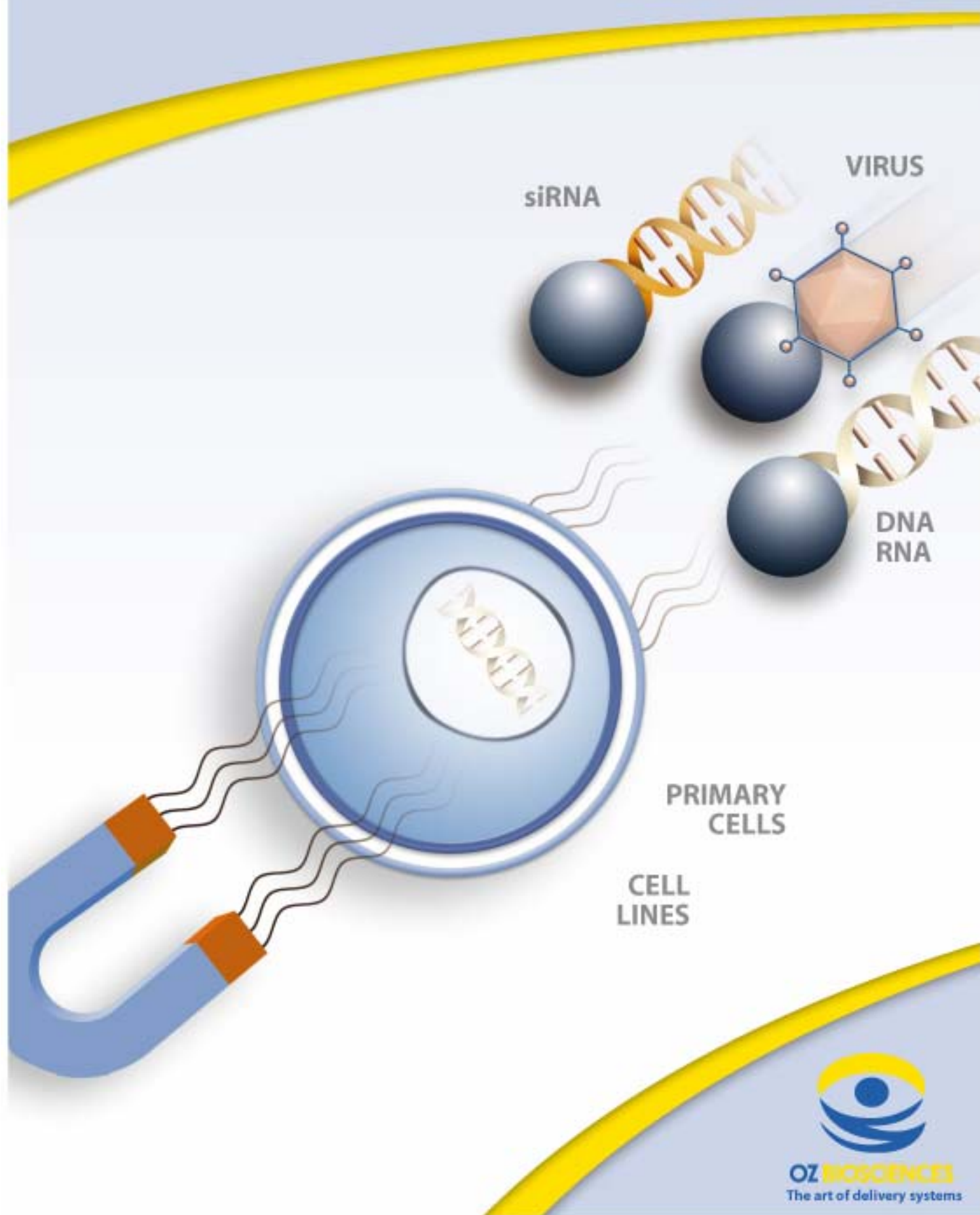


# Magnetofection™ : *PolyMag & CombiMag*

## INSTRUCTION MANUAL



# Magnetofection™: *PolyMag & CombiMag*

## 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 transfections / µg of DNA)	Number of transfections / 96 well plates
PN30100	PolyMag reagent	100	100	1000
PN30200	PolyMag reagent	200	200	2000
PN31000	PolyMag reagent	1000	1000	10000
CM20100	CombiMag reagent	100	100	1000
CM20200	CombiMag reagent	200	200	2000
CM21000	CombiMag reagent	1000	1000	10000
KM30200	Magnetofection Selection Kit <sup>1</sup>	200 (2 X 100)	200	2000
KC30296	Magnetofection Starting Kit <sup>2</sup>	200 (2 X 100)	200	2000
MF10096	Magnetic Plate	N/A	N/A	N/A

<sup>1</sup> Contains 1 vial of each reagent (*PolyMag* and *CombiMag*)

<sup>2</sup> 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 (phone, fax, email, website). For all other supplementary information, do not hesitate to contact our dedicated technical support ([tech@ozbiosciences.com](mailto:tech@ozbiosciences.com)) and/or to visit our website: [www.ozbiosciences.com](http://www.ozbiosciences.com).

#### OZ BIOSCIENCES

Parc Scientifique et Technologique de Luminy  
BP13

13273 Marseille Cedex 9, France

Tel: +33 (0) 4.91.82.81.72

Fax: +33 (0) 4.91.82.81.70

E-mail: [contact@ozbiosciences.com](mailto:contact@ozbiosciences.com)

