Transfection reagent

PolyMag CRISPR

For Genome Editing experiments using DNA and/or RNA

Protocol

We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

Magnetofection Technology

This reagent needs to be used with a magnetic plate

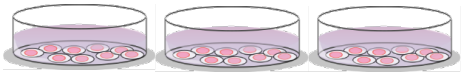
OZBIOSCIENCES

The art of delivery systems

PolyMag CRISPR Quick Protocol

To find the ideal conditions, PolyMag CRISPR 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 CRISPR (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

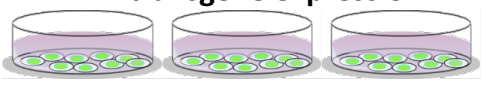
4 **Mix each tube of DNA (step 2) to each tube of PolyMag CRISPR (step 3)**

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.
- ✓ For co-transfection with 2 plasmids encoding for guide RNA and Cas9 endonuclease, prepare a mix of gRNA and Cas9 encoding plasmids in a 0.25:1 to 1:1 ratio (respectively gRNA to Cas9).
- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/PolyMag CRISPR 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; add the DNA solution into the PolyMag CRISPR tube.
- ✓ Dilute PolyMag CRISPR with deionized water for doses less than 1µL.
- ✓ 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 CRISPR Reagent | Specifications

Package content	PNC40200: 200 µL of PolyMag CRISPR reagent PNC41000: 1 mL of PolyMag CRISPR reagent KPC30200: 100µL of PolyMag CRISPR reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the PolyMag CRISPR transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase at the recommended storage temperature
Product Descriptions	PolyMag CRISPR is a magnetic nanoparticles formulation specifically optimized to deliver high level of plasmid DNA and/or mRNA expressing Cas9 and guide RNA (gRNA).
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 CRISPR Volume (µL)	Transfection volume
96 well	0.5 – 2 x 10^4	0.1 – 0.5	50	0.1 – 0.5	200µL
24 well	0.5 – 1 x 10^5	0.25 - 1	100	0.25 - 1	500µL
6 well	2 – 4 x 10^5	2 - 6	200	2 - 6	2mL

Table 1: Suggested transfection conditions

2. DNA/PolyMag CRISPR complexes preparation

a. *PolyMag CRISPR*: 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 CRISPR solutions by vigorous pipetting and incubate at room temperature for 20 minutes. Do not vortex.

3. Transfection

a. Add the PolyMag CRISPR / 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 minutes.

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 two plasmids respectively encoding for guide RNA and Cas9 endonuclease, mix the two plasmids and perform transfection as described above. We generally recommend using 0.25µg sgRNA encoding plasmid and 0.75µg Cas9 encoding plasmid with 1µL PolyMag CRISPR. However, depending on many parameters (promoters, read-out, cell type…) the stoichiometry of each DNA may vary.

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.

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 CRISPR* to nucleic acid
- Cell density
- Incubation time

1. Start by optimizing the ratio PolyMag CRISPR/ DNA. To this end, use a fixed amount of DNA. Vary the amount of PolyMag CRISPR from 0.25 to 5µL / µg of DNA. The ratio PolyMag CRISPR / 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 CRISPR / DNA that has been previously optimized. For this purpose, you can perform a serial dilution of a preformed magnetic vector complex.

3. Stoichiometry of each plasmid can also be investigated: vary the proportion of each plasmid DNA within the whole mix.

4. After having identified the correct quantities of PolyMag CRISPR 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 CRISPR/Cas9 Genome Editing

- **Pro-deliverIN CRISPR** for Cas9 protein delivery
- **RmesFect CRISPR** for mRNA transfection
- **ViroMag CRISPR** to enhance transduction efficiency of CRISPR/Cas9 viruses

Purchaser Notification

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Rev. 01/2019