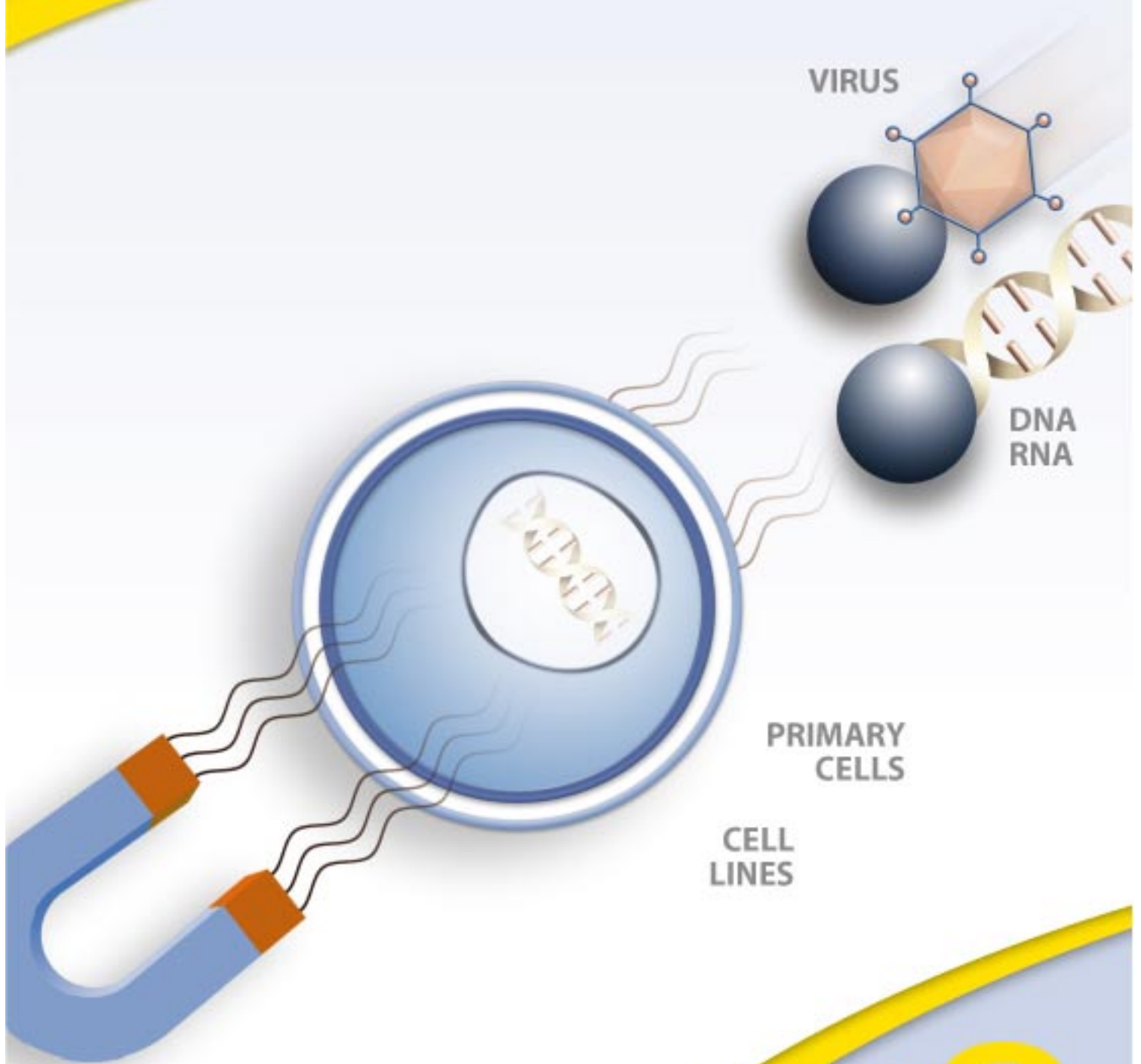


Magnetofection™ INSTRUCTION MANUAL



Magnetofection™ is a novel, simple and highly efficient *in vitro* and *in vivo* transfection method *

List of Magnetofection™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)	Number of transfections / 96 well plates
PN-30100	PolyMag II	100	100	1000
PN-30200	PolyMag II	200	200	2000
PN-31000	PolyMag II	1000	1000	10000
CM-20100	CombiMag	100	100	1000
CM-20200	CombiMag	200	200	2000
CM-21000	CombiMag	1000	1000	10000
KM-30200	Magnetofection Selection Kit ¹	200 (2 X 100)	200	2000
KC 30296	Magnetofection Starting Kit ²	200 (2 X 100)	200	2000
MF-10096	Magnetic Plate	N/A	N/A	N/A

¹ Contains 1 vial of each reagent (*PolyMag* and *CombiMag*)

² Contains 1 vial of each reagent (*PolyMag* and *CombiMag*) plus a Magnetic Plate

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us. For all other supplementary information, do not hesitate to contact our dedicated technical support.

