We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

Transfection reagent

PolyMag™

Polymer complex for all nucleic acids transfection

Protocol

Magnetofection Technology

This reagent needs to be used with a magnetic plate

OZBIOSCIENCES

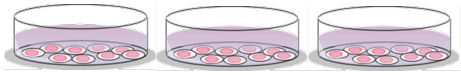
The art of delivery systems

PolyMag & PolyMag Neo Quick Protocol

To find the ideal conditions, PolyMag or PolyMag Neo must be tested at ratio **1 µL/µg** 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.*

1

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



2

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

96 well plate	24 well plate	6 well plate
0.0625µg/0.125µg/0.25µg in 50µL serum-free medium or buffer*	0.25µg/0.5µg/1µg in 100µL serum-free medium or buffer*	1µg/2µg/4µg in 200µL serum-free medium or buffer*

3

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

96 well plate	24 well plate	6 well plate
0.0625µL/0.125µL/0.25µL in an empty microtube	0.25µL/0.5µL/1µL in an empty microtube	1µL/2µL/4µL in an empty microtube

4

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

96 well plate			24 well plate			6 well plate		
DNA		*PolyMag*	*DNA*		*PolyMag*	*DNA*		*PolyMag*
0.0625µg	+	0.0625µL	0.25µg	+	0.25µL	1µg	+	1µL
0.125µg	+	0.125µL	0.5µg	+	0.5µL	2µg	+	2µL
0.25µg	+	0.25µL	1µg	+	1µL	4µg	+	4µL

5

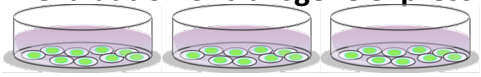
Incubate 20 min at room temperature

6

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

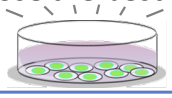
7

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



8

Choose the best conditions



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 µL, 400 µL 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 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 tube.

- ✓ Dilute PolyMag with deionized water.

- ✓ 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.

PolyMag Reagent | Specifications

Package content	PN30100: 100 µL of PolyMag reagent PN30200: 200 µL of PolyMag reagent PN31000: 1 mL of PolyMag 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 transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	PolyMag 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

Protocol | DNA or shRNA vectors transfection in adherent cells

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 Volume (µL)	Transfection volume
96 well	$0.5 – 2 \times 10^4$	0.1 – 0.5	50	0.1 – 0.5	200µL
24 well	$0.5 – 1 \times 10^5$	0.5 - 2	100	0.5 -2	500µL
6 well	$2 – 4 \times 10^5$	2 - 6	200	2 - 6	2mL

Table 1: Suggested transfection conditions

2. DNA/PolyMag complexes preparation

a. *PolyMag*: Vortex the reagent and place the appropriate amounts in an empty microtube (refer to Table 1).

b. *DNA*: Dilute the indicated quantity of DNA (see Table 1) in 50 to 200 µL of culture medium without serum and supplement.

c. Add the DNA solution to the PolyMag solutions by vigorous pipetting and incubate at room temperature for 15 to 20 min. Do not vortex.

3. Transfection

a. Add the PolyMag / 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.

b. Remove the magnetic plate.

c. Cultivate the cells at 37°C in a CO_2 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.

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 complexes preparation

The siRNA and PolyMag 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 per µg of siRNA.

a. *siRNA* solution. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to 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	
*Amount of siRNA (1 µM stock)**						
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 preparation*. Add 0.5 to 3 µL of PolyMag 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	*Amount of PolyMag (µL)*		
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 per nM of siRNA used

c. Add the siRNA solution onto the PolyMag 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 CO_2 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.

- Start with 50nM siRNA and test four amounts of PolyMag.

- 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. On day two, change your medium and repeat the treatment with 25nm siRNA / PolyMag.

Protocol | DNA or shRNA vectors transfection in suspension cells

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. DNA/PolyMag complexes preparation

a. *PolyMag*: 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 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 (see Table 4) in 50 to 200 µL of culture medium without serum and supplement.

c. Add the DNA solution to the PolyMag solutions by vigorous pipetting and incubate at Room Temperature for 15 to 20 min. Do not vortex or centrifuge.

3. Transfection

a. While PolyMag / DNA are incubating, 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.

i. Seed the cells on polyLysine-coated plates and use the protocol for adherent cells

OR

ii. Briefly, centrifuge the cells (2 min) to pellet them and use the protocol for adherent cells

OR

iii. 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

iv. 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 / 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.

Optimization Protocol

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* to nucleic acid
- Cell density
- Incubation time

1. Start by optimizing the ratio PolyMag / DNA. To this end, use a fixed amount of DNA. Vary the amount of PolyMag from 0.25 to 5µL / µg of DNA. The ratio PolyMag / 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 / 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 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.

Additional transfection reagents 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

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