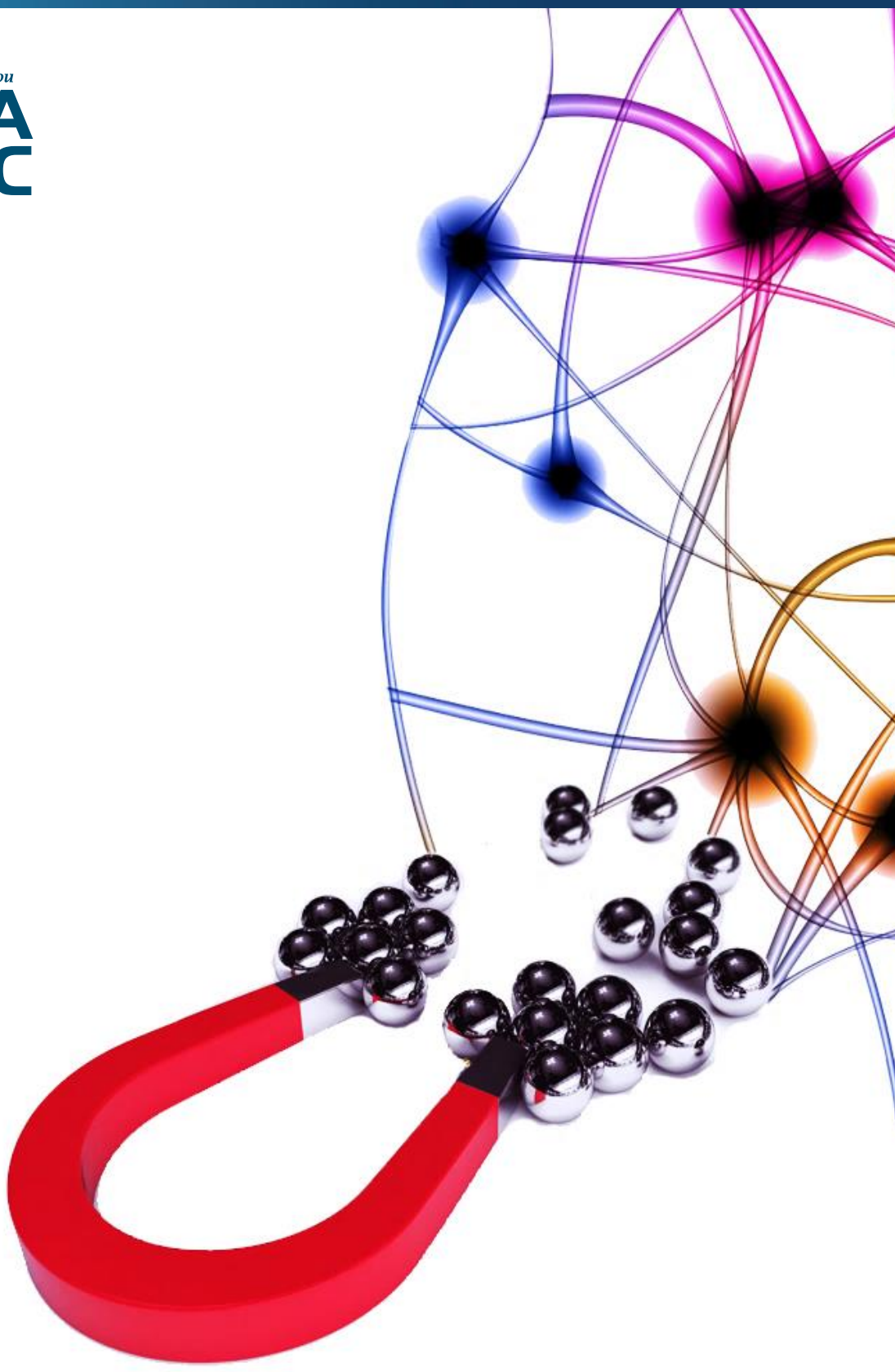
Glial-Mag Transfection Kit

INSTRUCTION MANUAL

We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC



OZBIOSCIENCES

The art of delivery systems

Glial-Mag

Instruction Manual

Glial-Mag is the ideal transfection reagent specific for **microglial cells** transfection.

