

Transfection reagent

M PolyMag Neo™

Polymer complex for all nucleic acids transfection

Protocol

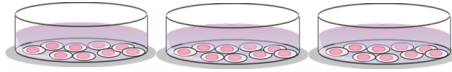
- M** Magnetofection Technology
This reagent needs to be used with a magnetic plate

PolyMag & PolyMag Neo Quick Protocol

To find the ideal conditions, PolyMag or PolyMag Neo must be tested at ratio **1 $\mu\text{L}/\mu\text{g}$** DNA. For the DNA quantity, we suggest **0.0625/0.125/0.25 μg** per well in 96-well, **0.25/0.5/1 μg** per well in 24-well and **1/2/4 μg** per well in 6-well.*

Seed cells to be at 70% confluent the day of transfection*

1



Prepare 3 tubes of DNA (with different amounts of nucleic acids)*

2



96 well plate

24 well plate

6 well plate

0.0625 μg /0.125 μg /0.25 μg in 50 μL serum-free medium or buffer*

0.25 μg /0.5 μg /1 μg in 100 μL serum-free medium or buffer*

1 μg /2 μg /4 μg in 200 μL serum-free medium or buffer*

Prepare 3 tubes of PolyMag or PolyMag Neo (with different amounts of magnetic beads)*

3



96 well plate

24 well plate

6 well plate

0.0625 μL /0.125 μL /0.25 μL in an empty microtube

0.25 μL /0.5 μL /1 μL in an empty microtube

1 μL /2 μL /4 μL in an empty microtube

Mix each tube of DNA (step 2) to each tube of PolyMag or PolyMag Neo (step 3)



4

96 well plate

24 well plate

6 well plate

DNA + *PolyMag*

DNA + *PolyMag*

DNA + *PolyMag*

0.0625 μg + 0.0625 μL

0.25 μg + 0.25 μL

1 μg + 1 μL

0.125 μg + 0.125 μL

0.5 μg + 0.5 μL

2 μg + 2 μL

0.25 μg + 0.25 μL

1 μg + 1 μL

4 μg + 4 μL

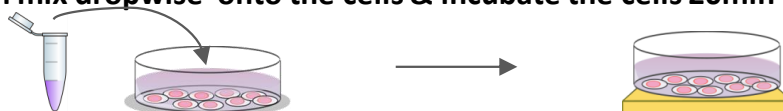
Incubate 20 min at room temperature

5



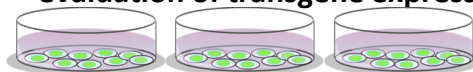
Distribute each mix dropwise onto the cells & incubate the cells 20min on the magnetic plate

6



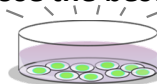
Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene expression*

7



Choose the best conditions

8



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 μ L, 400 μ L and 2 mL of complete culture medium.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/PolyMag Neo complexes preparation. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ We recommend respecting the order of addition of reagents: add the DNA solution into the PolyMag Neo tube.
- ✓ Dilute PolyMag Neo with deionized water for doses less than 1 μ L.
- ✓ 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. This can be done immediately after the 20min incubation on the magnetic plate while keeping the cells onto the magnetic device, or 4 to 6h post-Magnetofection. Alternatively, the cells may be kept in serum-free medium during Magnetofection (up to 4h). In this case, a medium change will be required after Magnetofection.

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications



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Any questions?



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Package content	PG60100: 100 µL of PolyMag Neo reagent PG60200: 200 µL of PolyMag Neo reagent PG61000: 1 mL of PolyMag Neo reagent KC30200: 100 µL of PolyMag reagent + 100µL of PolyMag Neo reagent + 100µL CombiMag reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the PolyMag Neo transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase at the recommended storage temperature
Product description	PolyMag Neo is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of primary cells, hard-to-transfect cells and cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

1. 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 (refer to Table 1). For suspension cells, use the specific protocol given below. Immediately preceding transfection, the medium can be replaced with fresh medium (optionally without serum).

Tissue Culture Dish	Adherent Cell Number	DNA amount (μg)	Dilution volume (μL)	PolyMag Neo Volume (μL)	Transfection volume
96 well	$0.5 - 2 \times 10^4$	0.1 - 0.5	50	0.1 - 0.5	200 μL
24 well	$0.5 - 1 \times 10^5$	0.5 - 2	100	0.5 - 2	500 μL
6 well	$2 - 4 \times 10^5$	2 - 6	200	2 - 6	2mL

Table 1: Suggested transfection conditions

2. DNA/PolyMag Neo complexes preparation

- PolyMag Neo*: Vortex the reagent and place the appropriate amounts in an empty microtube (see Table 1).
- DNA*: Dilute the indicated quantity of DNA (refer to Table 1) in 50 to 200 μL of culture medium without serum and supplement.
- Add the DNA solution to the *PolyMag Neo* solutions by vigorous pipetting and incubate at room temperature for 15 to 20 min. Do not vortex.

3. Transfection

- Add the *PolyMag Neo* / *DNA* complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution. Place the cell culture plate on the magnetic plate during 30 min.
- Remove the magnetic plate.
- Cultivate the cells at 37°C in a CO₂ incubator under standard conditions until evaluation of transgene expression (from 24h up to 7 days).

NOTES:

- In case of cells very sensitive to transfection, the medium can be changed right after the Magnetofection procedure
 - keep cells onto the magnetic plate and replace the transfection medium with fresh pre-warmed complete culture medium.
- Some cell types need medium change 2 - 4h after transfection.

