Transfection reagent

Glial-Mag™

For Microglial cells

Protocol

We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

Magnetofection Technology

This reagent needs to be used with a magnetic plate

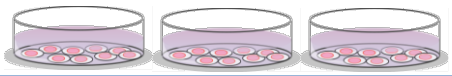
OZBIOSCIENCES

The art of delivery systems

Glial-Mag™ Quick Protocol

To find the ideal conditions, Glial-Mag must be tested at ratios **2 µL/µg**, , **3.0 µL/µg**, **3.5 µL/µg** and **4 µL/µg** (µL of Glial-Mag / µg of DNA). For the DNA quantity, we suggest **0.1 µg** per well in 48-well, **0.2 µg** per well in 24-well and **0.6 µg** per well in 6-well.

1 **Seed cells to be at 70% confluent the day of transfection – For primary cells, if needed change only 50% of the culture medium 24h before transfection***



2 **Prepare 4 identical tubes of DNA**

48 well plate	24 well plate	6 well plate
0.1 µg in 25µL of serum-free medium or buffer* x 4	0.2 µg in 50µL of serum-free medium or buffer* x 4	0.6 µg in 250µL of serum-free medium or buffer* x 4

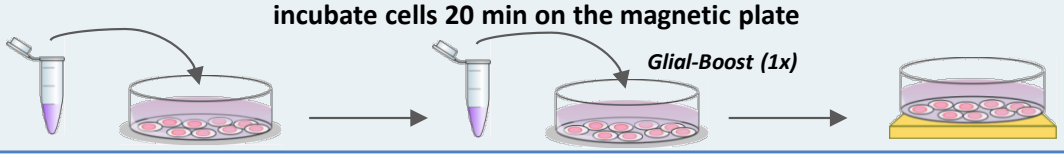
3 **Prepare 4 tubes of Glial-Mag™ (with 4 different amounts of reagent)**

48 well plate	24 well plate	6 well plate
0.2µL/0.3µL/0.35µL/0.4µL in an empty microtube	0.4µL/0.6µL/0.7µL/0.8µL in an empty microtube	1.2µL/1.8µL/2.1µL/2.4µL in an empty microtube

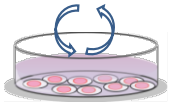
4 **Mix gently each tube of DNA (step 2) to each tube of Glial-Mag (step 3)**

5 **Incubate 20 min at room temperature**

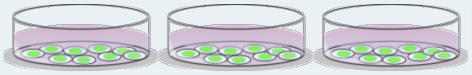
6 **Distribute each mix drop by drop onto the cells to insure uniform distribution; add Glial-Boost (1X final) & incubate cells 20 min on the magnetic plate**



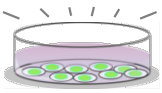
7 **Remove the cells from the magnet, incubate 2h at 37°C and perform a medium change***



8 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression**



9 **Choose the best ratio DNA: Glial-Mag™**



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

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ Cells Preparation:
  - For Primary µGlial and Glial cells, the cell density is a critical parameter to achieve good transfection with low toxicity; the suitable cell density will depend on the growth rate and the conditions of the cells. Higher cell confluency is preferable than low cell density. We recommend optimizing the cell culture density according to your experimental conditions.
  - For cell lines, 24h before transfection seed the cells 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.

- ✓ For primary cells, avoid medium change within the 24h before Magnetofection. If needed, replace 50% of the culture medium with fresh pre-warm complete culture medium 24h before transfection.

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/Glial-Mag complexes preparation. Culture medium such as MEM, 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 Glial-Mag magnetic nanoparticles.

- ✓ For doses of Glial-Mag less than 1µL, dilute the reagent 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 2h post-Magnetofection (as suggested in the protocol).