List of Glial-Mag kits

Catalog Number	Description	Glial-Mag Volume (µL)	Glial-Boost Volume	Number of transfections[1]
GL00250	Glial-Mag transfection kit	250 µL	3 mL	350
GL00500	Glial-Mag transfection kit	500 µL	6 mL	700
KGL00250	Glial-Mag Starting Kit [2]	250 µL	3 mL	350

[1] Values are given for transfections in a 24-well plate using the recommended transfection conditions (0.2 µg DNA/well)

[2] Contains 1 vial of each reagent and a Magnetic plate (250µL of Glial-Mag reagent + 3 mL of Glial-Boost + Super Magnetic plate MF10000)

1. Technology

1.1. Description

Glial-Mag is the ideal transfection reagent specifically developed for microglial cells transfection with high efficiency. **Glial-Mag kit** is an optimized specific magnetic nanoparticles formulation, issued from our Magnetofection™ technology associated to **Glial-Boost**, designed to boost transfection efficiency.
Magnetofection™ is a highly efficient gene delivery method that exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the nucleic acids towards and into- the target cells. In this manner, the complete applied nucleic acid dose gets concentrated onto the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose.

Glial-Mag transfection reagent principal advantages:

- Designed for microglial cells & highly efficient with BV2 lineages
- Low nucleic acid amount - minimized toxicity
- High level of nucleic acid compaction
- Easy and straightforward protocol
- Compatible with any culture medium.

1.2. Kit Contents, Stability and Storage

Contents
Kits content varies according to their size:
• 1 tube containing 250 µL of Glial-Mag and 3 mL of Glial-Boost good for up to 350 assays in a 24-well plate.
• 1 tube containing 500 µL of Glial-Mag and 6 mL of Glial-Boost good for up to 700 assays in a 24-well plate
• 1 tube containing 250 µL of Glial-Mag and 3 mL of Glial-Boost good **and a super magnetic plate**.

Stability, Storage and Shipping
Stability: Glial-Mag and Glial-Boost are stable for at least 18 months at the recommended storage temperature.

Storage: Upon reception and for long-term use, store the Glial-Mag transfection reagent and Glial-Boost at -20°C.

Shipping condition: Room Temperature

Glial-Mag transfection reagent is stable for several days at room temperature or at +4°C without losing activity. The numbers of freeze and thaw cycles do not affect the efficiency of the reagent.

2. Applications

Glial-Mag has been developed specifically for DNA transfection into microglial cells (primary and cell lines). This transfection reagent is serum compatible and is used for transient as well as stable transfection. This product is very stable, ready-to-use and intended for research purpose only.

3. General Protocol

3.1. General Considerations / Important Guidelines

The instructions given below represent standard protocol. Optimal conditions may vary depending on the plasmid, cell lineage, clone, size of cell culture dishes and conditions of culture. As a starting point, use **3.5 µL of Glial-Mag per µg of DNA.** Refer to the optimization procedure to find optimal transfection conditions.

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. Use regularly passaged microglial cell lines or freshly prepared primary cells at confluence between 60 and 80% (visual confluence). Do not use cells that have been cultured for too long (> 2 months).

- **Nucleic Acids** should be as pure as possible and free of contaminants. We suggest avoiding long storage of the diluted nucleic acid solution before the addition of Glial-Mag to circumvent any degradation or surface adsorption. We recommend using pVectOZ-GFP plasmid for an efficient transfection control.

- **Culture Medium**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the Glial-Mag transfection reagent.

3.2. Cells Preparation

Cell culture prior to transfection: one day before transfection prepare the cells according to the table below.

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 (see the suggested cell number in the 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.

Table 1: Cell number suggested (per well).