Site Internet: [www.ozbiosciences.com](http://www.ozbiosciences.com)

\* Patent Pending



# 1. Technology

## 1.1. Description

Congratulations on your purchase of the **Magnetofection™** reagent!

**Magnetofection™** is an original, 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 non-viral vectors)
- Universal (primary cells and cell lines)
- Non toxic & economical

## 1.2. Available Reagents

OZ Biosciences offers two types of ready-to-use Magnetofection™ transfection 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. **CombiMag** has been used successfully with plasmid DNA, antisense oligonucleotides, mRNA and siRNAs

Two other Magnetofection™ based reagents have been developed by OZ Biosciences: **SilenceMag** specifically design for siRNA delivery and **ViroMag** specially develop for all viral applications. Further detailed information on these two specific reagents can be found in our website: [www.ozbiosciences.com](http://www.ozbiosciences.com)

## 1.3. Kit Contents

**Kit contents** differ according to their size

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

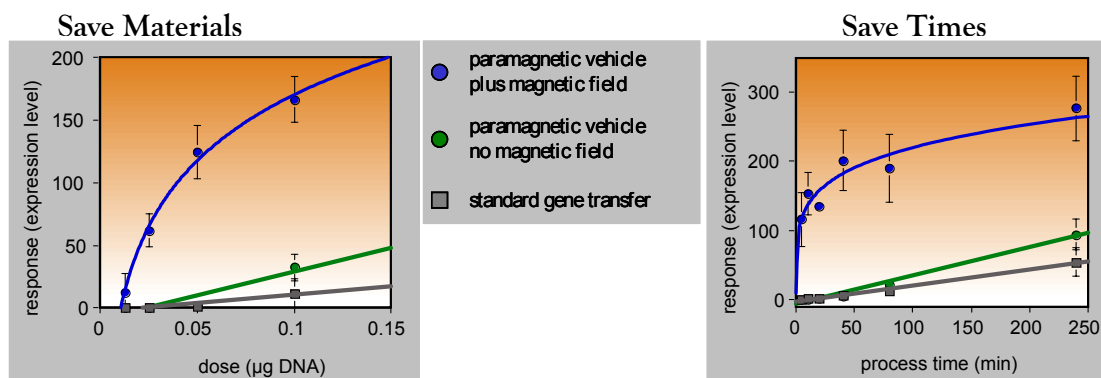
Storage +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. Magnetofection kits are stable for at least one year at the recommended storage temperature.

