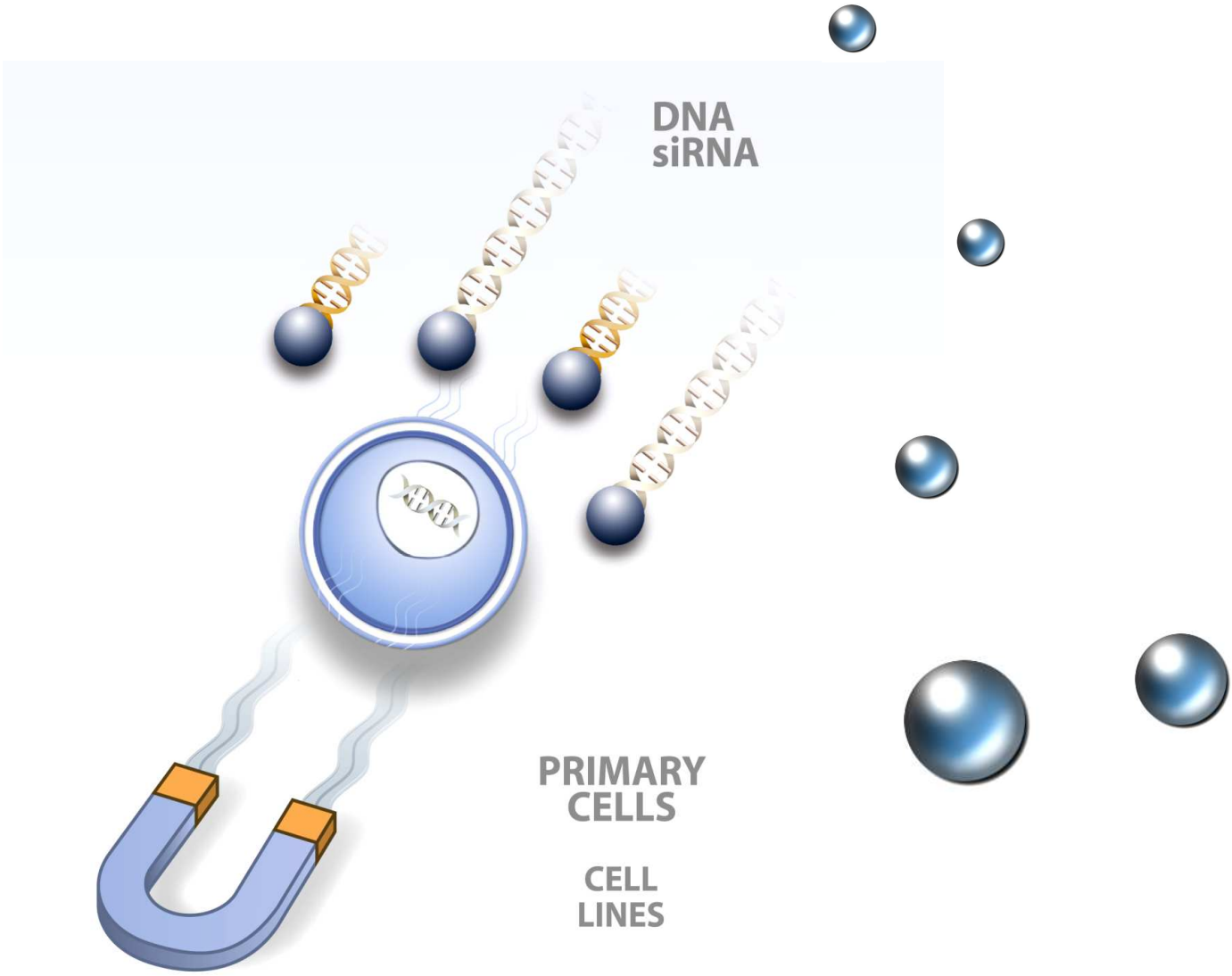
Magnetofection™ CombiMag

INSTRUCTION MANUAL



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Magnetofection™*: *CombiMag*

Instruction Manual

Magnetofection™ is a 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
CM20100	CombiMag reagent	100	100	1000
CM20200	CombiMag reagent	200	200	2000
CM21000	CombiMag reagent	1000	1000	10000
MF10000	Super Magnetic Plate	N/A	N/A	N/A
MF14000	Mega Magnetic Plate	N/A	N/A	N/A
MF10096	96-Magnets, Magnetic Plate	N/A	N/A	N/A

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1. Technology

1.1. Description

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 onto 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 transfected cells compared to standard transfection.
2. Up to several thousand folds increased levels of transgene expression compared to standard transfection.
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 acids and non-viral vectors)
- Universal (primary cells and cell lines)
- Non toxic & economical

1.2. Available Reagents

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 siRNA
CombiMag is also available *in vivo* grade (***in vivo* DogtorMag**) for your targeted gene delivery *in vivo*.