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

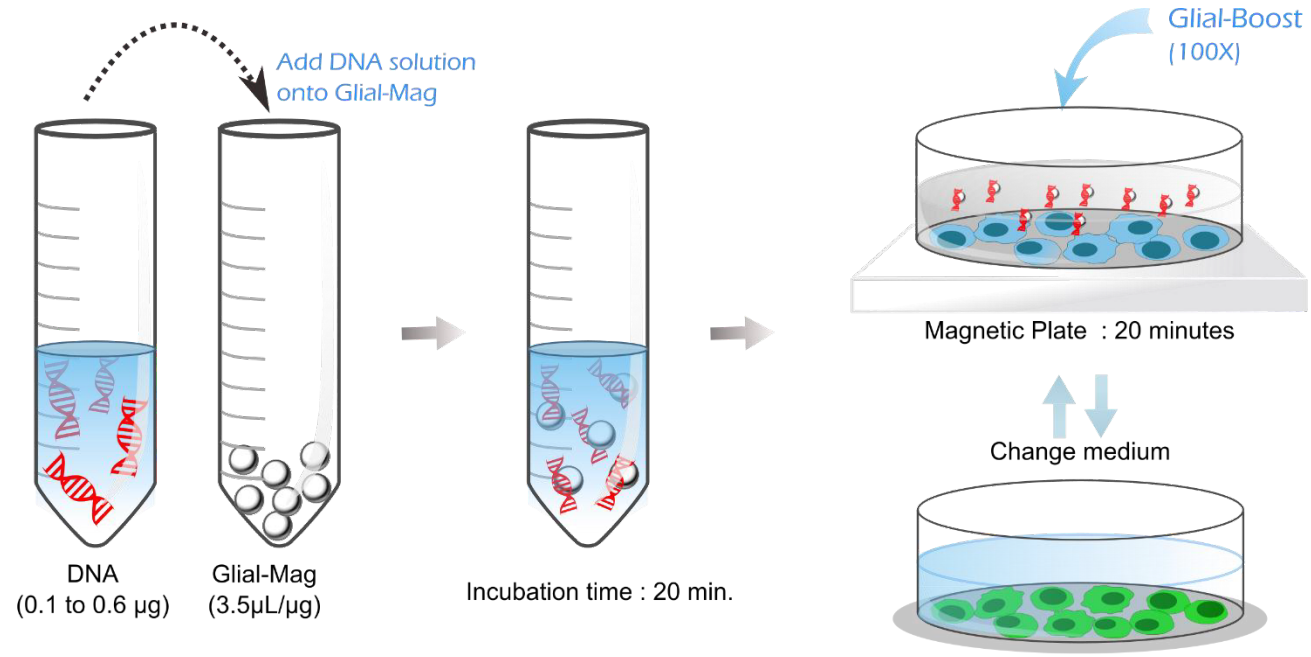
[1] Surfaces area may vary depending on the manufacturer.

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

3.3. DNA Transfection Protocol

Use the following procedure to transfect DNA into microglial cells. The Table 2 shows optimized transfection conditions according to different cell culture formats (all amounts are given on per-well basis).
Use this rapid protocol : **3.5 µL of Glial-Mag per µg of DNA**.

Note: We suggest beginning with the recommended ratios and optimize it, if required.



1) **Reagents preparation.** Allow reagents to reach room temperature before beginning.
   a. *DNA solution.* Dilute **0.1** to **0.6 µg** of DNA in **50** to **250 µL** of culture medium without any supplement (SVF, antibiotics, growth factors...) or PBS (refer to table 2).
   b. *Glial-Mag solution.* Mix the reagent gently before use. Prepare a tube containing Dilute **3.5 µL** of Glial-Mag per µg DNA.

Table 2: DNA amount, Glial-Mag volume and transfection conditions suggested (per well).

Tissue Culture Dish format	DNA Quantity (µg)	Glial-Mag Volume (µL)[1]	Dilution Volume (µL)[2]	Glial-Boost (µL)	Total culture medium Volume
48 wells	0.1	0.35	50	2.5 µL	250 µL
24 wells	0.2	0.7	100	5 µL	500 µL
6 wells	0.6	2.1	250	20 µL	2 mL

[1] For low volumes, to ensure a correct pipetting, we recommend preparing dilution of Glial-Mag in sterile culture-grade H2O.
[2] Volumes of dilution medium for step 1.