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OZ BIOSCIENCES
The art of delivery systems

* Patent Pending

1. Technology

1.1. Description

Congratulations on your purchase of the Magnetofection™ reagent!

Magnetofection™ is a novel, simple and highly efficient method to transfect cells in culture and in vivo. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the vectors towards, possibly even into, the target cells. In this manner, the complete applied vector dose 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:

1. Greatly improved transfection rates in terms of percentage of cells transfected compared to standard transfections.
2. Up to several thousand folds increased levels of transgene expression compared to standard transfections.
3. High transfection rates and transgene expression levels are achievable with extremely low vector doses, which allow saving expensive transfection reagents.
4. Extremely short process time in comparison to standard procedures. A few minutes of incubation of cells with gene vectors are sufficient to generate high transfection efficiency.

Based upon a validated and recognized magnetic drug targeting technology this innovative method is:

- Efficient
- Simple & rapid
- Multipurpose (for all types of nucleic acid and viral & non-viral vectors)
- Universal (primary cells and cell lines)
- Non toxic & economical

1.2. Available Kits

OZ Biosciences offers two types of ready-to-use Magnetofection™ reagents.

1. **PolyMag** is a universally applicable magnetic particle preparation for high efficiency nucleic acid delivery. Nucleic acids to be transfected and the magnetic particles are mixed in a one-step procedure **PolyMag** has been used successfully with plasmid DNA, phosphorothioate antisense oligonucleotides and siRNAs.
2. **CombiMag** is a magnetic particle preparation designed to be combined with any commercially available transfection reagent such as cationic polymers and lipids and can be associated with viruses. **CombiMag** has been used successfully with plasmid DNA, antisense oligonucleotides, siRNAs adenovirus and retrovirus

1.3. Kit Contents

Kit contents varies according to their size

- One tube containing 100 µL of particle suspension good for 100 transfections with 1 µg of DNA
- One tube containing 200 µL of particle suspension good for 200 transfections with 1 µg of DNA
- 1 tube containing 1000 µL of particle suspension good for 1000 transfections with 1 µg of DNA

Stability and Storage

Storage +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. Magnetofection kits are stable for at least one year at the recommended storage temperature.

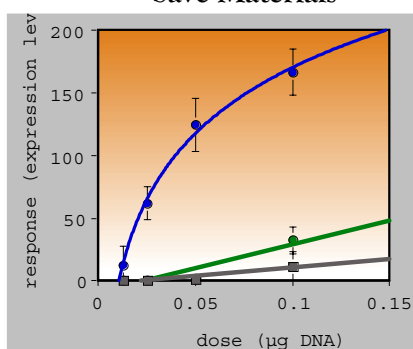
- DO NOT FREEZE THE MAGNETIC NANOPARTICLES!
- DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!

Shipping condition Room Temperature

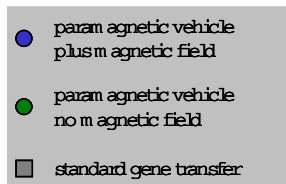
2. Applications

2.1. Nucleic Acids Dose Response and Transfection Kinetics

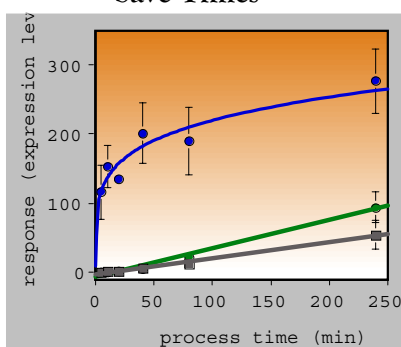
Save Materials



DNA dose response profile. NIH-3T3 cells were transfected with a commercial transfection reagent L +/- **CombiMag** with and without the magnetic field for 15 min. Luciferase expression was assayed after 24 hours.



Save Times



Transfection kinetics NIH-3T3 cells were incubated with a commercial transfection reagent G ± **CombiMag** with and without positioning on the magnetic plate for the indicated time spans. Luciferase expression was assayed after 24 hours.