Glial-Mag Reagent | Specifications

Package content	GL00250: 250 µL of Glial-Mag reagent + 3 mL of Glial-Boost GL00500: 500 µL of Glial-Mag reagent + 6 mL of Glial-Boost KGL0250: Glial-Mag Starting Kit - 250 µL of Glial-Mag reagent + 3 mL of Glial-Boost + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the Glial-Mag transfection reagent and Glial-Boost at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	Glial-Mag is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of microglial cell lines and primary cells.
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA or shRNA vectors

1. Cells Preparation

It is recommended to plate the cells the day prior transfection* in classical culture medium. Cells should be 60-80 % confluent at the time of transfection (refer to Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and protein expression analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Tissue Culture Dish format	Surface area per well[1]	Cell Number
48 wells	1 cm^2	0.2 – 0.4 x 1.10^5
24 wells	2 cm^2	0.5 – 0.8 x 1.10^5
6 wells	10 cm^2	2 – 4 x 1.10^5

[1] Surfaces area may vary depending on the manufacturer.

Table 1: Suggested cell number (per well)

* Some primary cells require being prepared 48h before transfection; change half of the culture medium 24h transfection.

2. DNA/Glial-Mag complexes preparation

a. *Glial-Mag*: Mix the reagent gently before use and place the appropriate amounts in a microtube (refer to Table 2).

Tissue Culture Dish	DNA Quantity (µg)	Dilution Volume (µL)	Glial-Mag Volume (µL)	Gial-Boost (µL)	Transfection Volume
48 well	0.1	50	0.35	2.5	250 µL
24 well	0.2	100	0.7	5	500 µL
6 well	0.6	250	2.1	20	2 mL

Table 2: Suggested DNA amount, Glial-Mag and Glial-Boost volume and transfection conditions

b. *DNA*: Dilute the indicated quantity of DNA (see Table 2) in 50 to 250 µL of culture medium without serum and supplement.

c. Combine the DNA and the Glial-Mag solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 20min. **Do not vortex or centrifuge.**

3. Transfection

a. Add the Glial-Mag / DNA complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Add Glial-Boost (1X final) directly onto the cells.

c. Place the cell culture plate on the magnetic plate during 30 minutes.

d. Remove the magnetic plate and incubate your cells under standard conditions for 2h.

e. Perform a medium change 2h after transfection.

f. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression (from 24h up to 72h).

NOTE: please refer to Figure 1 for schematic representation of transfection with GlialMag.

