

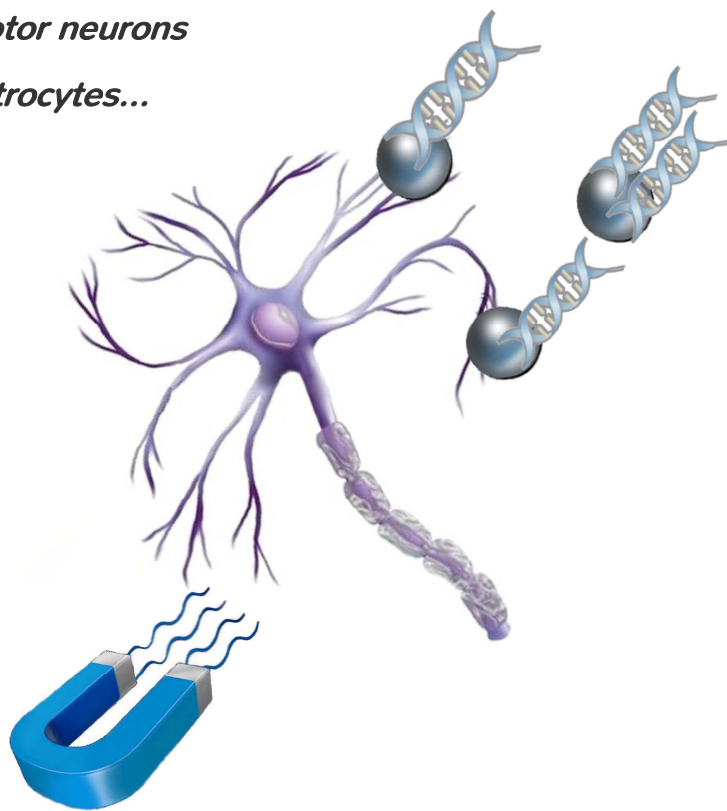
INSTRUCTION MANUAL

Primary Neurons

Neuronal & Glial Cell Lines

Motor neurons

Astrocytes...



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Magnetofection™: NeuroMag

Instruction Manual

NeuroMag

Achieve highly efficient transfection of primary neurons and neuronal cell lines.

List of NeuroMag Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
NM50200	NeuroMag	200	50-70
NM50500	NeuroMag	500	125-175
NM51000	NeuroMag	1000	250-350
MF10096	Magnetic Plate with 96-magnets	-	
MF10000	Super Magnetic Plate	-	
MF14000	Mega Magnetic Plate	-	
KC30800	NeuroMag Starting Kit ¹	200	50-70
KC30896	NeuroMag Starting Kit ²	200	50-70

¹ Contains 1 vial of NeuroMag (NM50200) and a Super Magnetic Plate (MF10000).

² Contains 1 vial of NeuroMag (NM50200) and a Magnetic Plate with 96-magnets (MF10096).

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

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1. Technology

1.1. Description

Congratulations on your purchase of our Magnetofection™ reagent: **NeuroMag!**

Magnetofection™ is a novel, simple and highly efficient method to transfect cells *in vitro* and *in vivo*. This technology exploits magnetic force to drive the nucleic acids associated with magnetic particles towards and into the target cells. In this way, the complete applied dose of nucleic acids gets concentrated on the cells within few minutes so that 100% of the cells get in contact simultaneously with all DNA or RNA doses. **NeuroMag** is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of primary neurons and neuronal cell lines. In this way, **NeuroMag** is the ideal transfection reagent for neurosciences applications.

NeuroMag main features are:

1. High transfection efficiency for primary neurons and neuronal cell lines
2. Concentrate the entire nucleic acids dose on the cells very rapidly
3. Suitable for primary neurons cultured from 7 up to 21 days (div)
4. Not toxic and serum compatible
5. Higher and longer transgene expression level (expression up to 7 days post-transfection)
6. Target/confine transduction to specific area (magnetic targeting)
7. Versatile: for all nucleic acids

1.2. Kit Contents

Kit contents vary according to their size:

- One tube containing 200 µL of NeuroMag nanoparticles good for 50-70 transfections with 1 µg of DNA
- One tube containing 500 µL of NeuroMag nanoparticles good for 125-175 transfections with 1 µg of DNA
- One tube containing 1000 µL of NeuroMag nanoparticles good for 250-350 transfections with 1 µg of DNA

Stability and Storage

Storage: Upon receipt and for long-term use, store the reagent at **-20°C**. Magnetofection kits are stable for at least 2 years at the recommended storage temperature. **Shipping condition:** Room Temperature.

- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**

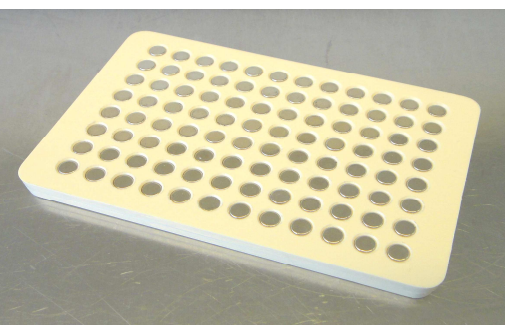
2. Applications

2.1. Application Areas and Cell Types

NeuroMag has been developed for very efficient transfections of various types of nucleic acids such as DNA, RNA or oligonucleotides in primary neurons and immortalized neuronal cells. It is particularly efficient with co-culture of primary hippocampal or corticoid neurons. An updated list of transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need. NeuroMag is serum compatible and can be used for transient and stable transfection. This product is very stable, ready-to-use and intended for research purpose only.

2.2. Magnetofection Apparatus

In addition to suitable magnetic nanoparticles, Magnetofection™ requires an appropriate magnetic field. Three magnetic plates (96-magnets Plate, Super Magnetic Plate and Mega Magnetic Plate) especially designed for Magnetofection, are available. Their special geometry produces a strong magnetic field that is suitable for all cell culture dishes (T-75 flasks, 60 & 100 mm dishes, 6-, 12-, 24-, 48-, and 96-well plates).



Magnetic plate 96 magnets



Super Magnetic Plate



Mega Magnetic Plate

3. General Protocols

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of primary neurons preparation and different neuronal cell lines. Optimal conditions may vary depending on the nucleic acid, neuronal cell types and conditions, size of cell culture dishes and presence or absence of serum. Therefore, the amounts and ratio of the individual components (DNA and **NeuroMag**) may have to be adjusted to achieve the best results. We suggest you to optimize the various transfection parameters as described in section **3.6** (Optimization Protocol) or to refer to the “**Protocol for optimizing transfection with NeuroMag**” at the end of this document. The following recommendations can be used as guidelines to quickly achieve very good transfection and high transgene expression level. As a starting point, we recommend to use **1, 2, 3 and 3.5 µL of NeuroMag per µg of DNA**. **NeuroMag** can be used in the presence or in the absence of serum.

- **Primary cells.** We recommend using primary neurons from embryo of E18 and prepared as described below in mixed culture. The optimal transfection efficiency is then achieved between 10-15 div depending on cell culture conditions. Cultures are always good when neurons are plated in reasonable density.
- **Cell lines** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The cell confluency is a critical parameter and the optimal confluency has to be adjusted according to the cells used.
- **Nucleic acids** should be as pure as possible. Endotoxins levels must be very low since they interfere with transfection efficiencies. Moreover, we suggest avoiding long incubation time of the DNA/RNA solution in buffers or serum free medium before the addition in the **NeuroMag** reagent to circumvent any degradation or surface adsorption. We highly recommend the use of endotoxin-free plasmid preparation kits.
- **Antibiotics.** 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 **NeuroMag** reagent and this effect is cell type dependent and usually small.
- **Materials.** We recommend using polypropylene tubes to prepare the DNA and transfection reagent solutions but glass or polystyrene tubes can also be used.

A protocol used for other transfection reagents should never be employed for **NeuroMag** and inversely. Each transfection reagent has its own molecular structure, biophysical properties and concentration, which have an important influence on their biological activity.

