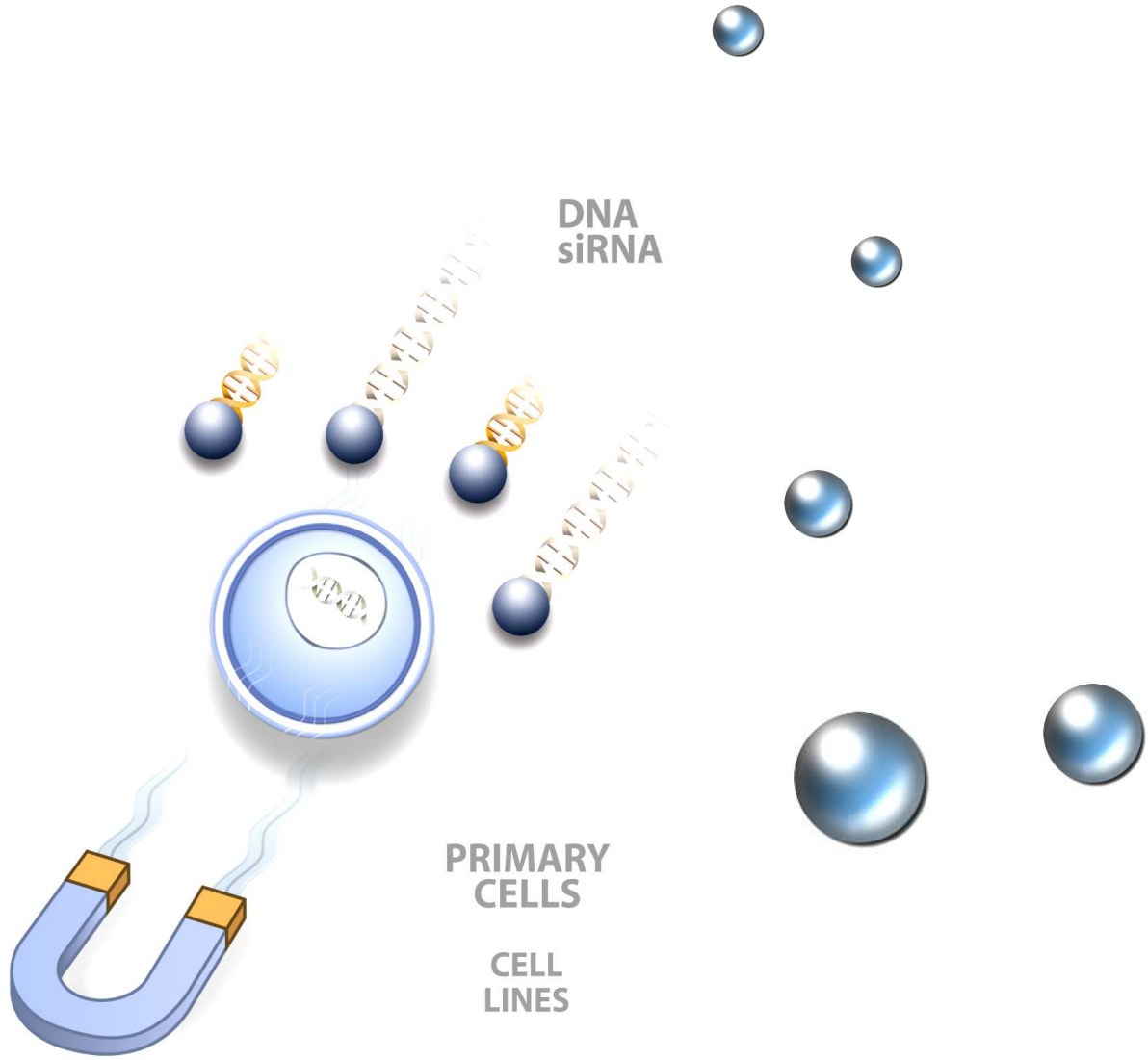
Magnetofection™
PolyMag & PolyMag Neo

INSTRUCTION MANUAL



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Magnetofection™: *PolyMag, PolyMag Neo*

