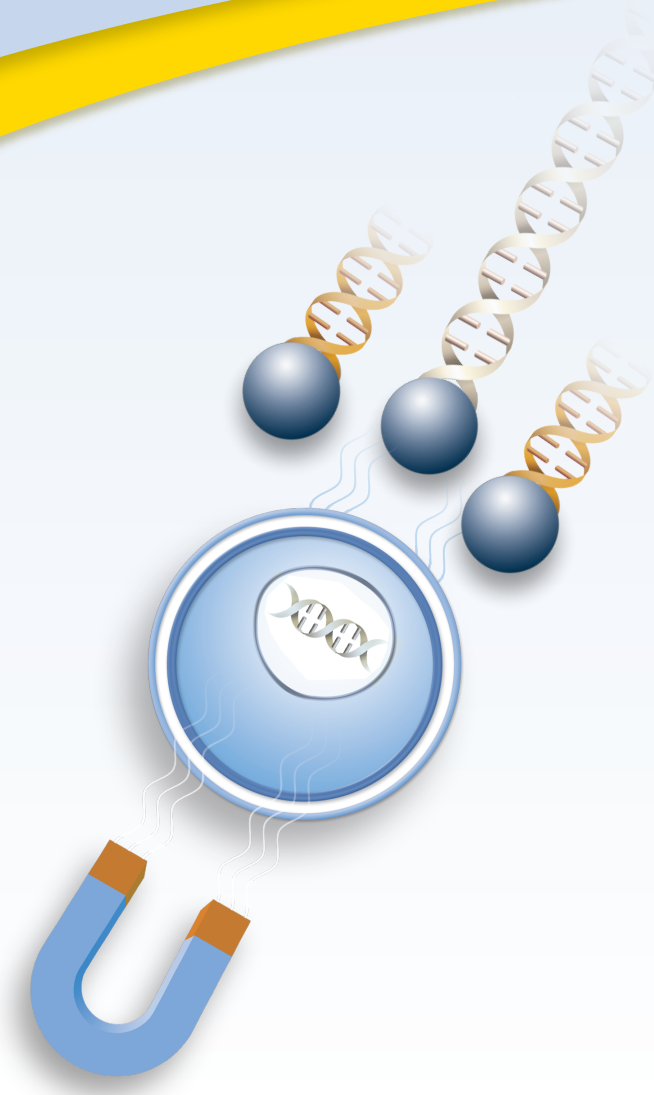
Magnetofection™ : PolyMag Neo / PetakaG3™

INSTRUCTION MANUAL



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Celartia
Transforming the art of cell culture into scientific engineering

PolyMag Neo / PetakaG3™

Instruction Manual

PolyMag Neo / PetakaG3™

The powerful Magnetofection-based transfection reagent, PolyMag Neo, from OZ Biosciences is now associated with the most advanced cell culture device, PetakaG3™, from Celartia to propose a unique transfection solution.

List of Magnetofection™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfections / µg of DNA)	Number of transfections / 96 well plates
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
KC30400	Super Starting Kit [2]	200 + 3 x 100	200	400 - 2000
KC30296	Magnetofection Starting Kit [3]	3 x 100	200	2000
KC30496	Super Starting Kit [4]	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*) plus a Super Magnetic Plate (MF10000)
[2] Contains 1 vial of each reagent (*SilenceMag, PolyMag, PolyMag Neo* & *CombiMag*) plus a Super Magnetic Plate (MF10000)
[3] Contains 1 vial of each reagent (*PolyMag, PolyMag Neo* and *CombiMag*) plus a 96-magnets Magnetic Plate (MF10096)
[4] Contains 1 vial of each reagent (*SilenceMag, PolyMag, PolyMag Neo* & *CombiMag*) plus a 96-magnets Magnetic Plate (MF10096)

List of PetakaG3™ cell culture device

Please refer to: www.celartia.com or www.petaka.com for the appropriate reference and catalog number

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

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1. Technology

1.1. Description

OZ Biosciences SAS and Celartia Ltd. are delighted to announce the association of their most powerful reagents for transfection with the most convenient cell culture device.
OZ Biosciences has developed specifically for PetakaG3 ™ cell culture device, an optimized procedure for transfecting cells. Two methods were developed: 1) Magnetofection: magnetic assisted transfection of cultured cells and 2) Lipofection: lipid-based transfection. These processed enables powerful transfection of cells in a more economically and efficiently manner. Transfection can be achieved on one or both sides of PetakaG3 ™ cell culture device, with the same or different plasmids allowing very sophisticated experiments on cell interactions through soluble factors and contact factors.
Two transfection technologies are available combining either PolyMag Neo to experience the powerful Magnetofection-based transfection reagent or DreamFect Gold™, the effective lipid-based reagent. Each of those is suitable for very high transfection efficiency.

