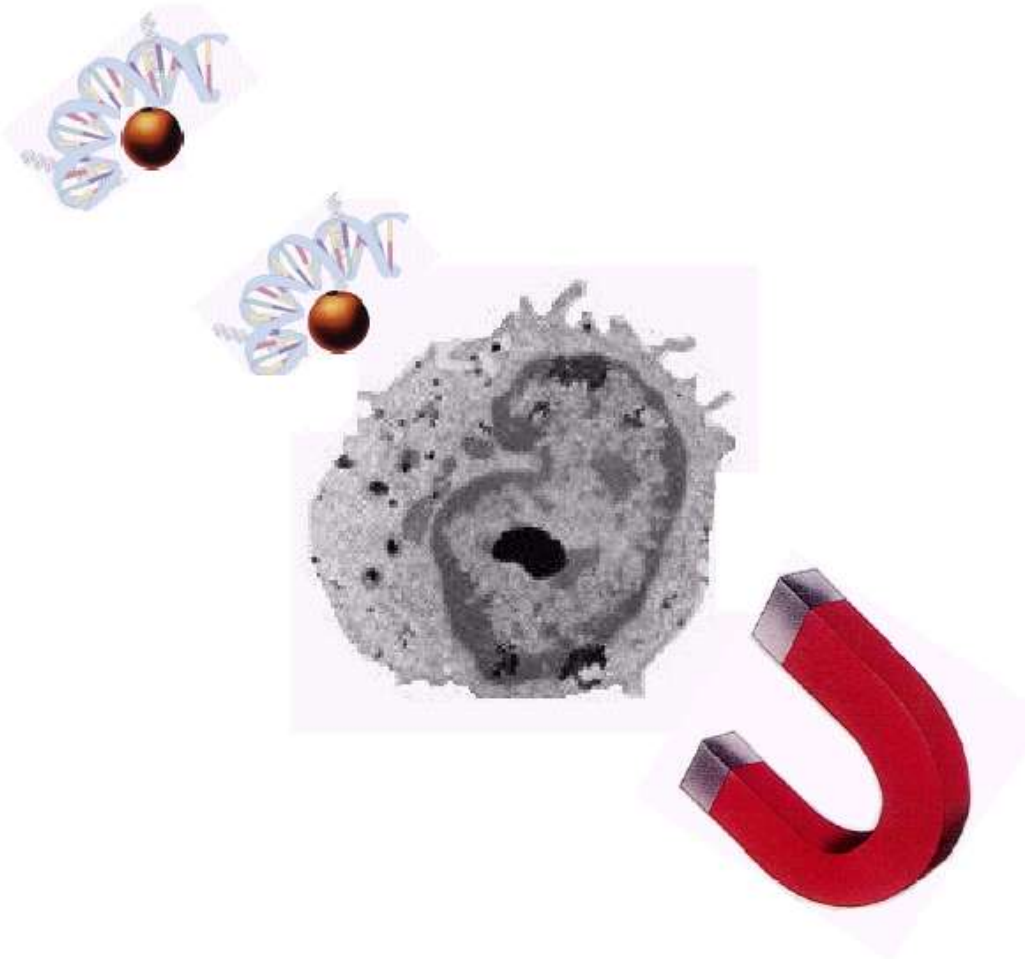
the new gene transfection technology

Magnetofection™ is a novel, simple and highly efficient method to transfect cells in culture.