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
PN30100	PolyMag reagent	100	100	1000
PN30200	PolyMag reagent	200	200	2000
PN31000	PolyMag reagent	1000	1000	10000
PG60100	PolyMag neo reagent	100	100	1000
PG60200	PolyMag neo reagent	200	200	2000
PG61000	PolyMag neo reagent	1000	1000	10000
KC30200	Magnetofection Starting Kit [1]	3 x 100	200	2000
KC30300	SiRNA Starting kit [2]	200 +2x100		
KC30400	Super Starting Kit [3]	200 + 3 x 100	200	400 - 2000
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

[1] Contains 1 vial of each reagent (*PolyMag, PolyMag Neo* and *CombiMag*) + one Super Magnetic Plate (MF10000)
[2] Contains 1 vial of each reagent (*SilenceMag, PolyMag PolyMag Neo*) + one Super Magnetic Plate (MF10000)
[3] Contains 1 vial of each reagent (*PolyMag, PolyMag Neo*, *CombiMag and SilenceMag*) + one Super Magnetic Plate (MF10000)

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). Several kits containing different Magnetofection reagents are also available, please contact us (contact@ozbiosciences.com) for a more detailed offer. For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com) and/or to visit our website: www.ozbiosciences.com.

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1. Technology

1.1. Description

Magnetofection™ is an original, simple and highly efficient method to transfect cells *in vitro* 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

OZ Biosciences offers different ready-to-use Magnetofection™ transfection reagents for *in vitro* applications:

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, antisense oligonucleotides and siRNA.
2. **PolyMag Neo** is an optimized formulation of PolyMag for a higher gene expression level in primary, hard-to-transfect and cell lines.

PolyMag is also available *in vivo* grade (***in vivo* PolyMag**) for your targeted gene delivery *in vivo*. Further detailed information on Magnetofection™ reagents can be found at: www.ozbiosciences.com

1.3. Kit Contents, Stabiblity and Storage

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 reagents 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!**

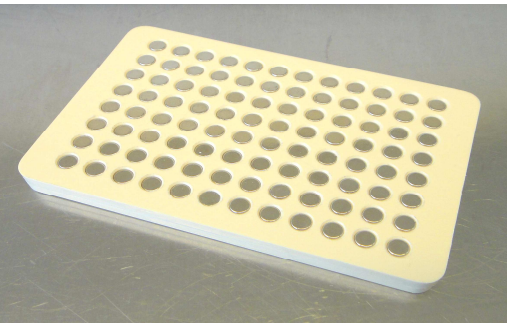
Shipping condition: Room temperature

2. Applications

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. An updated list of cells successfully tested as well as product citations is available on the website: www.ozbiosciences.com.

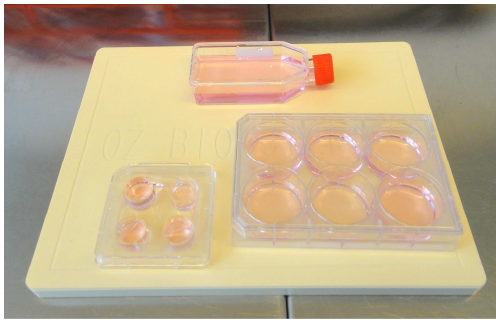
3. Magnetofection™ Apparatus

Besides suitable magnetic nanoparticles, Magnetofection™ requires an appropriate magnetic field 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. Protocols

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 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 starting point to achieve good transfection.

