



Transfection reagent

M Glial-Mag™

For Microglial cells

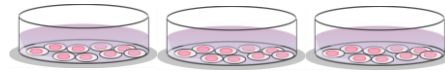
Protocol

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

Glial-Mag™ Quick Protocol

To find the ideal conditions, Glial-Mag must be tested at ratios **2 $\mu\text{L}/\mu\text{g}$** , **3.0 $\mu\text{L}/\mu\text{g}$** , **3.5 $\mu\text{L}/\mu\text{g}$** and **4 $\mu\text{L}/\mu\text{g}$** (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 50 μL of serum-free medium or buffer* x 4

0.2 μg in 100 μL of serum-free medium or buffer* x 4

0.6 μg in 250 μL 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

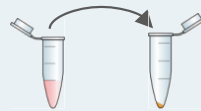
6 well plate

0.2 μL /0.3 μL /0.35 μL /0.4 μL in an empty microtube

0.4 μL /0.6 μL /0.7 μL /0.8 μL in an empty microtube

1.2 μL /1.8 μL /2.1 μL /2.4 μL in an empty microtube

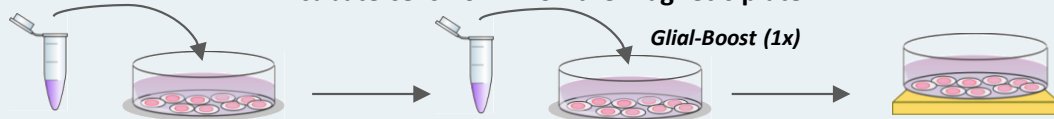
4 **Mix gently each tube of DNA (step 2) to each tube of Glial-Mag (step 3)**



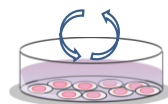
5 **Incubate 20 min at room temperature**



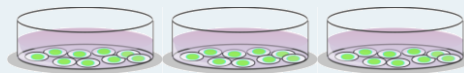
6 **Distribute each mix drop by drop onto the cells to insure uniform distribution; add Glial-Boost (1X final) & incubate cells 20 min on the magnetic plate**



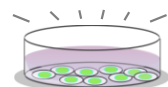
7 **Remove the cells from the magnet, incubate 2h at 37°C and perform a medium change***



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 μ Glial 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 μ L, 400 μ L 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 1 μ L, 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).

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



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Any questions?



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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 description	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

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 CO₂ 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.

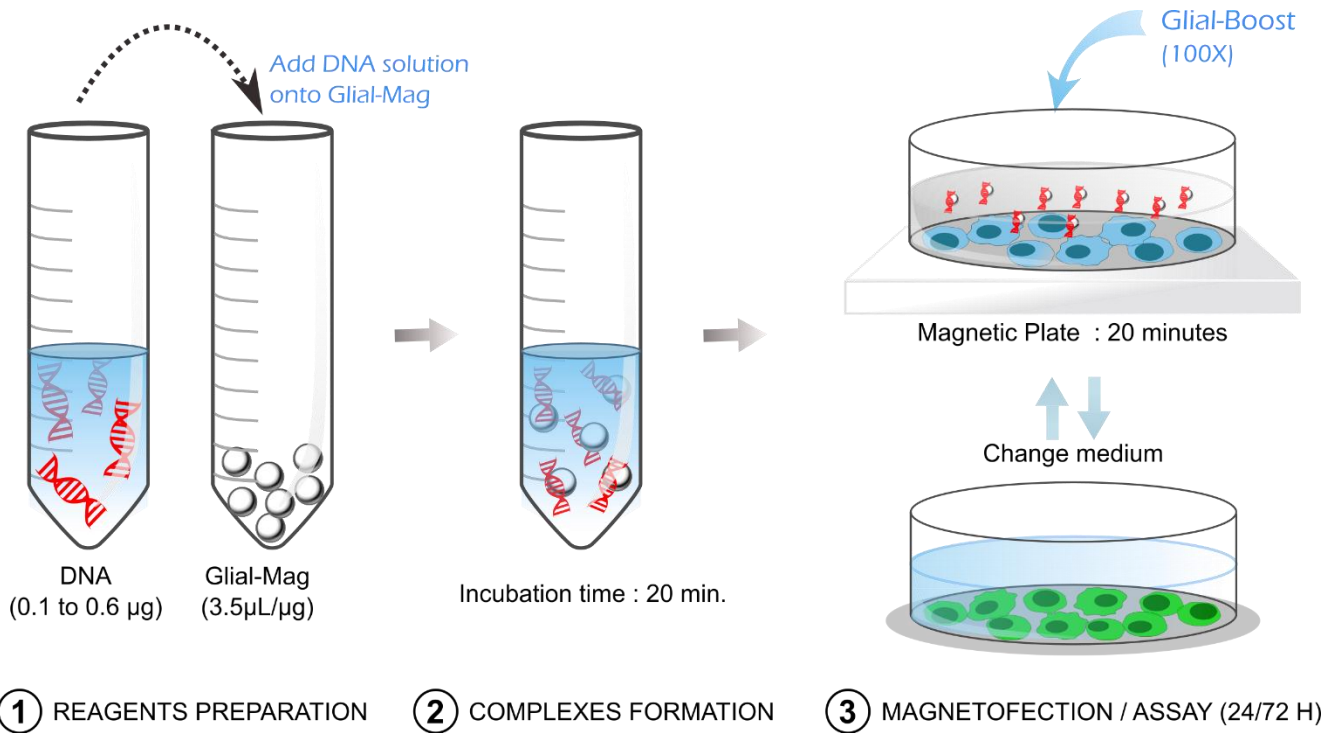


Figure 1: Glial Mag transfection steps

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 H₂O
- 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

Purchaser Notification

Limited License

The purchase of the Glial-Mag 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 Glial-Mag 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 Glial-Mag kit reagents and documentation to OZ Biosciences, or by destroying all Glial-Mag components. Purchasers are advised to contact OZ Biosciences with the notification that a Glial-Mag 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 Glial-Mag 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

Glial-Mag 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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