2.2. Nucleic Acids Types and Vectors

The **CombiMag** reagent can be combined with any nucleic acid, any cationic polymer-based and lipid-based transfection reagent and also with virus (adenovirus or retrovirus).

<i>Nucleic Acid or Virus Type</i>	<i>PolyMag</i>	<i>CombiMag</i>
DNA (plasmid)	√	√
Antisense Oligonucleotides	√	√
siRNA	√	√
Adenovirus	n.d.	√
Retrovirus	n.d.	√

Magnetofection™ is generally applicable on numerous cell types. This technology has been tested successfully on a variety of immortalized cell lines as well as primary cells (see table below). If a particular cell type or cell line is not listed below, this does not imply that Magnetofection™ is not going to work. OZ Biosciences is going to maintain an updated list of cells successfully tested that is available on the website: www.ozbiosciences.com. For the cells listed, some reagents have not been tested so far, as indicated by "n.d." (not determined).

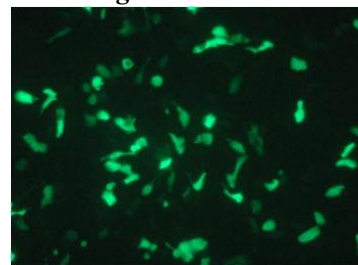
Primary Cells

Confluent Primary Human Keratinocytes
Transfected with a commercial reagent L or **PolyMag**
Reporter Gene: *GFP**

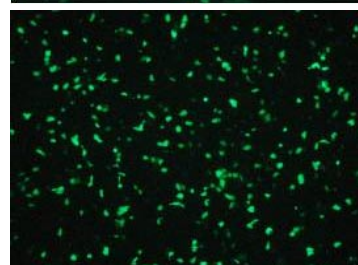
Standard Transfection



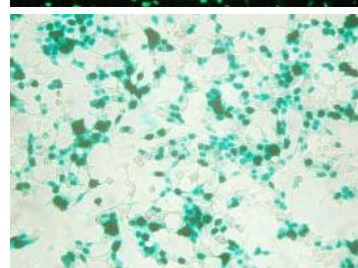
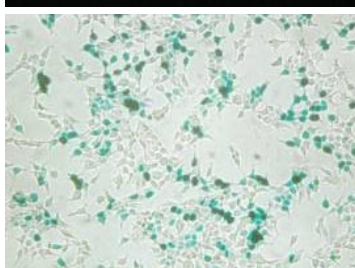
Magnetofection™



Primary Human Keratinocytes
Transfected with a commercial reagent F +/- **CombiMag**
Reporter Gene: *GFP**



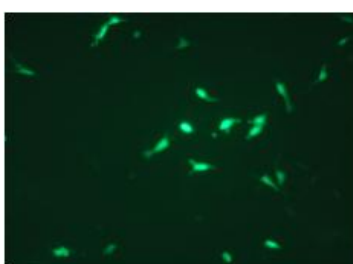
Primary Chondrocytes / Pig
Transfected with a commercial reagent F +/- **CombiMag**
Reporter Gene: *βgalactosidase**



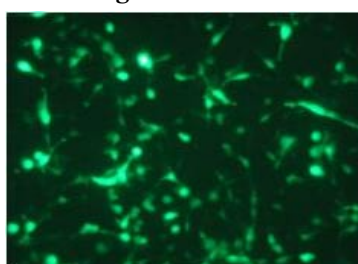
Cell Lines

CT-26 Colon Carcinoma / Mouse
Transfected with a commercial reagent D or **PolyMag**
Reporter Gene: *GFP**

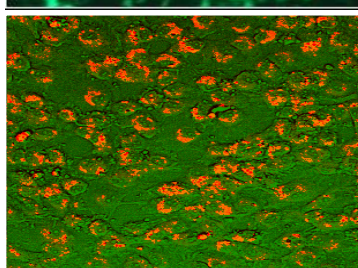
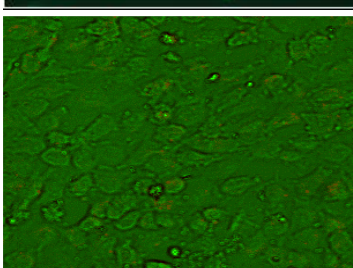
Standard Transfection



Magnetofection™



HUVEC-C (Primary) / Human
Transfected with a commercial reagent E +/- **CombiMag**
Fluorescent Oligonucleotides **