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Technology

)a Description

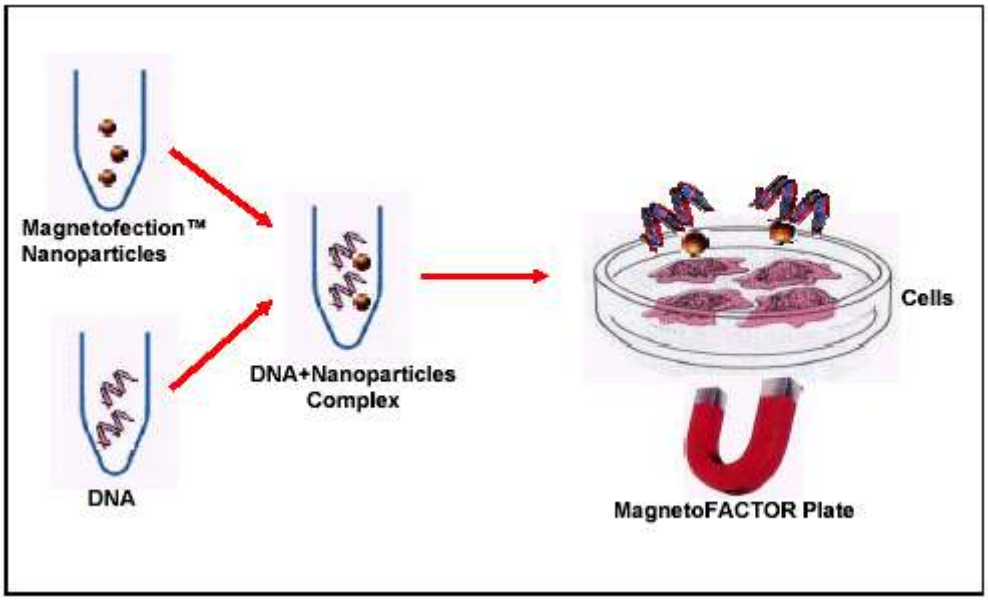
Magnetofection™ is a novel, simple and highly efficient method to transfect cells in culture. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to draw the vectors towards, possibly even into, the target cells. In this manner, the full vector dose applied gets concentrated on the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose. This has several important consequences:

• Greatly improved transfection rates in terms of percentage of cells transfected compared to standard transfection.

• Up to several thousand fold increased levels of transgene expression compared to standard transfections upon short-term incubation.

• High transfection rates and transgene expression levels are achievable with extremely low vector doses, which allows to save expensive transfection reagents.

• Extremely short process time. A few minutes of incubation of cells with gene vectors are sufficient to generate high transfection efficiency, compared to several hours with standard procedures.



)b Magnetofection™ Reagents

As the manufacturer of the Magnetofection™ technology, Interbiotech offers two types of ready-to-use Magnetofection™ reagents.

PolyMAG is a universally applicable magnetic particle preparation for high efficiency nucleic acid delivery. It is mixed in a one-step procedure with the nucleic acid to be transfected and has been used successfully with plasmid DNA, antisense oligonucleotides and siRNAs.

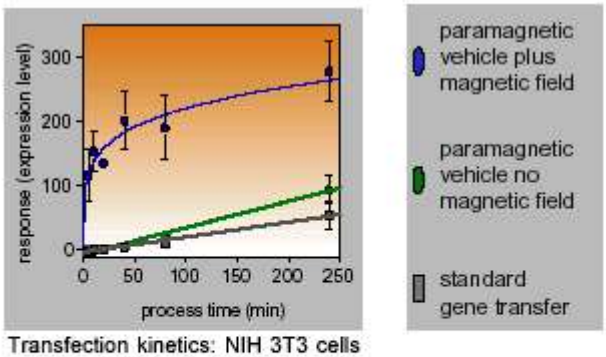
CombiMAG is a magnetic particle preparation designed to be combined with any commercially available transfection reagent such as polycations and lipids and can be associated with plasmid DNA, antisense oligonucleotides, siRNAs or viruses. It allows you to create your own magnetic gene vector based on your favourite transfection reagent.

Purchaser Notification

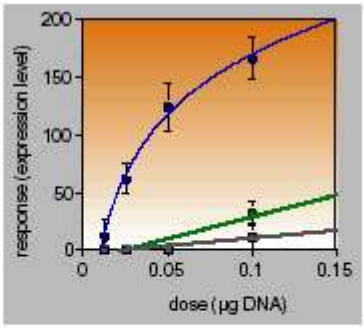
The Magnetofection™ reagents and all of its components are developed, designed, intended and sold for research use only. They are not to be used for human diagnostic or any drug intended.

Magnetofection™ are registered trademark

)c Nuclec Acids Dose Response and Transfection Kinetics



Transfection kinetics: NIH 3T3 cells were incubated with GenePorter™ (Gene Therapy Systems) ± CombiMAG with and without positioning on the Magneto-FACTOR plate for the indicated time spans. Luciferase expression was assayed after 24 hours.