3.2. Neuronal Cell Lines and Primary Neurons Preparation

3.2.1 Primary Neurons Preparation

Primary neurons preparation is a crucial step in order to transfect them efficiently. You will find below the protocol used to prepare hippocampal neurons. In addition, you can find some important tips and tricks in the publication of *Buerli, T., Pellegrino, C., Baer, K. et al, "Efficient transfection of DNA or shRNA vectors into neurons using Magnetofection" Nature Protocol 2007 accepted for publication.*

A- Solution preparation:

- 1- **Dissection solution:** HBSS (without calcium and magnesium) containing 0.25% D-Glucose, keep at 4°C. Prepare fresh solution each time.
- 2- **Culture medium:** MEM supplemented with 10% Nu serum, 15mM HEPES pH 7.2, 0.45% glucose, 1mM sodium pyruvate, 2mM L-glutamine, 10 IU/ml penicillin-streptomycin.
- 3- **Culture feeding medium:** MEM supplemented with 2% B27, 15mM HEPES, 0.45% glucose, 1mM sodium pyruvate, 2mM glutamine.

B- Tissue culture vessel preparation:

- 1- Dissolve Poly-L-lysine (Sigma P-1520) at 0.1 mg/mL in water (aliquot and store at -20°C).
- 2- Cover the coverslip or dish with the Poly-L-lysine solution.
- 3- Incubate over-night at 37°C.
- 4- Rinse twice in water.
- 5- Let dry under a sterile laminar hood.

C- Cell preparation:

Prepared cells from (E)18-19 hippocampal neurons. Use glia-neuron co-culture using defined media and not Banker type cultures nor glia feeder layer. NeuroMag reagent is also suitable with neurobasal media.

- 1- Rinse hippocampi twice in cooled (0°C, max 2-3°C) dissection solution.
- 2- Dissect hippocampi free of meninges in cooled (2-3°C) dissection solution.
- 3- Incubate hippocampi solution with trypsin solution (at 0.25 % final concentration) for 15 min at 37°C in one 15 mL Falcon tube. 10 to 20 hippocampi are generally used in 5 ml of solution in 15 ml falcon tube.
- 4- Stop the trypsin action by washing two times with HBSS or MEM.
- 5- Resuspend in a final volume of 1 to 3 mL of MEM containing 10 % fetal calf serum.
- 6- Triturate tissue using a P1000 micropipette (10 times).
- 7- Wait 1-2 minutes until non-dissociated tissue goes to the bottom of the tube. Transfer dissociated cells into new tube, add 2 mL of HBSS to the remaining non-dissociated clusters and push it (5-10 times) through Pasteur pipette until complete dissociation of tissue. Transfer the dissociated cells to the tube containing the first dissociated cells.

- 8- Count the number of cells.
- 9- Dilute the dissociated neurons to the desired concentration with culture medium. Plate the cells to the polylysine coated dish or coverslip (see the table 1 below for the suggested cell amount). We recommend optimizing the number of cells relative to the dish/coverslip used. For example, 2mL of cells at a density of 300 000 or 400 000 cells per mL can be plated in 35 mm dish. Cell density will also vary according to the desired time point of transfection (immature or mature).
- 10- Grow the cells in 5% CO₂ and 37°C in the absence of a glial feeder cell layer in 10% Nu tissue culture medium until the desired time point for transfection.
- 11- From 10 DIV in culture, change 50 % of old medium with fresh culture feeding medium every 3 days and the day before Magnetofection.

Note that optimal neuronal growth prior to Magnetofection is critical. It depends on the addition of B27 in the culture medium and on the cell density.

3.2.2 Neuronal Cell Lines

Neuronal cell lines. 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 conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) 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 transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous. (See section 3.3 for procedure)