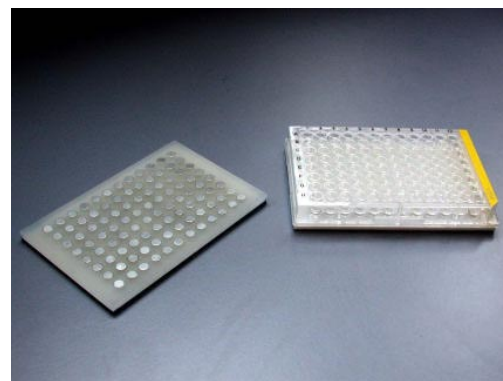
We are grateful to the laboratories of * Dr. C. Plank (Technical University, Munich) and of ** Dr. F. Kroetz (Ludwig-Maximilians University, Munich) for kindly providing these data.

<i>Cell Line</i>	<i>Cell Type</i>	<i>Source</i>	<i>PolyMag</i>	<i>CombiMag</i>
293, HEK-293, 293-T, -EBNA	Transformed Embryonic Kidney	Human	√	√
16HBE14o	Airway Epithelium	Human	√	√
181RDB	Pancreatic Cells	Human	n.d.	√ ²
A549	Non-small cell lung carcinoma	Human	n.d.	√ ^{1, 2}
B16F10	Melanoma	Mouse	√	√
CHO-K1	Epithelial-like (Ovary)	Hamster	√	√
COS-1	Fibroblast (Kidney)	Green Monkey	√	√
COS-7	Fibroblast (Kidney)	Green Monkey	√	√
CT-26	Colon Carcinoma	Mouse	√	√
CV-1	Fibroblast-like (Kidney)	Monkey	√	√
HeLa	Cervical Epithelial Carcinoma	Human	√	√
HepG2	Hepatoma	Human	√	√
HUVEC	Endothelial Cells (primary)	Human	√	√
K-562	Myelogenous Leukemia	Human	n.d.	√ ¹
L929	Fibrosarcoma	Mouse	√	√
MCF-7	Breast Adenocarcinoma	Human	√	√
MDCK	Normal -Kidney	Canine	√	√
NIH3T3	Fibroblasts	Mouse	√	√
PC-12	Pheochromocytoma (adrenal)	Rat	√	√
SAOS	Osteosarcoma	Human	n.d.	√ ²
U937	Histiocytic Lymphoma	Human	n.d.	√ ²
Primary Airway Epithelium		Pig	n.d.	√
Primary Aortic Endothelial Cells (PAEC)		Human Bovine	√	√ ¹
Primary Carotid Artery Smooth Muscle Cells		Bovine	√	n.d.
Primary Chondrocytes		Pig	√	√ ¹
Primary Fibrochondrocytes		Pig	√	√
Primary Keratinocytes		Human Mouse	n.d.	√ ¹
Primary Nasal Airway Epithelium		Human	√	√
Primary Peripheral Blood Lymphocytes		Human Mouse	n.d.	√ ^{1,2}

¹ Successfully tested in combination with several commercially available transfection reagents (F, L, D, E...) and ² adenovirus. *, ** For more information see the bibliographic references at the end of this manual.

3. Magnetofection™ Apparatus

Apart from suitable magnetic nanoparticles, Magnetofection™ requires appropriate magnetic fields. A magnetic plate especially designed for Magnetofection is provided to exert these specific magnetic fields. 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 flasks, 60 & 100 mm dishes, 6-, 12- and 24-well plates). In the larger plate formats, the magnetic plate will produce a pattern of higher and lower densities of transfected cells according to the geometry of the magnetic field lines.



4. Example Protocols

4.1. 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 and are dependent on the nucleic acid, transfection reagent or virus used. Consequently, the amounts and ratio of the individual components (DNA and reagents) may have to be adjusted to achieve best results. Therefore, we advise you to optimize the various transfection or infection parameters (components concentration, cell number, incubation time...). Several protocol optimizations are available in the Appendix. The following recommendations can be used as guidelines to achieve good transfection with minimal incubation times.

4.2. General Protocol

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should be 60-90 % confluent at the time of Magnetofection (see the suggested cell number in the table below). For suspension cells, use the specific protocol given below or seed the cells on polylysine-coated plates (0.1 - 2x10⁶ cells/96 - 6 well plates) and use the protocol for adherent cells. Immediately preceding transfection, the medium can be replaced with fresh medium (optionally without serum) if necessary.