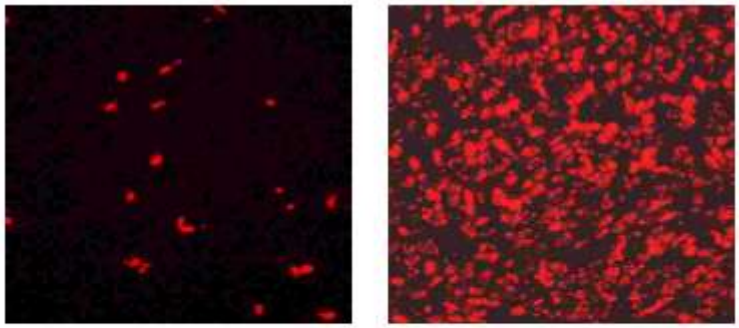
Dose response profile in NIH 3T3 cells using Lipofectamine™ (Invitrogen) ± CombiMAG with and without positioning on the MagnetoFACTOR plate for 15 min. Luciferase expression was assayed after 24 hours.

)d Application

Magnetofection™ is generally applicable for adherent cells and has been tested with a variety of immortalized cell lines and primary cells listed below. If a particular cell type or cell line is not listed this does not mean that Magnetofection™ would not work. Also for the cells listed, some reagents have not been tested so far, as indicated by “n.d.” (not determined).

The **CombiMAG** reagent can be combined with any polycationic and lipidic transfection reagent, and also with adenoviral and retroviral vectors. In some cases, references are made in the footnotes to very successful combinations with commercially available reagents that have been tested so far.

Type of nucleic acid / virus	PolyMAG	CombiMAG
Plasmid DNA	✓	✓
Antisense Oligonucleotides	✓	✓
SiRNA	✓	✓
Adenovirus	n.d	✓
Retrovirus	n.d	✓



Primary human umbilical vein endothelial cells positioned on the MagnetoFACTOR plate were incubated for 15 min with a Cy3 fluorescence-labeled antisense-oligonucleotide complexed with Effectene™ (Qiagen; left) or Effectene™ + CombiMAG (right).

(Data kindly provided by F. Kroetz. Ludwig-Maximilians University Munich)

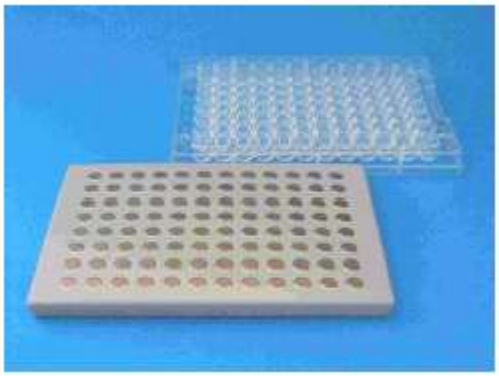
Cell Line	Cell Type	PolyMAG	CombiMAG
K-562	Myelogenous leukemia	**n.d**	✓
293	Kidney transformed	✓	✓
HeLa	Epithelial	✓	✓
COS-7	Fibroblasts	✓	✓
CHO	Epithelial-like	✓	✓
NIH3T3	Fibroblasts	✓	✓
HepG2	Hepatoma	✓	✓
16HBE14o	Human airway epithelium	✓	✓
CT-26	Colon Carcinoma	✓	✓
HUVEC	Primary endothelial cells	✓	✓
B16F10	Mouse Melanoma	✓	✓
USOS	Human osteosarcoma	**n.d**	✓
A549	Human non-small cell lung carcinoma	**n.d.**	✓
181RDB	Pancreatic cells	**n.d.**	✓
Primary keratinocytes		**n.d.**	✓
Primary chondrocytes		✓	✓
Primary fibrochondrocytes		✓	✓
Primary peripheral blood lymphocytes		**n.d**	✓
Primary carotid artery smooth muscle cells		✓	**n.d.**
Primary aortic endothelial cells (PAEC)		✓	✓
Primary porcine airway epithelium		**n.d**	✓
Primary human nasal airway epithelium		✓	n.d

Utensils for Magnetofection™

)a MagnetoFACTOR plate

Apart from suitable magnetic nanoparticles, Magnetofection™ requires appropriate magnetic fields. These are provided by the MagnetoFACTOR plate, especially designed for Magnetofection™.

The MagnetoFACTOR-96 plate its special geometry not only produces strong magnetic fields under each well of 96-well plates but is also applicable for other plate formats T-75 culture flasks, 6- and 12-well plates. In the larger plate formats, the MagnetoFACTOR plate will produce a pattern of higher and lower densities of transfected cells according to the geometry of the magnetic field lines. The MagnetoFACTOR-24 plate is special designed for the 24-well format. The MagnetoFACTOR plates are compatible for well plates of the most manufacturer.