Protocol | Co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. For example, if you have two DNA plasmids, mix 0.5 µg of each plasmid, complex the 1 µg of DNA with 1 µL of PolyMag Neo.

Option for Co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Protocol | siRNA

1. siRNA/PolyMag Neo complexes preparation

The siRNA 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 1.5 or 2 µL of PolyMag Neo per µg of siRNA.

- a. *siRNA solution.* Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100µL (see Table 2) of culture medium WITHOUT serum and antibiotics. We recommend starting with a final siRNA concentration of 50nM.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
<i>Amount of siRNA (1 µM stock)*</i>						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 2: Suggested dilution procedure and amount of siRNA to test

- b. *PolyMag Neo preparation.* Add 0.5 to 3 µL of PolyMag Neo in an empty microtube (refer to Table 3).

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50 µL		50 µL		100 µL	
Final transfection Volume	200 µL		500 µL		2 mL	
Final siRNA concentration	<i>Amount of PolyMag Neo (µL)</i>					
10 nM	0.5		0.5		0.5	
20nM	0.5		0.5		1	
≥ 50 nM	0.5		1		3	

Table 3: Recommended amount of PolyMag Neo per nM of siRNA used

- c. Add the siRNA solution onto the PolyMag Neo reagent. Mix gently by carefully pipetting up and down and incubate the mixture for 15-20 min at room temperature.

Do not vortex or centrifuge!

2. Transfection

- a. Add the mixture drop by drop directly onto the cells and gently rock the plate to ensure a uniform distribution.
- b. Place the cell culture plate upon the magnetic plate during 30 min
- c. Remove the magnetic plate
- d. Cultivate the cells at 37°C in a CO₂ incubator under standard conditions until evaluation of gene silencing. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.

NOTES:

- Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection
- For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- If cells are very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation.

IMPORTANT OBSERVATIONS

- Ensure to avoid the presence of serum when preparing the transfection reagent/siRNA complexes. Use a medium well pH (some old medium can turn pink or purple instead of being orange or red) which could influence complexes formation and siRNA stability.
- Avoid incubating your diluted siRNA too long in your serum-free medium; prepare first your transfection reagent, dilute your siRNA and quickly transfer the diluted siRNA into the PolyMag Neo tube.
- Start with 50nM siRNA and test four amounts of PolyMag Neo.
- The gene silencing is highly dependent on your protein half-life and consequently it will be good to analyze your protein expression by western at 48h, 72h and 96h.
- Treating your cells twice with 25nM siRNA instead of once with 50nM can enhance significantly siRNA effects. Basically, on day one, incubate your cells with 25nm siRNA /PolyMag Neo. On day two, change your medium and repeat the treatment with 25nm siRNA / PolyMag Neo.

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10⁵ cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. DNA/PolyMag Neo complexes preparation

- a. *PolyMag Neo*: Vortex the reagent and place the appropriate amounts in an empty microtube (refer to table 4).

Tissue Culture Dish	Suspension Cell Number	DNA Quantity (µg)	PolyMag Neo Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.5 – 1 x 10 ⁵	0.5	0.5	2 x 25	100 µL
24 well	2 - 4 x 10 ⁵	1	1	2 x 50	250 µL
6 well	10 - 15 x 10 ⁵	4	4	2 x 100	1 mL

Table 4: Suggested transfection conditions for suspension cells.

- b. *DNA*: Dilute the indicated quantity of DNA (refer to Table 4) in 50 to 200 µL of culture medium without serum and supplement.
- c. Add the DNA solution to the PolyMag Neo solutions by vigorous pipetting and incubate at room temperature for 15 to 20 min. Do not vortex or centrifuge.

3. Transfection

- a. While PolyMag Neo / DNA are incubating, dilute the cells to be transfected to 5 x 10⁵ - 1 x 10⁶ / 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.
- Seed the cells on polyLysine-coated plates and use the protocol for adherent cells
OR
 - Briefly, centrifuge the cells (2 min) to pellet them and use the protocol for adherent cells
OR
 - Mix cell suspension with 30 µL of *CombiMag* (from OZ Biosciences) reagent per mL of cell suspension.
Incubate for 10 - 15 min.
Distribute cells to your culture dish placed upon the magnetic plate.
Incubate for 15 min
OR
 - Incubate the cells in serum free medium during 2h prior Magnetofection. The absence of serum allows some cells to adhere onto the plastic dish surface.
- b. Add the resulting mixture of PolyMag Neo / DNA to the cells while keeping the cell culture plate on the magnetic plate.

- c. Incubate for 15-20 min.
- d. 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.
- e. Remove culture plate from magnetic plate.
- f. Continue to cultivate cells as desired until evaluation of transgene expression.

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 Neo* to nucleic acid
 - Cell density
 - Incubation time
1. Start by optimizing the ratio *PolyMag Neo* / DNA. To this end, use a fixed amount of DNA. Vary the amount of *PolyMag Neo* from 0.25 to 5 μ L / μ g of DNA. The ratio *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 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 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.

NOTES

Additional products for primary and hard-to-transfect cells experiments:

- **SilenceMag** for siRNA transfection applications
- **NeuroMag** dedicated to neurons transfection
- **In vivo PolyMag** for *in vivo* transfection of all nucleic acids

Purchaser Notification

Limited License

The purchase of the PolyMag Neo kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). 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 PolyMag Neo kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all PolyMag Neo kit reagents and documentation to OZ Biosciences, or by destroying all PolyMag Neo components. Purchasers are advised to contact OZ Biosciences with the notification that a PolyMag Neo 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 PolyMag Neo kit 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

PolyMag Neo kit 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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