Cell Number and Transfection Volume Suggested

Tissue Culture Dish	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	2 - 4 x 10 ⁵	2 - 6	2 mL
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

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 to 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 change 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.

4.3. PolyMag ©

The protocol is as simple as follows: Use 1 μL of *PolyMag* per μg of DNA.

- 1) Before each use, vortex the *PolyMag* material. Add 1 to 10 μL of *PolyMag* (according to the DNA amount) to a microtube or to a microwell (U-bottom well is preferred to get a better mixing). If required and for doses less than 1 μL in your protocol, predilute *PolyMag* with deionized water.
- 2) Dilute 1 to 10 μg of DNA to 200 μL with serum and supplement-free culture medium (such as DMEM).
- 3) Add the ~200 μL DNA solution to the *PolyMag* solution and mix immediately by vigorous pipetting.
- 4) After 20 to 30 minutes of incubation, add the 200 μL of complexes to the cells. The total transfection volumes per well (culture medium + *PolyMag* mixture) are suggested in the table above. Note: to transfect cells in duplicate prepare your DNA/*PolyMag* complexes as described previously and transfer 100 μL of the resulting mixture to each well containing the cells to be transfected.
- 5) Place the cell culture plate upon the magnetic plate for 5 to 20 minutes.
- 6) Remove the magnetic plate. Optionally perform a medium change.
- 7) Cultivate the cells under standard conditions until evaluation of transgene expression.

4.4. CombiMag ©

Until now, a universal method allowing to enhance the efficiency of synthetic (non-viral) and viral gene delivery systems was lacking. Magnetofection™ is the only existing method answering these needs. The conducted studies have shown that Magnetofection:

- Increases the efficiencies of commercial transfection reagents & reduces the required DNA doses.
- Significantly improves the efficiencies of all types of nucleic acids delivered.
- Improves viral infectious capacity.
- Extend the host tropisms of viral vectors to non-permissive cells.

Transfection Reagents. A number of suppliers sell transfection reagents. All of these can be associated with *CombiMag* reagent by simple mixing in order to generate magnetic delivery system. The resulting combination leads to strong efficiency improvements for commercial transfection reagents. This solution allows you to create your magnetic gene vector.

There are two strategies of using *CombiMag*:

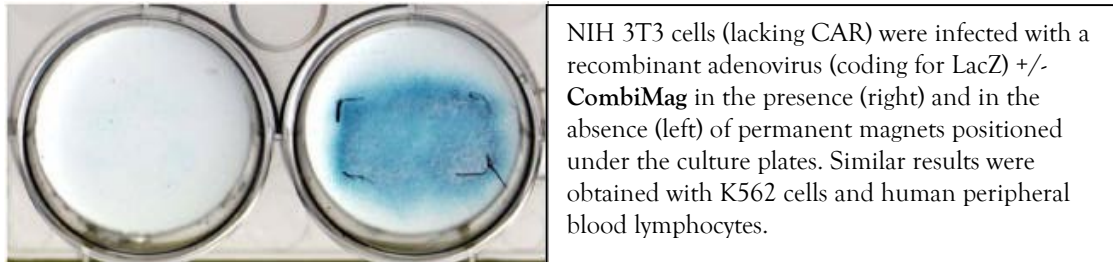
- One is to prepare a standard complex of DNA and a commercial transfection reagent according to the instructions of the manufacturer, followed by mixing with *CombiMag*.
- The second strategy is to first mix DNA and *CombiMag* followed by immediate mixing with the transfection reagent. In this case, the manufacturer's instructions are used except that instead of DNA alone, a mixture of DNA and *CombiMag* is added to the transfection reagent.

Depending on the transfection reagent used, the mixing order of components may influence the final transfection efficiency of Magnetofection™. It is recommended to use 1 or 2 µL of *CombiMag* per µg 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.