2) **Complexes formation.** Combine the DNA and the Glial-Mag solutions. Mix gently by carefully pipetting up and down and incubate the mixture for 20 minutes at room temperature. Do not vortex or centrifuge!

   Note: Proceed to step 3 within 30 minutes

3) **Transfection**.
   a. Add the complexes in a dropwise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.
   b. Add **2.5** to **20 µL** of Glial-Boost (1X final) directly onto the cells.

4) **Magnetofection**. Place the cells upon the specific magnetic plate for 20-30 min

5) Remove the magnetic plate and cultivate cells under standard conditions for 2h.

6) **Medium Change**. Perform a medium change 2h after transfection

7) **Assay.** Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein expression. We recommend performing assay from 24 to 72h.

Reverse transfection. Prepare the complexes as described above, then transfer them into an empty culture dish or well and finally and directly add the cells at twice the recommended cell density.

Other protocols for optimization or co-transfection are also available on our website at www.ozbiosciences.com or by contacting our technical support department (tech@ozbiosciences.com).

OZ Biosciences offers plasmids coding for CAT (#PL00010), **GFP** (#PL00020), **LacZ** (#PL00030), **LUC** (#PL00040) **and SEAP** (#PL00050) **as transfection controls**. These control plasmids are recommended to set up optimization procedure.

3.4. Optimization protocol for DNA transfection

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

- Vary the Glial-Mag (µL) / DNA (µg) ratio from 3/1 to 4.5/1.
  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.

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

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

Refer to detailed optimization protocols for 24-well plates the end of this document (section 7).

4. Appendix

4.1 Quality Controls

To assure the performance of each lot of Glial-Mag reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 15 days.
Biological Activity	Transfection efficacies on BV2 cells. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions

Low transfection efficiency	1- **Optimization of Glial-Mag/ DNA ratio.** See section 3.4. 2- **DNA amount.** Use different quantities of DNA with the optimized ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 60 to 80% but most favorable cell density may vary according to the cell subtype; preferably mid-log growth phase. 4- **DNA quality.** Nucleic acid should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins. 5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Use pVectOZ plasmids as controls for transfection. 6- **Cell condition.** 1) Cells in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing DNA / transfection reagent complexes**. It is critical to use serum-free medium or buffer (HBS, PBS) during the complexes preparation. 8- **Culture medium composition.** 1) In some cases, transfection efficiency can be increased in absence of serum. Transfect these cells in serum-free medium during the first 4h. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 18h depending on the readout and the cell. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared each time to avoid aggregation. 11- **Transfection reagent temperature.** Reagents should be at ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials. 2- **Protein expression is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a control plasmid. 3- **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously.

5. Related Products

MAGNETOFECTION TECHNOLOGY

Super Magnetic Plate *(standard size for all cell culture support)*

+ Transfection reagents:
PolyMag Neo - *for all nucleic acids*
Magnetofectamine™ (Lipofectamine™ 2000 + CombiMag) - *for all nucleic acids*
NeuroMag - *dedicated for neurons*
SilenceMag - *for siRNA application*

PLASMIDS PVECTOZ

pVectOZ-LacZ, Luc, CAT, GFP, SEAP

ASSAY KITS

Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)

BIOCHEMICALS

D-Luciferin, K^+ and Na^+
X-Gal powder 1g / G-418, Sulfate

6. Purchaser Notification

Limited License

The purchase of this product grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the purposes 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 this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us.

Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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 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

This product 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.

7. Optimization Procedure

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.

1) 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 **2 h**
- Change medium using pre-warmed complete culture medium
- Incubate the cells under standard culture conditions for 24 to 72 h.

2) 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 **2 h**
- Change medium using pre-warmed complete culture medium
- Incubate the cells under standard culture conditions for 24 to 72 h.