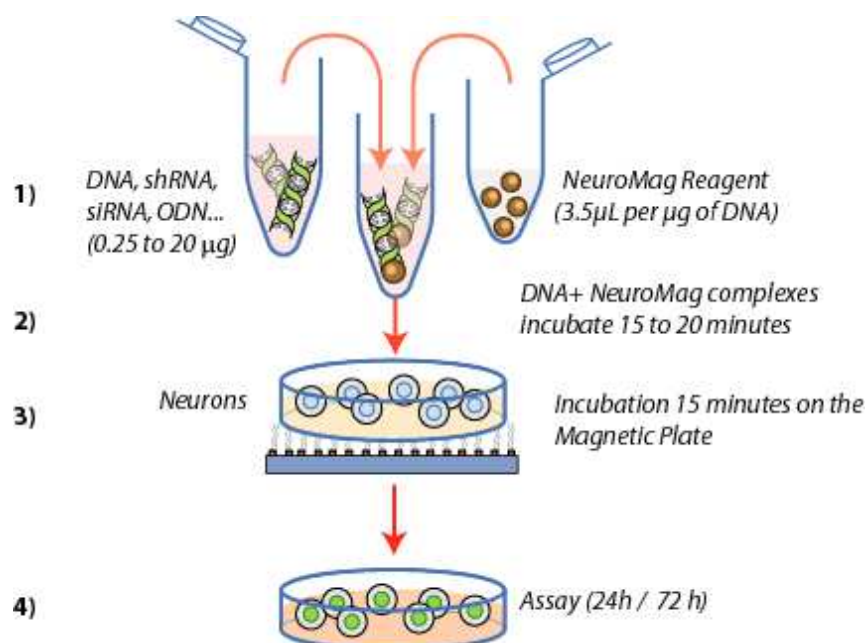
Table 1: Cell number suggested.

Tissue Culture Dish	Neuronal cell lines	Primary neurons
96 well	0.05 – 0.2 x 10 ⁵	-
24 well	0.5 – 1.5 x 10 ⁵	0.5 – 1.5 x 10 ⁵
12 well	1 – 3 x 10 ⁵	0.5 – 2 x 10 ⁵
6 well	2 – 8 x 10 ⁵	4 – 8 x 10 ⁵
60 mm dish	5 – 18 x 10 ⁵	8 – 18 x 10 ⁵
90 - 100 mm	10 – 50 x 10 ⁵	25 – 50 x 10 ⁵
T-75 flask	15 – 60 x 10 ⁵	30 – 60 x 10 ⁵

3.3. Transfection Protocol of DNA or shRNA vectors

Table 2: DNA amount, NeuroMag volume and transfection conditions suggested.

Tissue Culture Dish	DNA Quantity (µg)	NeuroMag Volumes (µL)	Final Dilution Volume (µL)	Transfection Volume
96 well	0.5	0.5 – 1 – 1.5 – 1.75	50	200 µL
24 well	1	1 – 2 – 3 – 3.5	100	500 µL
12 well	2	2 – 4 – 6 – 7	100	1 mL
6 well	4	4 – 8 – 12 – 14	200	2 mL
60 mm dish	10	10 – 20 – 30 – 35	300	4 mL
90 - 100 mm	12	12 – 24 – 36 – 42	500	8 mL
T-75 flask	15	15 – 30 – 45 – 52.5	700	10 mL



The DNA and NeuroMag solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 1, 2, 3 and 3.5 µL of NeuroMag per µg of DNA. We suggest beginning with these ratios and optimize it, if required, by following section 3.6 or by referring to the optimization protocol.

1. Cells.

1.1. Primary Neurons. Mixed cultured cells were used for this procedure. 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. Primary neurons have been transfected from 7 to 21 DIV. The best results were achieved with cells cultured for 10-15 days *in vitro* (day of transfection) depending on cell culture conditions.

Exchange 50% of the culture media 24 hours before transfection

1.2. Cell lines. The day prior transfection, prepare the cells as described in section 3.2.

2. DNA/NeuroMag complexes preparation

2.1. NeuroMag: Vortex the reagent and place the appropriate amounts in a microtube (see Table 2).

2.2. DNA: Dilute the indicated quantity of DNA (see Table 2) in 50 to 700 µL of culture medium without serum and supplement.

2.3. Add the DNA solution to the NeuroMag solutions by vigorous pipetting or brief vortexing and incubate at room temperature for 15 to 20 minutes.

3. Transfection

3.1. Add the NeuroMag / DNA complexes onto cells [growing in culture feeding medium if > 10 DIV or culture medium if <10 DIV] drop by drop and gently rock the plate to ensure a uniform distribution. Place the cell culture plate on the magnetic plate during 15 minutes.