- 1) Before each use, vortex the tube of *CombiMag*. Add 1 or 2 µL of *CombiMag* per µg of DNA to be transfected to a microtube. For DNA doses of less than 1 µg predilute an aliquot of *CombiMag* reagent with deionized water and use the volume required for your DNA dose.
- 2) Prepare the DNA / transfection reagent complexes according to the reagent's manufacturer instructions, but omit the usual final incubation step after mixing DNA & reagent and immediately proceed to step 3.
- 3) Add the DNA / transfection reagent complex solution into the *CombiMag* suspension and mix immediately by vigorous pipetting.
- 4) Incubate for 15 - 30 minutes.
- 5) Add the resulting mixture to the cells to be transfected. Note: to transfect cells in duplicate prepare your DNA/transfection reagent complexes as described above. If the complexes have been prepared in 200 µL, then transfer 100 µL of the resulting mixture in each well containing the cells to be transfected. The total transfection volumes/ per well (culture medium + *CombiMag* mixture) are suggested in the table above.
- 6) Place the cell culture plate upon the magnetic plate for 5 to 20 minutes.
- 7) Remove the magnetic plate. Optionally perform a medium change.
- 8) Cultivate the cells under standard conditions until evaluation of transgene expression.
- 9) Depending on the commercial transfection reagent used, this protocol may have to be adapted.

Virus. Viral infection is highly cell surface receptor-dependent. For instance, adenoviruses are dependent on cells to express CAR (Coxsackie's and adenovirus receptor) and HIV on cells to express CD4. Unfortunately, many important and interesting target tissues for fundamental research and gene therapy are non-permissive to viral gene delivery (tumor tissues and apical surface of lung epithelium may express variable, little or none of the required receptors).

- The association of viral vectors with *CombiMag* is sufficient to force infection of non-permissive cells as shown with adenovirus.
- Magnetofection also increases retroviral infectious capacity.



Viral Magnetofection is carried out in the same manner as standard transductions with the exceptions

- That virus preparations are mixed with *CombiMag* prior transduction
- That the cell culture plate is positioned upon the magnetic plate during transduction
- That polybrene or other additives are NOT used for retroviral transductions.

- 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, which require cell division for infection. Cells must be plated the day prior transfection.
- 2) Provide a suitable amount (see examples below) of *CombiMag* in a tube large enough to contain the volume of virus preparation added in step 3.
- 3) Add your virus preparation (e.g. retroviral supernatant or purified adenovirus diluted in HBS, PBS or cell culture medium) to the tube(s) containing *CombiMag* and mix immediately by pipetting or gentle vortexing. Thereafter, incubate 20 minutes at room temperature.
- 4) The ratios virus / *CombiMag* should be adjusted according to the viral titers and cell types used. For optimization, we suggest as a starting point to use 1.5 μL , 3 μL , 6 μL , and 12 μL of *CombiMag* with a fixed quantity of virus preparation / supernatant.

For example:

- For K562, adenovirus (200 MOI) / 6 μL of *CombiMag*
- For human PBL, adenovirus (500 MOI) / 3 - 6 μL of *CombiMag*
- For NIH-3T3, adenovirus (200 MOI) / 3 - 6 μL of *CombiMag*
- For NIH-3T3, retrovirus ($1-5 \times 10^3$ Xgal CFU/ml) / 3 - 6 μL of *CombiMag*

- 5) Add the mixture prepared in step 3 to the cells in duplicate or triplicate.
- 6) Place the cell culture plate upon the magnetic plate for 30 minutes.

- 7) Remove the magnetic plate. Optionally perform a medium change.
- 8) Cultivate the cells under standard conditions until evaluation of transgene expression.
- 9) Depending on the viral vector type, the quantity of virus and the cell types used, this protocol would have to be adjusted.

4.5. Magnetofection™ of suspension cells

1. The composition and preparation of *PolyMag* / DNA or *CombiMag* / transfection reagent or virus are performed exactly as described above from steps 1 to 3.
2. While *PolyMag* / DNA or *CombiMag* / transfection reagent or virus incubate (step 4 above), dilute the cells to be transfected to 5×10^5 - 1×10^6 / mL in medium (with or without serum- or supplement; depending on cell type and sensitivity of cells towards serum-free conditions) and perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles.
 - a. Seed the cells on polylysine-coated plates and use the protocol for adherent cells.
OR
 - b. Briefly, centrifuge the cells (2 minutes) in order to pellet them and use the protocol for adherent cells
OR
 - c. Mix cell suspension with 30 μ L of *CombiMag* reagent per 1 ml of cell suspension.
 - i. Incubate for 10 - 15 minutes.
 - ii. Distribute cells to your tissue culture dish placed upon the magnetic plate (volume of culture medium containing cells depends on the culture dish size; see suggested transfection volume in table above as indication).
 - iii. Incubate for 15 minutes
3. Add the resulting mixture of *PolyMag* / DNA or *CombiMag* / transfection reagent or virus to the cells while keeping the cell culture plate on the magnetic plate.
4. Continue to incubate for 15 minutes.
5. Carefully remove the medium supernatant from the cells and replace with fresh complete medium while the culture plate remains positioned on the magnetic plate. Be careful not to aspirate the magnetically sedimented cells.
6. Remove culture plate from magnetic plate.
7. Continue to cultivate cells as desired until evaluation of transgene expression.