MagnetoFACTOR-96 plate

MagnetoFACTOR-24 plate

)e Kits contents

Product Number	**Discription**	**Size** (sufficient for n µg DNA)	**Number of transfections** (96-well format)
BC3012	PolyMAG-100	100	1000
BC3014	PolyMAG-500	500	5000
BC3015	PolyMAG-1000	1000	10.000
BC3042	CombiMAG-100	100	2000
BC3043	CombiMAG-500	500	10.000
BC3044	CombiMAG-1000	1000	20.000
BC3052	MagnetoFACTOR plate 96		

Storage:
All components of the Magnetofection™ kit should be stored at room temperatur (20-25°C). After first use store the kit at +4°C.
• Do not freeze the magnetic nanoparticles
• Do not add anything to the stock solution of magnetic nanoparticles
• Shipping Conditions: Room Temperatur

)f General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cell lines. Optimal conditions do vary from cell line to cell line. Therefore, the amount of DNA used and the ratios of the individual components may have to be adjusted to achieve best results. The following recommendations can be used as guidelines to achieve good transfection with minimal incubation times.

)g General Protocol

Adherent cells are seeded such that they reach 60-80% confluency at the time of Magnetofection™.
Suspension cell lines (0.1-2x106 cells/96 – 6 well) are suitably seeded in polylysinecoated plates or use the special protocol 4.4. (Suspension cells) for Magnetofection™. Immediately preceeding transfection, the medium is replaced with 150 µl of fresh medium (optionally without serum).

Tissue Culture dishes	Cell Number	DNA Quantity (µg)	Transfection Volume
96 well	0.5-2 x 10^4	0.1- 0.5	200 µl
24 well	0.5 - 1 x 10^5	0.5 - 2	500 µl
12 well	1- 2 x 10^5	2 - 4	1 ml
6 well	1- 4 x 10^5	2- 6	2ml
60 mm dish	5 - 10 x 10^5	6 - 8	5 ml
90-100 mm dish	10 - 20 x 10^5	8 - 12	10 ml
T-75 flask	20 - 50 x 10^5	15 - 25	15 - 20 ml

Cell Number and Transfection Volume Suggested

Protocol

The same protocol can be used to produce stably transduced cells except that 48 hours post transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours before exposing the transduced cells to selection media.

Vectors are prepared in medium without serum and supplement or in physiological saline because serum may interfere with vector assembly. According the standard Magnetofection™ protocol, the serum and supplement-free vector cocktail is added to the cells that are covered with complete medium. Therefore, the addition of the transfection cocktail will result in the dilution of supplements such as serum,antibiotics or other additives of your standard culture medium. Although a medium changes after Magnetofection™ is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration. Alternatively, the cells may be kept in serum-free medium during Magnetofection™. In this case, a medium change will be required after Magnetofection™.

)a Magnetofection in T-75 Culture Flasks, 6-, 12- or 24-well Plate Formats

As a rule of thumb, a DNA dose of 50 to 300 ng per square centimeter culture dish will yield good results. However, it is emphasized that every cell line requires optimization with respect to DNA dose and vector formulation.

A useful DNA dose in the 6-well format is 1 or 2 micrograms of DNA. The ratio of magnetic particles and DNA remains the same as in the general protocol.

The easiest way to generate the complexes is to provide the required amount of magnetic particles in an Eppendorf tube. To this you add DNA which has been diluted with serum-free medium (e.g. DMEM) to 2 or 4 micrograms per ml. After 20 min incubation, add 500 microliters of this to the cells. Position the culture plate on the magnetic plate for up to 15 min, then perform a medium change.

Adding complexes to the cells: You can either have the cells in 1 to 1.5 ml of their appropriate medium and add the 500 microliters of complexes. Otherwise you can completly remove the medium, add the complexes, incubate while the culture dish is postioned on the magnetic plate and then perform a medium change.

As always, DNA dosage and exact conditions depend on potential toxicity etc. If toxicity is high, reduce the dose and possibly also the duration of incubation with complexes.

Similar procedures apply for the other culture dish formats. A useful DNA dose for T-75 culture flasks is 10 micrograms, for 12- and 24-well plates 200 nanograms up to one microgram.

In the latter case, provide the DNA dose in a volume of 200 microliters, otherwise proceed as described above for the 6-well plate format.

For the T-75 flask format provide the DNA in a volume of 0.5 to 1 ml medium,and mix with the required amount of magnetic particles.