3.2. Remove the magnetic plate.

4. Cultivate the cells at 37°C in a CO₂ incubator under standard conditions until evaluation of transgene expression (from 24h up to 7 days).

3.4. Optimization Protocol*

** Please refer to last pages for complete optimization protocol*

Although high transfection efficiencies can be achieved with the rapid protocol, some optimization may be needed in order to obtain the maximum efficiency in particular cell lines or primary cells culture. Several parameters can be optimized:

- Dose of nucleic acid used
- Ratio of NeuroMag to nucleic acid
- Cell density
- Incubation time

We recommend that you optimize one parameter at a time. It is required to keep the other parameters (cell number, dose of nucleic acid...) constant while one parameter is being optimized.

1) Quantity of DNA:

To achieve the optimal transfection efficiency, the amount of DNA used can be increased or decreased. It is important to always keep the number of cells and the incubation time constant during your optimization procedure. Adjust the best amount of DNA required by maintaining a fixed ratio of **NeuroMag** reagent to DNA (**3.5 µL / µg DNA**), and vary the DNA quantity over the suggested range (table 3).

2) NeuroMag / DNA ratio:

For optimization, first maintain a fixed quantity of DNA (according to the size of your culture dish, cell number and previous optimization) and then vary the amount of **NeuroMag** reagent from 2 to 5 µL per 1 µg of DNA.

3) Cell number:

The cell number (density) is also a critical parameter to achieve good transfection efficiency and the optimal confluency has to be adjusted according to the cells used. 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.

Table 3: Suggested range of DNA amounts for optimization.

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.15 – 1	200 µL
24 well	0.2 – 2	500 µL
12 well	0.4 – 4	1 mL
6 well	1 – 8	2 mL
60 mm dish	2 – 16	4 mL
90 – 100 mm dish	5 – 30	8 mL
T-75 flask	6 – 35	10 mL

4) Incubation time:

The optimal time range between transfection and assay for gene activity varies with cells, promoter activity, expression product, etc. The transfection efficiency can be monitored after 24 hours up to several days (7) by analyzing the gene product. Reporter genes such as GFP, β-galactosidase, secreted alkaline phosphatase or luciferase can be used to quantitatively measure gene expression.

In addition, do not hesitate to contact our technical service at tech@ozbiosciences.com to request more detailed technical insights and applications update.

3.5. Co-Transfection Protocol

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above in section 3.3 or 3.4. For example, if you have two DNA plasmids, mix 1 µg of each plasmid, complex the 2 µg of DNA with 7 µL of **NeuroMag**.

Option for Co-transfection.

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4 h to 24 h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection (3.3 or 3.4). A medium changed can be also performed between the two transfections.

3.6. Important Observations

A. Primary Neurons preparation.

- Hippocampi removed from the brains must be dissected free of the meninges in cooled HBSS without Ca²⁺ and Mg²⁺ and incubated with 0.25% trypsin for 15 min at 37°C. It is essential to remove all meninges from the hippocampi and to respect the time of incubation for the trypsin treatment.
- The temperature of dissection solution is critical; it should not be warmer than 2-3°C.
- Triturate tissue using fire polished Pasteur pipette: 10 times.
- Wait 1-2 minutes until non-dissociated tissue goes to the bottom of the tube. Transfer dissociated cells into new tube, add 2 mL of HBSS to the remaining non-dissociated clusters and push it (5-10 times) through Pasteur pipette until complete dissociation of tissue. Transfer dissociated cells into the tube with first portion of the neurons.
- Test different concentrations of cells in culture dish.
- Culture medium composition is critical (see section 3.2)
- The batch of Nu-serum might vary and so it is essential to test different Nu-serum concentrations (10-20%).
- On day 10 of culture, 50% of the medium must be changed with fresh culture feeding medium
- If you need good culture for electrophysiology and transfections, never use AraC.

B. Ensure to avoid the presence of serum when preparing the NeuroMag / DNA complexes. Use a medium with a correct pH (some old medium can turn pink or purple instead of being orange or red) which could influence complexes formation and DNA stability.