- **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



**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 24h.

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

### 2.2. Nucleic Acids Types and Vectors

The **CombiMag** reagent can be combined with any nucleic acid and all transfection reagents.

Nucleic Acid or Virus	PolyMag	CombiMag	SilenceMag	ViroMag
DNA (plasmid)	√	√	NA	NA
Antisense Oligonucleotides	√	√	NA	NA
mRNA	√	√	NA	NA
siRNA	√	√	√	NA
Viruses	NA	√	NA	√

### 2.3. Cell Types

Magnetofection™ is applicable with numerous cell types and has been successfully tested on a variety of immortalized cell lines as well as primary cells (see examples in table below and website). If a particular cell type or cell line is not listed below, this does not imply that Magnetofection™ is not going to work. An updated list of cells successfully tested as well as product citations is available on the website: [www.ozbiosciences.com](http://www.ozbiosciences.com). For the cells listed, some reagents have not been tested so far, as indicated by "n.d." (not determined).

## 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). The magnetic plate design allows producing a heterogeneous magnetic field that magnetizes the nanoparticles in solution, forms a very strong gradient and covers all the surface of the plate.



<b>Cell Line</b>	<b>Cell Type</b>	<b>Source</b>	<b>PolyMag</b>	<b>CombiMag<sup>1</sup></b>
16HBE14o	Airway Epithelium	Human	√	√
181RDB	Pancreatic Cells	Human	n.d.	√
293, HEK-293, 293-T, -EBNA	Transformed Embryonic Kidney	Human	√	√
3T6	Embryonic fibroblast	Mouse	√	√
A549	Non-small cell lung carcinoma	Human	n.d.	√
B16F10	Melanoma	Mouse	√	√
BEAS-2B	Bronchial epithelial cells	Human	√	√
BHK-21	Kidney	Hamster	√	√
BIU-87	Bladder cancer	Human	√	√
C6	Glioma	Rat	√	√
CHO-K1	Epithelial-like (Ovary)	Hamster	√	√
COS-1, COS-7	Fibroblast (Kidney)	Green Monkey	√	√
CALU 3	Lung adenocarcinoma (epithelial)	Human	√	√
CT-26	Colon Carcinoma	Mouse	√	√
CV-1	Fibroblast-like (Kidney)	Monkey	√	√
FaDu	Head-&-neck squamous carcinoma	Human	√	n.d.
HBL-100	Transformed breast	Human	n.d.	√
HCT-116	Colon adenocarcinoma	Human	n.d.	√
HeLa	Cervical Epithelial Carcinoma	Human	√	√
Hep2	Laryngeal epithelium	Human	√	√
HepG2	Hepatoma	Human	√	√
HT1080	Fibrosarcoma	Human	√	√
HUVEC	Endothelial Cells (primary)	Human	√	√
L929	Fibrosarcoma	Mouse	√	√
MCF-7	Breast Adenocarcinoma	Human	√	√
MDCK	Normal -Kidney	Canine	√	√
N2A	Neuroblastoma	Mouse	√	√
NIH3T3	Fibroblasts	Mouse	√	√
NS20Y	Neuroblastoma	Mouse	√	√
PC-12	Pheochromocytoma (adrenal)	Rat	√	√
RAW	Macrophage (monocyte)	Mouse	n.d.	√
β-TC	Pancreatic Islet β cells	Mouse	√	√
Primary Airway Epithelium		Pig	n.d.	√
Primary Aortic Endothelial Cells (PAEC)		Human, Bovine	√	√
Primary Carotid Artery Smooth Muscle Cells		Bovine	√	n.d.
Primary Chondrocytes		Pig	√	√
Primary Embryonic Fibroblast (MEF)		Mouse	n.d.	√
Primary Fibrochondrocytes		Pig	√	√
Primary Gastric Gland Cells		Human	√	√
Primary Glial cells		Human, Rat Mouse	√	√
Primary Hepatocytes		Rat	n.d.	√
Primary Hippocampal Neurons		Mouse, Rat	√	√
Primary HUVEC		Human	√	√
Primary Keratinocytes		Human, Mouse	n.d.	√
Primary Nasal Airway Epithelium		Human	√	√
Primary Smooth Muscle Cells (SMC)		Porcine	n.d.	√
Primary Stroma - Endotrium		Human	√	n.d.
Primary Trophoblastic cells		Human	n.d.	√

<sup>1</sup> Successfully tested in combination with several commercially available transfection reagents including **Dreamfect™**.

