solid support (Universal, 96 and 24 Magnet Bar Plates can be combined individually) for multi-user parallel use.

Universal magnet plate (8 x 13 cm) with collection of different culture plates and MATra reagents.

96 Magnet Bar Plate (8 x 12 cm) with 96well culture plate.

The MATra technology is compatible with robots for high-throughput transfections!

Selected References for Magnet Assisted Transfection

Technology

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Applications

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MATra assay formats

Formats	Volume of MATra-A or MATra-si Reagent recommended per well [µl]	Transfections per 200 µl vial
96 well plate	0.1	2000
48 well plate	0.3	667
24 well plate	0.6	333
12 well plate	1.2	167
6 well 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

STORAGE WARNING: All MATra reagents with magnetic beads have to be stored distant (> 10 cm) from strong magnetic fields like the Magnet Plates at all times!!! Otherwise agglutination of the beads may lead to complete loss of transfection function.

Order information for MATra reagents

product	amount	cat. no.
MATra-A Reagent	200 µl (for 200 µg nucleic acids)	7-2001-020
MATra-A Reagent	1 ml (for 1000 µg nucleic acids)	7-2001-100
MATra-si Reagent	200 µl (for 200 µg nucleic acids)	7-2021-020
MATra-si Reagent	1 ml (for 1000 µg nucleic acids)	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	1 ml (for up to 1000 µg nucleic acids)	7-2003-100
IBAfect	500 µl	7-2005-050
IBAfect	1 ml	7-2005-100
IBAfect	5 ml	7-2005-500

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

Further Magnet Bar Plate Set combinations or 384 Magnet Bar Plates on request.

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