Main ***PolyMag Neo*** features are:

1. Highly efficient & Biodegradable
2. Achieve superior transgene expression level than any other reagents
3. Multipurpose (various types of nucleic acids)
4. Universal (cell lines and primary cells)
5. Simple, Ready-to-use and Rapid
6. Serum compatible

Main ***PetakaG3™*** features are:

1. Easiness of cell culture conditions
2. Protection against contamination – perfect for establishing stable transfection
3. Robust structure / Optimized cell transport
4. 8 times more media volume per incubator
5. 15 times more cell culture surface area per incubator
6. Optimized media and growth factors supplies
7. completely safe and versatile
8. Easy storage and tracking

1.2. Products Specifications

For PolyMag Neo transfection reagent, please refer to its instruction manual for the specifications (handling, shipping, storage, stability). http://www.ozbiosciences.com/polymag-neo-9.html
For PetakaG3 cell culture device, please refer to its instruction manual for the specifications (shipping, storage, stability). http://www.celartia.com/

2. Applications

2.1. Application Areas

Please refer to OZ Biosciences and Celartia to get full access to the range of applications for each product. The association of Magnetofection™ reagent, PolyMag Neo and PetakaG3™ cell culture device offer the unique opportunity to transport nucleic acids into cells seeded either on one or the two sides of the device with very high efficiency.

2.2. Cell Types

Magnetofection™ is applicable to numerous cell types and has been successfully tested on a variety of immortalized cell lines as well as primary cells. All cells successfully transfected with PolyMag Neo reagent in classic cell culture devices can be transfected on PetakaG3™ cell culture device. An updated list of transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

3. Protocols

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully. Optimal conditions may vary depending on the nucleic acid, cell types, and cell culture conditions. Therefore, the amounts and ratio of the individual components (DNA and PolyMag Neo) may have to be adjusted to achieve best results since each cell line has a particular optimal transfection reagent / nucleic acids ratio. As a result, we suggest optimizing the various transfection parameters as described in section **3.4).** The following recommendations can be used as guidelines to quickly achieve very good transfection and high transgene expression level. As a starting point, we recommend to use **1 µL of PolyMag Neo / 1 µg of DNA.**

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used. We recommend using regularly passaged cells for transfection and avoid cells that have been cultured for too long (> 2 months). Generally, siRNA transfection requires lower cell density than DNA transfection.
- **Nucleic acids** should be as pure as possible (endotoxin-free). Moreover, we suggest avoiding long incubation time of the DNA/RNA solution in buffers or serum free medium before the addition of DreamFect Gold reagent to circumvent any degradation or surface adsorption.
- **Antibiotics**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the PolyMag reagent and this effect is cell type dependent and usually small.
- **Materials**. We recommend using polypropylene tubes to prepare the DNA and transfection reagent solutions but glass or polystyrene tubes can also be used.

3.2. 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 conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of transfection (see the suggested cell number in the Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous. (See section 3.3 for procedure)

Please refer to Celartia website for more information on the cell preparation in PetakaG3™ cell culture device www.celartia.com

Table 1: Cell number, DNA amount, DreamFect Gold volume and transfection conditions suggested.

Number of PetakaG3 sides	Adherent Cell Number	DNA Quantity (µg)	PolyMag Neo Volume (µL)	Dilution Volume (µL)	Transfection Volume
One Side	20 – 50 x 10^5	10-20	10-20	250	20 mL
Two Sides	20 – 50 x 10^5	20-40	20-40	500	20 mL

3.3. Transfection protocol

This protocol has been optimized to transfect with high efficiency 1 or 2 sides of the PetakaG3™ cell culture device. Magnetofection™ allows to selectively transfect cells seeded on 1 or 2 faces of PetakaG3. It is the only technology offering the possibility to exclusively transfect cells on each side of the cell culture device with two different plasmids. The instructions provided here are guidelines to rapidly reach high transfection efficiency.

The DNA and PolyMag Neo solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 1 µL of PolyMag Neo per µg of DNA. We suggest beginning with this ratio and optimize it, if required, by following section 3.4.

A) Preparation of cells in PetakaG3 cell culture device

1) One side: Seed the cells on one according to Celartia protocol and incubate 6 hours in horizontal position.

2) Two sides: flip the PetakaG3 device and seed the cells on the other side as recommended by Celartia.

B) Magnetofection Procedure (side one)

1) PolyMag Neo solution. Before each use, vortex the *PolyMag Neo* reagent. Add 10 to 20 µL of *PolyMag Neo* (according to the DNA amount) to a microtube.