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 NANOPARTICLES!**

Shipping condition: Room temperature

2. Applications

2.1. Nucleic Acids Types and Vectors

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

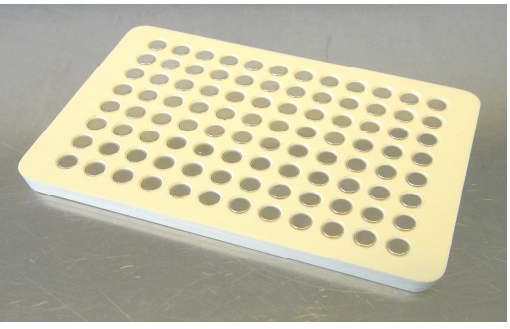
Nucleic Acid or Virus	*CombiMag*
DNA (plasmid)	✓
Antisense Oligonucleotides	✓
mRNA	✓
siRNA	✓

2.2. 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 list on website). If a particular cell type or cell line is not listed, this does not imply that Magnetofection™ is not going to work.

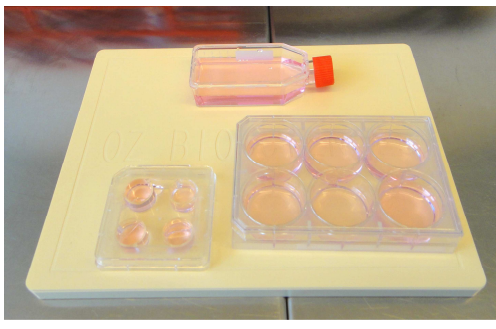
3. Magnetofection™ Apparatus

Besides suitable magnetic nanoparticles, Magnetofection™ requires an appropriate magnetic fields generated by a magnetic plate especially designed for Magnetofection. Its special geometry not only produces strong magnetic fields under each well of 96-well plates but is also applicable to other plate formats (T-75 flasks, 60 & 100 mm dishes, 6-, 12- and 24-well plates). Super Magnetic Plate suits for all cell culture supports and Mega Magnetic Plate is designed to hold up to 4 culture dishes at one time.



Magnetic plate 96 magnets

Super Magnetic Plate



Mega Magnetic Plate

4. Protocol

4.1. General Considerations

Instructions given below represent sample protocols that were successfully applied to a variety of cell lines. Optimal conditions do vary from cell line to cell line and are dependent on nucleic acid or transfection reagent used. Consequently, the amounts and ratio of the individual components (DNA and reagent) may have to be adjusted to achieve best results. Therefore, we advise you to optimize the various transfection 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 as a starting point to achieve good transfection.

4.2 General Protocol

It is recommended to seed the cells the day prior transfection. The suitable cell density will depend on the growth rate and the cells conditions. 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 (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	4 mL
90 - 100 mm dish	10 – 20 x 10^5	8 - 12	8 mL
T-75 flask	20 – 50 x 10^5	10 - 20	12 mL

4.3. Procedure

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.

6) Place the cell culture plate upon the magnetic plate for 20 minutes (at room temperature or 37°C).

7) Optionally perform a medium change. Remove the magnetic plate.

NB: 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.

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

4.4. Magnetofection of suspension cells

1) The composition and preparation of DNA / transfection reagent / *CombiMag* are performed exactly as described above from steps 1 to 3.

2) While DNA / transfection reagent / *CombiMag* are incubating (step 4 above), dilute the cells to be transfected to 5 x 10^5 - 1 x 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 four 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 µL 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

   OR

   d. Incubate the cells in serum free medium during 2 hours prior Magnetofection. The absence of serum allows some cells to adhere onto the plastic dish surface.

3) Add the resulting mixture of DNA / transfection reagent / *CombiMag* to the cells while keeping the cell culture plate on the magnetic plate.

4) Incubate for 15 minutes.

5) Remove culture plate from magnetic plate.

6) 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 conditions in order to get the best out of Magnetofection™. Several parameters can be optimized:

- Nucleic acid dose used
- Ratio of *CombiMag* to nucleic acid
- 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 to optimize one parameter at a time and start from the experimental procedures described above in section 4.

1) Start by optimizing the ratio *CombiMag* / DNA or *CombiMag* / transfection reagent. To this end, use a fixed amount of DNA. Vary the amount of *CombiMag* from 0.25 to 5µL / µg of DNA.

2) Thereafter, change the nucleic acid dose with a fixed ratio of *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*, 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. Quality Controls

To insure 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
CombiMag	1. Quality and size homogeneity of the magnetic nanoparticles. 2. Stability of the magnetic nanoparticle formulations. 3. Transfection efficacies on NIH-3T3 and COS 7 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot
Magnetic Plate	1. Tests of solidity 2. Test of the magnetic field force

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6. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)*
Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
***In vivo* Magnetofection**
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g
G-418, Sulfate 1g
X-Gal powder 1g

Purchaser Notification

Limited License

The purchase of this product 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 purposes 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.

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