4.2 Cells Preparation

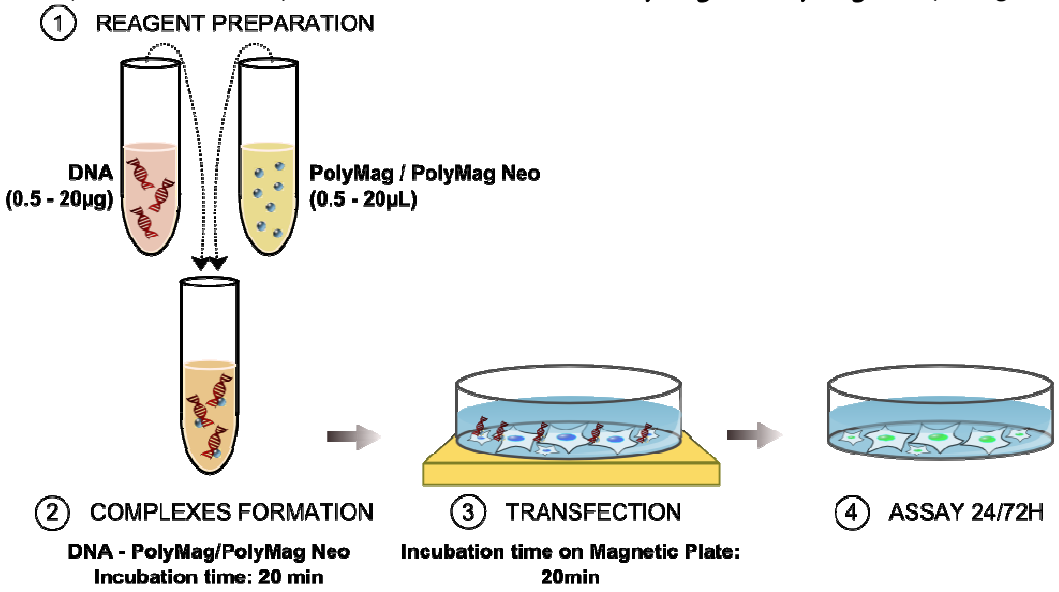
It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the cells conditions. Cells should be 60-90% confluent at the time of Magnetofection (see the suggested cell number in the table below). For suspension cells, use the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

Table 1: 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. Transfection protocol

Use the following procedure to transfect DNA into mammalian cells. The Table 1 shows transfection condition according to different cell culture formats (all amount are given on per-well basis). The DNA and the magnetofection reagent (PolyMag or PolyMag Neo) should be at room temperature and be gently vortexed prior to use. The protocol is as simple as follows: use 1 µL of ***PolyMag*** or ***PolyMag Neo*** per µg of DNA.



1) Before each use, vortex the ***PolyMag*** *or* ***PolyMag Neo*** tube. Add 1 to 10 µL of ***PolyMag*** *or* ***PolyMag Neo*** (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, predilute ***PolyMag*** or ***PolyMag Neo*** 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*** *or* ***PolyMag Neo*** solution and mix immediately by vigorous pipetting.

4) After 20 to 30 minutes of incubation, add the transfection mix (DNA + *PolyMag* or *PolyMag Neo*) to the cells. The total transfection volume per well (culture medium + ***PolyMag*** *or* ***PolyMag Neo*** mixture) is suggested in the Table 1.

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

6) Optionally perform a medium change and then remove the magnetic plate.

7) Cultivate the cells under standard conditions until evaluation of transgene expression

The same 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.

For most cell types, a medium change is not required after Magnetofection. However, 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 (up to 4 hours). In this case, a medium change will be required after Magnetofection.

4.4. Magnetofection of suspension cells

1) The composition and preparation of ***PolyMag*** *or* ***PolyMag Neo*** / DNA are performed exactly as described above from steps 1 to 3.

2) While ***PolyMag*** *or* ***PolyMag Neo*** / DNA 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 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 µL of *CombiMag* (from OZ Biosciences) reagent per mL of cell suspension.
      i. Incubate for 10 - 15 minutes.
      ii. Distribute cells to your culture dish placed upon the magnetic plate.
      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 ***PolyMag*** *or* ***PolyMag Neo*** / DNA to the cells while keeping the cell culture plate on the magnetic plate.

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

- Nucleic acid dose used
- Ratio of *PolyMag or PolyMag Neo* to nucleic acid
- Cell density
- Incubation time

OZ Biosciences team has investigated numerous factors during the course of Magnetofection reagent development. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedure described above in section 4.

1) Start by optimizing the ratio *PolyMag or PolyMag Neo* / DNA . To this end, use a fixed amount of DNA. Vary the amount of *PolyMag or PolyMag Neo* from 0.25 to 5µL / µg of DNA. The ratio *PolyMag* or *PolyMag Neo* / DNA can be changed by doubling or multiplying the volume of the reagent used. Reagent can be pre-diluted in deionized water.

2) Thereafter, change the nucleic acid dose with a fixed ratio of *PolyMag or PolyMag Neo* / DNA 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 quantities of *PolyMag* or *PolyMag Neo* and nucleic acid, you can 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
PolyMag, PolyMag Neo	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 a reference lot
Magnetic Plate	1. Tests of solidity 2. Test of the magnetic field force

5.3. 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
An updated list of products citations is available on the website: www.ozbiosciences.com

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)
In vivo SilenceMag *(for siRNA application)*
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

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

Purchaser Notification

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.

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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.
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