C. Avoid incubating your diluted DNA too long in your serum-free medium; prepare first your transfection reagent, dilute your DNA and quickly transfer the diluted DNA into the **NeuroMag** tube.

4. Appendix

4.1 Quality Controls

To guarantee the performance of each lot of **NeuroMag** produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
<i>Purity</i>	Silica Gel TLC assays. Every compound shall have a single spot.
<i>Sterility</i>	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
<i>Biological Activity</i>	Transfection efficiency on primary neurons and PC12 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	<p>1- DNA amount. Use different quantity of DNA with the recommended or optimized (above) transfection reagent / DNA ratio (3.5 μL / μg).</p> <p>2- NeuroMag / nucleic acid ratio. Optimize the reagent / nucleic acid ratio by using a fixed amount of DNA (μg) and vary the amount of NeuroMag as it is suggested in table 3.</p> <p>3- Cell density. A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 50 to 70% (true confluency, corresponding to 90% visual confluency) but most favorable cell density may vary according to the cell type; preferably mid-log growth phase.</p> <p>4- DNA quality. Nucleic acids should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies. Employ nuclease-free materials.</p> <p>5- Type of promoter. Ensure that DNA promoter can be recognized by the cells to be transfected. Another cells or viral-driven reporter gene expression can be used as a control.</p> <p>6- Cell condition. Cells should be healthy and neurons condition greatly influences transfection efficiency. The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency.</p> <p>7- Suboptimal age of cells at Magnetofection time point. The age of neurons at the time of transfection is a very critical point. Optimizing this parameter is a key element to achieve high transfection.</p> <p>8- Medium used for preparing DNA / transfection reagent complexes. It is critical that serum-free medium is used during the preparation of the complexes. Avoid any direct contact of pure nucleic acid solution with the plastic surface.</p> <p>9- Cell culture medium composition. The culturing medium plays an essential role to achieve high transfection rates of primary cells. Use the recommended culture medium. For some cell lines, transfection efficiency can be increased without serum or under reduced serum condition. Thus, transfect these cells in serum-free medium during the first 4h of incubation. The presence of antibiotics might affect cell health and transfection efficiency.</p> <p>10- 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 24 – 96 h by analyzing the gene product. Several reporter genes can be used to quantitatively monitored gene expression kinetics. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours.</p>
Low transfection efficiency	<p>11- Old transfection reagent / DNA complexes. The transfection reagent / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hour can be aggregated.</p> <p>12- Transgene detection assay. Ensure that your post-transfection assay is properly set up and includes a positive control.</p> <p>13- Transfection reagent temperature. Reagents should have an ambient temperature and be vortexed prior to use.</p>
Primary Neurons cell culture condition	<p>1- Neuronal cultures show unhealthy morphology. Suboptimal culturing conditions. Optimize the primary neuronal cultures from hippocampus and cortex. Control CO₂ and pH, control Nu-serum concentration.</p> <p>2- Too many glia cells / few neurons in the culture. Suboptimal culturing conditions. Vary</p>

	<p>amount of Nu-serum (increase up to 20%).</p> <p>3- Neurons in culture form clusters. Appearance of clusters after 6-10 DIV indicates a) inefficient coverslip coverage with poly-Lysine; b) insufficient washing of coverslips; c) inappropriate glass quality for neuronal cultures. The simplest way to identify the cause of cluster appearance is to verify the neurons growing on the plastic between coverslips. If the culture is homogeneous, it is required to prepare a new batch of coverslips.</p>
Cellular toxicity	<p>1- Unhealthy cells. a) Check cells for contamination, b) Use new preparation batch of cells, c) Cell density is too low.</p> <p>2- Transgene product is toxic. Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a DNA control.</p> <p>3- DNA quality - Presence of contaminants. Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Low quality DNA lead to cell death. DNA concentration as well as DNA constructs itself have an influence on efficiency and toxicity.</p> <p>4- Concentration of transfection reagent / nucleic acid too high. a) Decrease the nucleic acid amount. b) Optimize concentration of transfection reagent and ratios as outlined previously.</p> <p>5- Incubation time. Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4 h to 24 h.</p> <p>6- Key gene silencing. If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the shRNA this can lead to cell death.</p> <p>7- Medium changed. Usually, neuronal death after transfection is related to medium change and not to toxicity of reagents. Always, include non transfected cells to insure that transfection procedure healthiness.</p>
No or weak gene silencing effect	<p>1- shRNA design. The design of an efficient shRNA is a crucial step. Ensure to use a validated shRNA sequence encoded in the expression vector. If a validated shRNA cannot be used, assay your sequence in an easy to transfect cell line (if possible) in order to validate.</p> <p>2- Incubation time. Perform a time-course experiment to set up the optimal incubation time since gene silencing is dependent on the gene expression and the protein turnover rate.</p> <p>3- Old NeuroMag / shRNA vector complexes. The NeuroMag / shRNA vector complexes must be freshly prepared every time. Complexes kept for longer than 1 hour can be aggregated.</p>