## 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 and upon request by email. 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. 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 ( $\mu$ g)	Transfection Volume
96 well	$0.5 - 2 \times 10^4$	0.1 - 0.5	200 $\mu$ L
24 well	$0.5 - 1 \times 10^5$	0.5 - 2	500 $\mu$ L
12 well	$1 - 2 \times 10^5$	2 - 4	1 mL
6 well	$2 - 4 \times 10^5$	2 - 6	2 mL
60 mm dish	$5 - 10 \times 10^5$	6 - 8	4 mL
90 - 100 mm dish	$10 - 20 \times 10^5$	8 - 12	8 mL
T-75 flask	$20 - 50 \times 10^5$	10 - 20	12 mL

The same protocol can be used to produce stably transfected 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 transfected 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  $\mu$ L of *PolyMag* per  $\mu$ g of DNA.

- 1) Before each use, vortex the *PolyMag* material. Add 1 to 10  $\mu$ L 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  $\mu$ L in your protocol, predilute *PolyMag* with deionized water.
- 2) Dilute 1 to 10  $\mu$ g of DNA to 200  $\mu$ L with serum and supplement-free culture medium (such as DMEM).
- 3) Add the 200  $\mu$ L DNA solution to the *PolyMag* solution and mix immediately by vigorous pipetting.

- 4) After 20 to 30 minutes of incubation, add the 200  $\mu$ L 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  $\mu$ L 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.

For siRNA application, we recommend the use of ***SilenceMag***.

#### 4.4. CombiMag

Until now, a universal method enhancing the efficiency of synthetic (non-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.

A number of suppliers sell **transfection reagents**. They all can be associated with *CombiMag* by simple mixing in order to generate magnetic delivery system. The resulting mixture 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  $\mu$ L of *CombiMag* per  $\mu$ g 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  $\mu$ L of *CombiMag* per  $\mu$ g of DNA to be transfected to a microtube. For DNA doses of less than 1  $\mu$ g 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  $\mu$ L, then transfer 100  $\mu$ L of the resulting mixture in each well containing the cells to be transfected. The total transfection volume / 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.

**Note:** For certain cells (primary cells such as neurons), a medium change at this step, significantly improves the transfection efficiency and greatly minimizes potential cytotoxicity. To process the medium change, leave the cells

onto the magnetic plate, remove the culture medium and replace it with fresh culture complete medium. Thereafter, remove the magnetic plate and continue to step 8 below.

- 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.

For viral application, we recommend the use of **ViroMag**.

#### 4.5. Magnetofection™ of suspension cells

1. The composition and preparation of *PolyMag* / DNA or *CombiMag* / transfection reagent are performed exactly as described above from steps 1 to 3.
2. While *PolyMag* / DNA or *CombiMag* / transfection reagent incubate (step 4 above), dilute the cells to be transfected to  $5 \times 10^5$  -  $1 \times 10^6$  / 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) to pellet them and use the protocol for adherent cells.**OR**
  - c. Mix cell suspension with 30  $\mu$ L of *CombiMag* reagent per 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. Appendix

### 5.1. Protocol Optimization

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

- Nucleic acid dose used
- Ratio of *CombiMag* / *PolyMag* to nucleic acid
- 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. To this end, use a fixed amount of DNA and transfection reagent. Vary the amount of *CombiMag* / *PolyMag* from 0.25 to 5  $\mu\text{L}$  /  $\mu\text{g}$  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 with a fixed ratio of *PolyMag* / DNA or *CombiMag* / transfection reagent 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), 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  $\mu\text{L}$  medium per well.