2) DNA solution. Dilute 10 to 20 µg of DNA in 250 µL of culture medium without serum and antibiotics (such as DMEM; PBS* can also be used to dilute the DNA, see 3.4.3).
   - *Do not use serum-containing media for this step!*

3) Add the DNA solution in the 10 to 20 µL of PolyMag Neo, mix gently by carefully pipetting up and down 2-3 times and incubate the mixture for 15-20 minutes at room temperature. Do not vortex or centrifuge!
   - *The diluted solutions should be combined within 5 minutes.*

4) Add the DNA/PolyMag Neo complexes to at least 10 mL of complete growth culture medium (containing serum). Withdraw 11 mL culture medium from PetakaG3 device and inject the 10 mL containing complexes. Finally add 1 more mL to push complexes from the device circulation circuit.

5) Place the PetakaG3 device on magnetic plate in a horizontal position for 15 to 30 minutes.

6) Optionally, perform a medium change if the other side of the PetakaG3 is going to be transfected (see below step 8) or for sensitive cells.

7) For one side transfection, incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the transgene expression.

C) Magnetofection Procedure (side two)

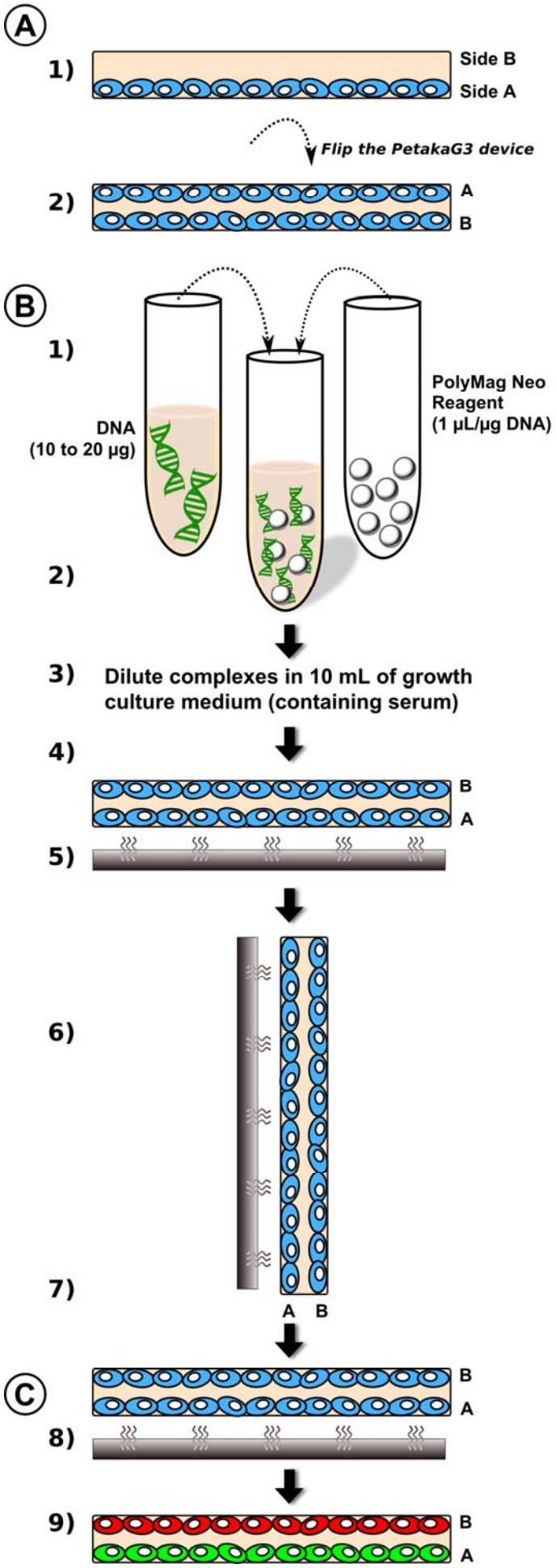
8) After incubation on the magnetic plate (step 5 above), place PetakaG3 in vertical position while keeping the magnetic plate against the transfected side during washing procedure. Wash and replace culture medium according to Celaratia instructions.

9) Optionally, incubate 5 to 10 minutes more on magnetic plate in horizontal position to ensure optimal cell uptake of the complexes on the first side.

10) Place PetakaG3 device 6 hours at 37°C until transfection of the other side.

11) Repeat steps B1 to B6 for the second side transfection.

12) Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the transgene expression.