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate (<i>standard size for all cell culture support</i>)
Mega Magnetic plate (<i>mega size to hold 4 culture dishes at one time</i>)
Transfection reagents:
PolyMag Neo (<i>for all nucleic acids</i>)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (<i>for all nucleic acids</i>)
NeuroMag (<i>dedicated for neurons</i>)
SilenceMag (<i>for siRNA application</i>)
Transfection enhancer:
CombiMag (<i>to improve any transfection reagent efficiency</i>)
Viral Transduction enhancers:
ViroMag (<i>to optimize viral transduction</i>)
ViroMag R/L (<i>specific for Retrovirus and Lentivirus</i>)
AdenoMag (<i>for Adenoviruses</i>)
Viral Capture & Concentration:
Mag4c kits (<i>for adeno & lenti viruses</i>)
In vivo Magnetofection
In vivo ViroMag (<i>for magnetic assisted viral infection</i>)
In vivo PolyMag (<i>polymer-based magnetic nanoparticles</i>)
In vivo DogtorMag (<i>lipid-based magnetic nanoparticles</i>)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (<i>siRNA transfection reagent</i>)
DreamFect Gold (<i>Transfection reagent for all types of nucleic acids</i>)
VeroFect (<i>for Vero cells</i>)
Ecotransfect (<i>Economical reagent for routine transfection</i>)
FlyFectin (<i>for Insect cells</i>)
i-MICST TECHNOLOGY
Viro-MICST (<i>to transduce directly on magnetic cell purification columns</i>)
3D TRANSFECTION TECHNOLOGY
3DfectIN (<i>for hydrogels culture</i>)
3Dfect (<i>for scaffolds culture</i>)
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (<i>for High Yield Protein Expression</i>)
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN (<i>delivery reagent for antibodies</i>)
Pro-DeliverIN (<i>delivery reagent for protein in vivo and in vitro</i>)
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K ⁺ and Na ⁺ 1g
G-418, Sulfate 1g
X-Gal powder 1g

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

6. Purchaser Notification

Limited License

The purchase of the **NeuroMag** Reagent 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 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 **NeuroMag** Reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

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

The **NeuroMag** Reagent 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.

For more information, or for any comments on the terms and conditions of this License, please contact:

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Protocol for optimizing transfection with NeuroMag

Key parameter before beginning the procedure:

- The DNA and transfection reagents solutions should have an ambient temperature and be gently vortexed prior to use.
- All the complexes must be prepared **in medium without serum and supplement** (DMEM or OPTIMEM).
- Cell culture conditions will vary with the DIV, the neuron territory (hippocampal, cortical, neural stem cells) or the species (rat, mouse...).

Storage:





















- NeuroMag nanoparticles must be stored at -20°C.

NeuroMag OPTIMIZATION

Original protocol: use **3.5 µL** of NeuroMag reagent (magnetic nanoparticles) per **µg** DNA (ratio 3.5:1).

This protocol is given for NeuroMag transfection reagent optimization in a 24 well plate culture format: 4 DNA quantities (0.5, 0.75, 1 and 1.5 µg) and 4 NeuroMag ratios (1:1, 2:1, 3:1 and 3.5:1) are tested according to the following layout.