1. In four tubes, dilute 7.2  $\mu\text{g}$  of DNA (or DNA-transfection reagent complex) each to 346  $\mu\text{L}$  with serum- and supplement-free medium (e.g. DMEM).
2. Provide 3.6, 7.2, 10.8 and 14.4  $\mu\text{L}$ , 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  $\mu\text{L}$  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  $\mu\text{L}$  with serum- and supplement-free medium (e.g. DMEM) by adding 10.4  $\mu\text{L}$  to A1, 6.8  $\mu\text{L}$  to A4 and 3.2  $\mu\text{L}$  to A7.

<b>Optimization Protocol</b>												
	1	2	3	4	5	6	7	8	9	10	11	12
A	●	○	○	●	○	○	●	○	○	●	○	○
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		
3-●	346 μL		346 μL				346 μL			346 μL		
4-●	10.4 μL		6.8 μL				3.2 μL			0 μL		
6-●	180 μL		180 μL				180 μL			180 μL		
7-☞	Serial dilution of 180 μL											
	<b>PolyMag or CombiMag</b>											
	DNA or transfection complex											
	Serumfree medium											
	Serumfree medium											

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.

### **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  $5 \times 10^5$  -  $1 \times 10^6$  / 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 µL of *CombiMag* reagent per 1 ml of cell suspension and follow steps C-E.
  - C. Incubate for 10 - 15 minutes.
  - D. Distribute 100 µL 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.

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

## 5.4. "Troubleshooting"

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. [tech@ozbiosciences.com](mailto: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.

An updated list of products citations is available on the website: [www.ozbiosciences.com](http://www.ozbiosciences.com)

## 6. Related Products

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

- **ViroMag** for all viral applications
- **SilenceMag** created specifically for all siRNA applications.

Description	Reference
Magnetofection™ siRNA starting Kit <sup>1</sup>	KC30396
Magnetofection™ Super Starting Kit <sup>2</sup>	KC30496
ViroMag Starting Kit <sup>3</sup>	KC30596
ViroMag – 200 µL	VM40200
ViroMag – 1000 µL	VM41000
SilenceMag – 200 µL	SM10200
SilenceMag – 1000 µL	SM11000
Magnetic Plate	MF10096
DreamFect™ – 1 mL	DF41000
DreamFect™ – 5 x 1 mL	DF45000
FlyFectin™ – 1 mL	FF51000
FlyFectin™ – 5 x 1 mL	FF55000
GeneBlaster™ Ruby	GB20011
GeneBlaster™ Sapphire	GB20012
GeneBlaster™ Topaz	GB20013
β-Galactosidase (ONPG) assay kits	GO10001
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal staining kit	GX10003
Bradford – Protein Assay Kit, B-PAK	BA00100

<sup>1</sup> Contain: SM10200 + MF10096

<sup>2</sup> Contain: PN30100 + CM20100 + SM10200 + MF10096

<sup>3</sup> Contain: VM40200 + MF10096

Please, feel free to contact us for all complementary information and remember to visit our website ([www.ozbiosciences.com](http://www.ozbiosciences.com)) to stay informed on the latest breakthrough technologies and updated on our complete product list.

### Limited License

The purchase of the Magnetofection™ Reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Magnetofection™ Reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all Magnetofection™ Reagent material and documentation to OZ Biosciences, or by destroying all Magnetofection™ Reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a Magnetofection™ Reagent kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of the Magnetofection™ Reagent research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

### Product Use Limitations

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.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development  
OZ Biosciences  
Parc Scientifique et Technologique de Luminy  
BP13  
13273 Marseille Cedex 9, France  
Tel: +33 (0)4.91.82.81.74  
Fax: +33 (0)4.91.82.81.70  
E-mail: [contact@ozbiosciences.com](mailto:contact@ozbiosciences.com)