After 20 min incubation add this to the cells which are covered with 5 to 10 ml of medium. Make sure to thoroughly mix the added vector dose with the medium covering the cells and then place the culture plate on the MagnetoFACTOR plate. For the rest, proceed as described above.

)h Cell Preparation – Suspension Cells

It is necessary for the Magnetofection™ that the cells are immobilizate on the well bottom. Therefore seed the suspension cells on polylysine-coated plates or use the following protocol for magnetic immobilization.

1. Dilute the cells to be transfected to 5 x 105 - 1 x 106 / ml in medium (serum- or supplement-containing or serum-free; depending on cell type and sensitivity of cells towards serum-free conditions).

2. Mix cell suspension with 30 µl of CombiMAG reagent per 1ml of cell suspension. Incubate for 10 - 15 min.

***Note**: vortex the CombiMAG before used.*

3.Distribute cells at 100 µl each per well of a flat-bottom 96-well plate placed upon the MagnetoFACTOR plate. Incubate for 15 min.

***Note**: If required in your setup, predilute CombiMAG with ddwater*

)i PolyMAG

1. Add 2.8 µl of PolyMAG to a microcentrifuge tube or a U-bottom well of a 96- well plate.

***Note**: vortex the PolyMAG before used.*

2. Dilute 2.8 µg of DNA to 175 µl with serum- and supplement-free medium (such as DMEM).

3. Add the 175 µl DNA solution to the 2.8 µl of PolyMAG and mix immediately by vigorous pipetting.

4. After 20 to 30 minutes add 12.5 µl or 25 µl or 50 µl each of the resulting mixture in triplicates to the cells (corresponding to 0.2 µg or 0.4 µg or 0.8 µg of DNA per well, respectively).

***Note**: The total transfection volumes/per well are suggested in the table above.*

5. Place the cell culture plate upon the MagnetoFACTOR plate for 5 to 20 minutes.

6. Remove the MagnetoFACTOR plate,

6a.Optionally perform a medium change.

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

***Note**: If required in your setup, predilute PolyMAG with ddwater*

)j CombiMAG

A number of suppliers sell efficient transfection reagents. All of these can be made a magnetofectin by simple mixing with CombiMAG, usually resulting in strong improvements of these reagents efficiencies.

There are two strategies of using CombiMAG:

• One is to prepare a standard DNA complex with a commercial transfection reagent according to the instructions of the manufacturer, followed by mixing with CombiMAG.

• The second strategy is to first mix DNA and CombiMAG followed by mixing with the transfection reagent.

Also in this case, the instructions of the manufacturer are used with the only exception that instead of DNA alone, a mixture of DNA and CombiMAG is added to the transfection reagent.

Depending on the transfection reagent, the mixing order of components may influence the final transfection efficiency. It is recommended to use 1 µl of CombiMAG per microgram of DNA in initial experiments.

However, depending on the cell line to be transfected and the commercial transfection reagent used, the optimal composition may be found above or below this ratio.

***Note**: If required in your setup, predilute CombiMAG with ddwater.*

)k Troubleshooting

Low Transfection Efficiency

- **Inappropriate buffer composition**

Serum-free buffer or medium has to be used for the formation of the Poly- or CombiMAG/DNA complex, otherwise proteins from the serum will bind to Poly- or CombiMAG; once the Poly- or CombiMAG/DNA complex is formed it can be applied to cells in the presence of serum.

- **Suboptimal ratio of Poly- or CombiMAG to nucleic acid or virus**

Determine the optimal ratio of Poly- or CombiMAG to DNA by using the optimization protocol for 96-well plate (see chapter 4.11.).

- **Correct handling of the MagnetoFACTOR plate**

Use the MagnetoFACTOR plate with the magnets facing up. After addition of the Poly- or CombiMAG/DNA complexes to the cells, position the cell culture plate on the MagnetoFACTOR plate.

- **Positive control**

Perform a positive control transfection experiment with a well-characterized reporter gene (e,g. GFP, Luciferase).

Cellular Toxicity

- **Cell density (% confluence) was not optimal at the time of transfection:**

Adherent cells are seeded such that they reach 60-80% confluency at the time of Magnetofection™. 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 (see chapter 4.4.).

- **Suboptimal amount of DNA (see chapter 4.11.):**

For the Magnetofection, one uses approximately 5x less DNA as for Lipofection.

- **Purity of transfecting molecule**

Check the purity of the molecule of interest to be delivered (lipopolysaccharides which are endotoxins will cause cell death).

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