A Preparation of cells in PetakaG3 cell cultre device

1) Seed the cells on one side of the PetakaG3 cell culture device according to Celartia protocol.

6 hours incubation

2) Seed the cells on the other side of the PetakaG3 cell culture device according to Celartia protocol.

B Magnetofection Procedure - Side 1

Allow Reagents to reach Room Temperature (R.T.)

1) Add diluted nucleic acids to PolyMag Neo transfection reagent

2) Incubate complexes 15 to 20 min at R.T.

3) Dilute complexes in 10 mL of growth culture medium (containing serum)

4) Replace culutre medium in Petaka Device with medium containing complexes

5) Incubate on magnetic plate

15 to 30 min.

6) Optionally perform a medium change

Keep the magnetic plate under PetakaG3 during Washing procedure

7a) **For one side transfection**, incubate the cells at 37°C in CO2 incubator under standard conditions until evaluation of the transgene expression.

C Magnetofection Procedure - Side 2

7b) **For two sides transfection**, incubate the cells at 37°C for 6 hours

8) Repeat steps B1 to B6 for the second side transfection

9) Incubate the cells at 37°C in CO2 incubator under standard conditions until evaluation of the transgene expression.

Assay (24h / 72h)

3.4. Optimization Protocol

Although high transfection efficiencies can be achieved in a broad range of cell types with the rapid protocol, some optimization may be needed in order to obtain the maximum efficiency in particular cells. For best results, we recommend optimization of the transfection protocol for each combination of plasmid and cell line used in order to get the best out of PolyMag Neo / PetakaG3. Several parameters can be optimized:

- Ratio of PolyMag Neo to nucleic acid (DNA/siRNA/RNA)
- Dose of nucleic acid used
- Cell type and cell density
- Culture medium composition (+/- serum) and reagent / nucleic acid complex medium
- Incubation time

We recommend optimizing one parameter at a time while keeping the other parameters (cell number, incubation time etc.) constant. The two most critical variables are the ratio of PolyMag Neo reagent to DNA and the quantity of DNA.

1) PolyMag Neo / DNA ratio:
This is an important optimization parameter. PolyMag Neo has to be used in slight excess compare to DNA but the optimal ratio will depend on the cell line. For optimization, first maintain a fixed quantity of DNA and then vary the amount of PolyMag Neo reagent. You can test 0.25; 0.5; 1; 1.5 and 2 per µg of DNA.

2) Quantity of DNA:
Thus, after optimization of the PolyMag Neo / DNA ratio, proceed to adjust the best amount of DNA required by maintaining a fixed ratio of PolyMag Neo reagent to DNA, and vary the DNA quantity. You can test for one side transfection 5; 10; 20 and 30 µg DNA.

3) PolyMag Neo / DNA complex medium:
PBS or serum- and antibiotic-free medium can be used to prepare the complexes. We recommend DMEM serum-free medium or PBS composition: 137mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4 and 6.5mM Na_2HPO_4 x 2 H_2O; pH7.4.

4) Cell culture conditions:
Thereafter, culture medium compositions, cell number, incubation times can also be optimized.

Do not hesitate to contact our technical service at tech@ozbiosciences.com to request any information or specific protocols.

4. Appendix

4.1. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **PolyMag Neo / nucleic acid ratio.** Optimize the reagent / nucleic acid ratio, please refer to optimization paragraph. 2- **DNA amount.** Use different quantity of DNA with the recommended or optimized (above) transfection reagent / DNA ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 50 to 70% (true confluency, corresponding to 90% visual confluency) but most favorable cell density may vary according to the cell type; preferably mid-log growth phase. 4- **Nucleic acid quality.** Nucleic acids should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies**.** Employ nuclease-free materials.

Low transfection efficiency	5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Another cells or viral-driven reporter gene expression can be used as a control. 6- **Cell condition.** 1) Cells that have been in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) Cells should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing DNA / transfection reagent complexes**. It is critical that serum-free medium or buffer (HBS, PBS) are used during the preparation of the complexes. Avoid any direct contact of pure PolyMag Neo and pure nucleic acid solution with the plastic surface. 8- **Cell culture medium composition.** 1) For some cells, transfection efficiency can be increased without serum or under reduced serum condition. Thus, transfect these cells in serum-free medium during the first 4h of incubation. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 24 – 96h by analyzing the gene product. Several reporter genes can be used to quantitatively monitored gene expression kinetics. 10- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hour can be aggregated. 11- **Transgene detection assay**. Ensure that your post-transfection assay is properly set up and includes a positive control. 12- **Transfection reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials 2- **Transgene product is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a nucleic acid control. 3- **Nucleic acid quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 5- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.

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. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.