5.1. Protocol Optimization

We strongly advise you to optimize your transfection and/or infection conditions in order to get the best out of Magnétofection™. Several parameters can be optimized:

- Nucleic acid or viral vector dose used
- Ratio of *CombiMag* / *PolyMag* to nucleic acid/virus
- Cell type and cell density
- Incubation time

OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedures described above (section 4).

- 1) Start by optimizing the ratio *PolyMag* / DNA or *CombiMag* / transfection reagent or virus. To this end, use a fixed amount of DNA and transfection reagent. Vary the amount of *CombiMag*/*PolyMag* from 0.25 to 5 μL / μg of DNA. The ratio *PolyMag* or *CombiMag* / DNA can be changed by doubling or multiplying the volumes of the reagents used. Similarly, the reagents can be pre-diluted in deionized water and aliquots of the resulting dilutions are incubated with DNA or pre-formed DNA complexes. Finally, the different components can be serially diluted to very low concentrations.
- 2) Thereafter, change the nucleic acid dose or viral MOI with a fixed ratio of *PolyMag* /DNA or *CombiMag* /transfection reagent or virus that has been previously optimized. For this purpose, you can perform a serial dilution of a preformed magnetic vector complex.
- 3) After having identified the correct quantity of *CombiMag*/*PolyMag*, nucleic acid, transfection reagent (commercial) or viral vector, you could pursue the process by optimizing the cell number as well as the incubation times for the complex formation and for the magnetic field application.

5.2. Protocol Optimization in a 96-well format

Adherent cells

For adherent cells, seed the cells at the desired density in a 96- well plate the day prior or at least several hours prior transfection in a total of 150 μL medium per well.

1. In four tubes, dilute 7.2 μg of DNA (or DNA-transfection reagent complex) each to 346 μL with serum- and supplement-free medium (e.g. DMEM).
2. Provide 3.6, 7.2, 10.8 and 14.4 μL , respectively, of *PolyMag* (in case of DNA) or *CombiMag* (in case of DNA-transfection reagent complex) in well A1, A4, A7 and A10, respectively, of a 96-well plate.
3. Add the 346 μL of DNA solution (or DNA-transfection reagent complex) from step 1 to wells A1, A4, A7 and A10, respectively, containing *PolyMag* or *CombiMag* and mix well by pipetting.
4. Fill up to 360 μL with serum- and supplement-free medium (e.g. DMEM) by adding 10.4 μL to A1,

6.8 μL to A4 and 3.2 μL to A7.

5. Incubate for 20 - 30 min at room temperature.
6. In the meantime, add 180 μL of serum- and supplement-free medium (e.g. DMEM) to the residual wells of columns 1, 4, 7 and 10 of the 96-well plate (B1 - H1, B4 - H4, B7 - H7, B10 - H10).
7. After the incubation in step 5 transfer 180 μL from well A1/A4/A7/A10 to B1/B4/B7/B10 using a multichannel pipet, mix by pipetting, transfer 180 μL from B1/B4/B7/B10 to C1/C4/C7/C10, mix by pipetting, from C1/C4/C7/C10 to D1/D4/D7/D10 and so on down to H1/H4/H7/H10.
8. Transfer 50 μL each in triplicates from column 1 to the columns 1, 2, and 3 of the cell culture plate where the cells to be transfected have been seeded, similarly from column 4 of the „dilution plate“ to columns 4, 5, and 6 of the culture plate, from column 7 „dilution plate“ to columns 7, 8, and 9 of the culture plate, and from column 10 „dilution plate“ to columns 10, 11, and 12 of the culture plate. Using a multichannel pipet for the transfer.
9. Place the culture plate on the magnetic plate for 15 min.
10. Remove the magnetic plate and continue to culture cells as desired. Optionally, perform a medium change, particularly if the transfection has been carried out in serum-free medium.