NOTE: transfection is compatible with neurons culture on glass coverslips.

		DNA quantities				
		0,5	0,75	1	1,5	
NeuroMag ratios	1:1					
	2:1					
	3:1					
	3,5:1					

- **Cell preparation**

Culture cells as usual, we recommend following culture conditions described by Buerli et al¹, Nature Protocols (2007) for long term cultures.

- **Reagent preparation** (the day of transfection)

DNA solutions. We recommend testing four DNA quantities, preparation for 5 wells

- 0.5 µg/well : Dilute 2.5 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
- 0.75 µg/well : Dilute 3.8 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
- 1.0 µg/well : Dilute 5.0 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
- 1.5 µg/well : Dilute 7.5 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.

Incubate 5 min at RT

NeuroMag dilution. Vortex the reagent before each use.

Prepare a 2X NeuroMag dilution in sterile culture grade water only:

2X: Add 2 µL NeuroMag to 2 µL of sterile culture grade water (final volume 4µL).

Mix well by pipetting up and down.

- **Complexes preparation**

Prepare 4 tubes of NeuroMag dilutions per DNA quantities:

For 0.5 µg DNA:	A1- add 1 µL NeuroMag (2X) to a new tube	(ratio 1:1)
	A2- add 1 µL NeuroMag to a new tube	(ratio 2:1)
	A3- add 1.5 µL NeuroMag to a new tube	(ratio 3:1)
	A4- add 1.8 µL NeuroMag to a new tube	(ratio 3.5:1)
For 0.75 µg DNA:	B1- add 1.5 µL NeuroMag (2X) to a new tube	(ratio 1:1)
	B2- add 1.5 µL NeuroMag to a new tube	(ratio 2:1)
	B3- add 2.3 µL NeuroMag to a new tube	(ratio 3:1)
	B4- add 2.7 µL NeuroMag to a new tube	(ratio 3.5:1)
For 1.0 µg DNA:	C1- add 1.0 µL NeuroMag to a new tube	(ratio 1:1)
	C2- add 2.0 µL NeuroMag to a new tube	(ratio 2:1)
	C3- add 3.0 µL NeuroMag to a new tube	(ratio 3:1)
	C4- add 3.5 µL NeuroMag to a new tube	(ratio 3.5:1)
For 1.5 µg DNA:	D1- add 1.5 µL NeuroMag to a new tube	(ratio 1:1)
	D2- add 3.0 µL NeuroMag to a new tube	(ratio 2:1)
	D3- add 4.5 µL NeuroMag to a new tube	(ratio 3:1)
	D4- add 5.3 µL NeuroMag to a new tube	(ratio 3.5:1)

Combine 100 µL of each DNA solutions to the corresponding NeuroMag dilutions (A to A1-A4), (B to B1-B4), (C to C1-C4) and (D to D1-D4).

Mix gently by pipetting up and down.

Incubate the mixtures for 20 minutes at room temperature.

- **Transfection**

- Add the complexes prepared previously dropwise onto the cells cultivated according to your experiment and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
- Then, incubate the cells on the magnetic plate at 37°C for 20 min.
- After 20 min of incubation, remove the magnetic plate.
- Incubate the cells under your standard culture conditions. No medium change is required during the incubation period but can be performed as an option.

NOTES :

- (1) Neurons are very "shock sensitive": avoid any physical shock that could activate the neurons and impair the transfection efficiency.
- (2) For transfection control experiments, we recommend using "transfection grade" plasmid such as pVectOZ-GFP (refer to our website for other transfection-grade control plasmids).



¹ Buerli T, Pellegrino C, Baer K, Lardi-Studler B, Chudotvorova I, Fritschy JM, et al. Efficient transfection of DNA or shRNA vectors into neurons using magnetofection. Nat Protoc 2007;2(12):3090-101.

² Sapet C, Laurent N, de Chevigny A, Le Gourrierc L, Bertosio E, Zelphati O, Béclin C. High transfection efficiency of neural stem cells with magnetofection. Biotechniques. 2011 Mar;50(3):187-9.

³ Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, et al. Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. Cell. 2012 May 11;149(4):923-35. Epub 2012 May 3.

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