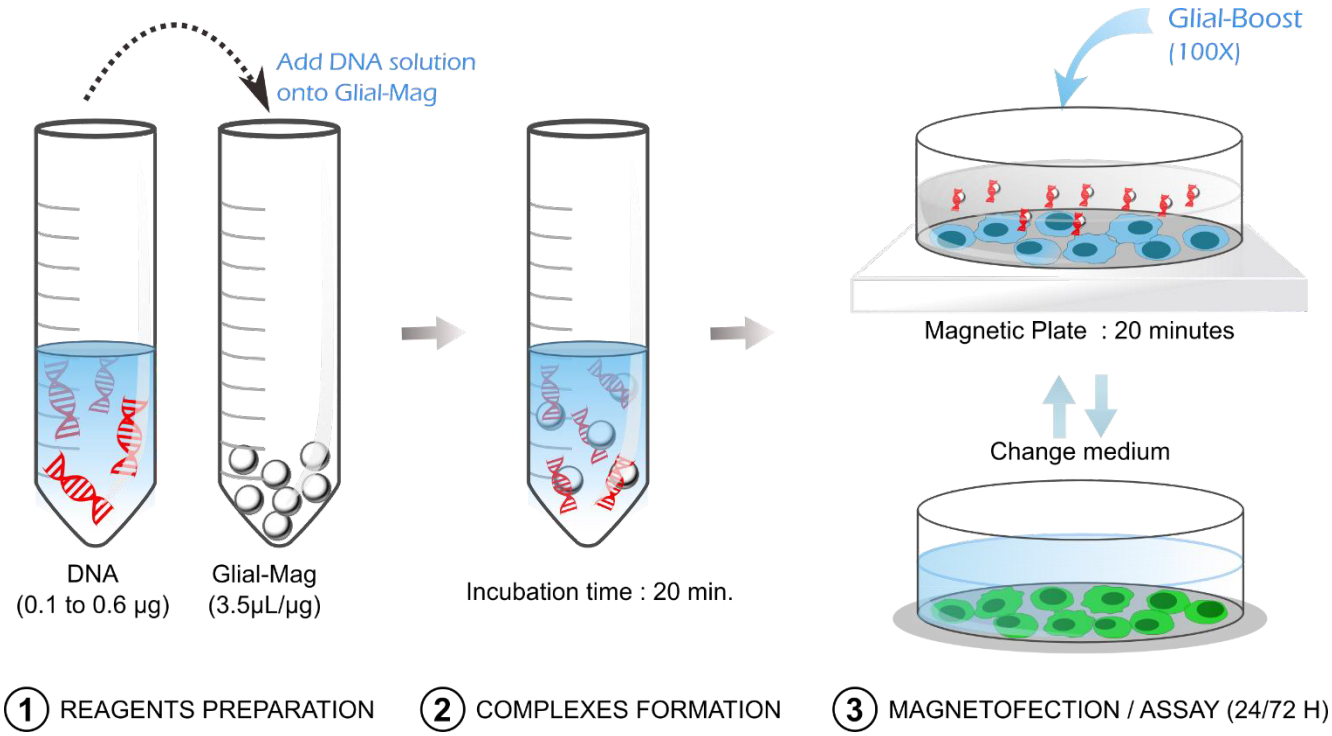


Figure 1: Glial Mag transfection steps

Optimization Protocol

1. General considerations

To achieve the highest efficiency, optimize the transfection conditions as follows:

- Vary the Glial-Mag (µL) / DNA (µg) ratio from 2/1 to 4/1.
  We recommend trying 2.0, 3.0, 3.5 and 4 µL Glial-Mag per µg DNA.
- Once the optimal Glial-Mag/DNA ratio is found, adjust the DNA quantity according to Table 3.
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Tissue Culture Dish	DNA Quantity (µg)
48 well	0.05 to 0.25
24 well	0.1 to 0.5
6 well	0.3 to 1.5

Table 3: Suggested range of DNA amounts for optimization (per well)

2. Optimization procedure in 24 well plate

Depending on the lab, culture medium, cell clone… conditions may be optimized to achieve the best transfection.

KEY PARAMETERS BEFORE BEGINNING

- For low volumes, to ensure a correct pipetting, we recommend preparing a 5X dilution of Glial-Mag in sterile culture-grade H_2O
- Discard remaining dilution after use.

a. Find the ideal Volume of Glial-Mag

Use fixed amount of DNA and vary volume of Glial-Mag.

- Prepare a 0.2 µg/well DNA solution enough for 5 wells: dilute 1 µg DNA in 250 µL medium w/o supplement.
- Prepare 4 tubes containing 2, 3, 3.5 and 4 µL of 5X diluted Glial-Mag corresponding to ratios of 2:1, 3:1, 3.5:1 and 4:1, respectively
- Add 50 µL of the DNA solution to each tube
- Incubate 20-30 min at RT
- Add the complexes onto the cells in a drop wise manner and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
- Add 5 µL of Glial-Boost per well and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Place the cells onto the magnetic plate and incubate 30 min at 37°C.
- Remove the Magnetic plate and incubate the cells under standard culture conditions for 2h
- Change medium using pre-warmed complete culture medium
- Incubate the cells under standard culture conditions for 24 to 72 h.

b. Find the ideal amount of DNA

Once the ratio is found, keep it unchanged and optimize conditions to find ideal DNA amount

- Prepare 4 DNA solutions containing 0.1, 0.2, 0.5 and 1 µg DNA in 50 µL medium w/o supplement.
- For each solution prepare a tube of Glial-Mag corresponding to the ratio found in (1).
- Add each solution of DNA to each Glial-Mag tube
- Incubate 20 min at RT
- Add the complexes onto the cells in a drop wise manner and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Add 5 µL of Glial-Boost per well and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Place the cells onto the magnetic plate and incubate 30 min at 37°C.
- Remove the Magnetic plate and incubate the cells under standard culture conditions for 2h
- Change medium using pre-warmed complete culture medium
- Incubate the cells under standard culture conditions for 24 to 72h.

Additional products for Brain experiments

- **NeuroMag** dedicated to neurons transfection
- **BrainFectIN** for *in vivo* transfection in central nervous system

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