		1	2	3	4	5	6	7	8	9	10	11	12	
	A	●	○	○	●	○	○	●	○	○	●	○	○	Optimization Protocol
	B	●	○	○	●	○	○	●	○	○	●	○	○	
	C	●	○	○	●	○	○	●	○	○	●	○	○	
	D	●	○	○	●	○	○	●	○	○	●	○	○	
	E	●	○	○	●	○	○	●	○	○	●	○	○	
	F	●	○	○	●	○	○	●	○	○	●	○	○	
	G	●	○	○	●	○	○	●	○	○	●	○	○	
	H	●	○	○	●	○	○	●	○	○	●	○	○	
	1	2	3	4	5	6	7	8	9	10	11	12		
2- ●	3.6 μL		7.2 μL		10.8 μL		14.4 μL		<i>PolyMag or CombiMag</i>					
3- ●	346 μL		346 μL		346 μL		346 μL		DNA or transfection complex					
4- ●	10.4 μL		6.8 μL		3.2 μL		0 μL		Serum-free medium					
6- ●	180 μL		180 μL		180 μL		180 μL		Serum-free medium					
7- ↺	Serial dilution of 180 μL													

Suspension cells

1. The composition and dilution series are performed exactly as described above from steps 1 to 5.
2. While *PolyMag / CombiMag* and DNA incubate (step 5 above) perform the following steps:
 - A. Dilute the cells to be transfected to $5 \times 10^5 - 1 \times 10^6 / \text{mL}$ in medium (with or without serum and /or supplement; depending on cell type and cell sensitivity towards serum-free conditions).

- B. Seed the cells on polylysine-coated plates OR centrifuges the cells (2 minutes) in order to pellet them and use the protocol for adherent cells. OR Mix cell suspension with 30 μ L of *CombiMag* reagent per 1 ml of cell suspension and follow steps C-E.
- C. Incubate for 10 - 15 minutes.
- D. Distribute 100 μ L of cells / well of a flat-bottom 96-well plate placed upon the magnetic plate.
- E. Incubate for 15 minutes.

In the meantime continue the vector dilution series by carrying out steps 6 and 7 as above.

3. Perform step 8 as above while keeping the cell culture plate on the magnetic plate.
4. Continue to incubate for 15 minutes.
5. Carefully remove the medium supernatant from the cells and replace with fresh complete medium while the culture plate remains positioned on the magnetic plate. Be careful not to aspirate the magnetically sedimented cells.
6. Remove culture plate from magnetic plate and continue to cultivate cells as desired.

5.3. Quality Controls

To assure the performance of each lot of Magnetofection™ produced, we qualify each component using rigorous standards. The following assays are conducted *in vitro* to qualify the function, quality and activity of each kit component.

Components	Standard Quality Controls
<i>PolyMag</i> or <i>CombiMag</i>	<ol style="list-style-type: none"> 1. Quality and size homogeneity of the magnetic nanoparticles. 2. Stability of the magnetic nanoparticle formulations. 3. Transfection efficacies on NIH-3T3, COS 7 and K562 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot
<i>Magnetic Plate</i>	<ol style="list-style-type: none"> 1. Tests of solidity 2. Test of the magnetic field force

5.4. “Troubleshooting”

Our dedicated and specialized (drug delivery systems) technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com

5.5. Bibliographic References

1. Scherer F, Anton M, Schillinger U, Henke J, Bergemann C, Kruger A, Gansbacher B, and Plank C. *Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo*. Gene Ther. 2002 Jan;9(2):102-9.
2. Krotz F, Wit C, Sohn HY, Zahler S, Gloe T, Pohl U, and Plank C. *Magnetofection-A highly efficient tool for antisense oligonucleotide delivery in vitro and in vivo*. Mol Ther. 2003 May;7(5):700-10.
3. Plank C, Schillinger U, Scherer F, Bergemann C, Remy JS, Krotz F, Anton M, Lausier J, and Rosenecker J. *The magnetofection method: using magnetic force to enhance gene delivery*. Biol Chem. 2003 May;384(5):737-47.
4. Plank C, Anton M, Rudolph C, Rosenecker J, and Krotz F. *Enhancing and targeting nucleic acid delivery by magnetic force*. Expert Opin Biol Ther. 2003 Aug;3(5):745-58.

6. Related Products

Description	Reference
β -Galactosidase assay kit with ONPG	GO-10001
β -Galactosidase assay kit with CPRG	GC-10002
X-Gal staining kit	GX-10003
DreamFect™ 0.5mL	DF-40500
FlyFectin™ 0.5mL	FF-50500
GeneBlaster™ SelectionKit	GB-20010

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

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The Magnetofection™ Reagent 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 included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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