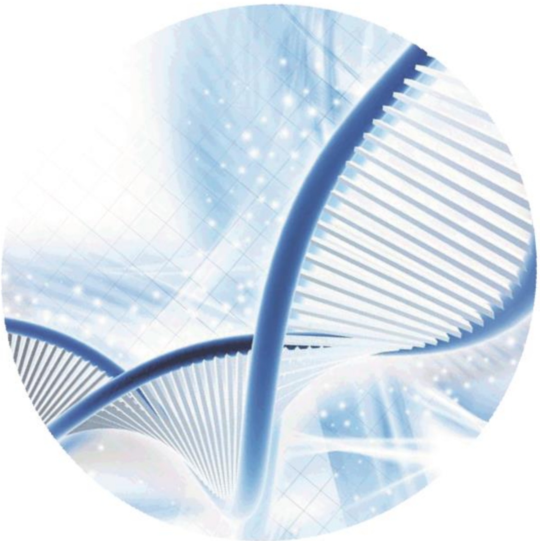


Transfection reagent

NeuroMag™

For Neurons

Primary Neurons, Neuronal cell lines, Motor Neurons, Astrocytes...

Protocol

Magnetofection Technology

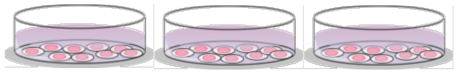
This reagent needs to be used with a magnetic plate

OZBIOSCIENCES
The art of delivery systems

NeuroMag Quick Protocol

To find the ideal conditions, NeuroMag™ reagent must be tested at ratios **1 µL/µg**, **2 µL/µg**, **3 µL/µg** and **3.5 µL/µg** (µL of NeuroMag/µg of DNA). For the DNA quantity, we suggest **0.5 µg** per well in 96-well, **1 µg** per well in 24-well and **4 µg** per well in 6-well.

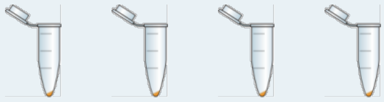
1 **Seed cells to be at 50-70% confluent the day of transfection - Do not change your medium 24h before transfection or if needed change only 50% ***



2 **Prepare 4 identical tubes of DNA**

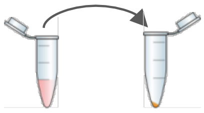
96 well plate	24 well plate	6 well plate
0.5µg in 50µL of serum-free medium or buffer* x 4	1µg in 100µL of serum-free medium or buffer* x 4	4µg in 200µL of serum-free medium or buffer* x 4

3 **Prepare 4 tubes of NeuroMag™ (with 4 different amounts of reagent)**

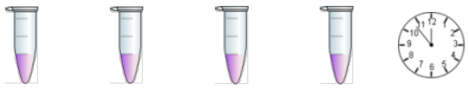


96 well plate	24 well plate	6 well plate
0.5µL/1µL/1.5µL/1.75µL in an empty microtube	1µL/2µL/3µL/3.5µL in an empty microtube	4µL/8µL/12µL/14µL in an empty microtube

4 **Mix gently each tube of DNA (step 2) to each tube of NeuroMag™ (step 3)**

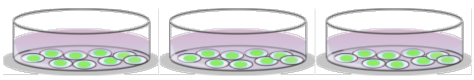


5 **Incubate 15 to 20 min at room temperature**

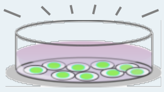


6 **Distribute each mix drop by drop onto the cells to insure uniform distribution & then place the cells 20 min. on the magnetic plate**

7 **Remove the cells from the magnetic plate & incubate cells for 24 to 72h at 37°C until evaluation of transgene expression***



8 **Choose the best ratio DNA: NeuroMag™ ratio**



These conditions might require some further optimizations depending on your cells, DNA, RNA, types of neurons, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ Cells preparation

    - For Primary Neurons. 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 1 to 22 DIV.
    - 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.

- ✓ 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/NeuroMag complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. NeuroMag is suitable with Neurobasal media. In contrast, we do not recommend RPMI for preparing the complexes.

- ✓ We recommend respecting the order of addition: add the DNA solution onto NeuroMag magnetic nanoparticles.

- ✓ For doses of NeuroMag 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 4 to 6 h post-Magnetofection.

Package content	NM50200: 200 µL of NeuroMag reagent NM50500: 500 µL of NeuroMag reagent NM51000: 1 mL of NeuroMag reagent KC30800: NeuroMag Starting Kit - 200 µL of NeuroMag reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the NeuroMag transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	NeuroMag is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of primary neurons and neuronal cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA or shRNA vectors

1. Cells Preparation

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 (see Table 1). Primary neurons have been transfected from 1 to 22 DIV. The best results were achieved with cells cultured for 10-15 days *in vitro* (day of transfection) depending on cell

Important tips and tricks

Buerli T., et al., *"Efficient transfection of DNA or shRNA vectors into neurons using Magnetofection"* ***Nature Protocols*** 2, 3090–3101 (2007)

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 (refer to 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

The day prior transfection, prepare the cells as described in Table 1.

Tissue Culture Dish	Neuronal cell lines	Primary neurons
96 well	$0.05 – 0.2 \times 10^5$	-
24 well	$0.5 – 1.5 \times 10^5$	$0.5 – 1.5 \times 10^5$
6 well	$2 – 8 \times 10^5$	$4 – 8 \times 10^5$
60 mm dish	$5 – 18 \times 10^5$	$8 – 18 \times 10^5$

Table1: Recommended Cell number

2. DNA/NeuroMag complexes preparation

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

b. *DNA*: Dilute the indicated quantity of DNA (see Table 2) in 50 to 200 µL of culture medium <u>without</u> serum and supplement.

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

Tissue Culture Dish	DNA Quantity (µg)	NeuroMag Volume (µL)	Dilution Volume (µL)	Transfection Volume (mL)
96 well	0.5	0.5 – 1 – 1.5 – 1.75	50	0.200
24 well	1	1 – 2 – 3 – 3.5	100	0.500
6 well	4	4 – 8 – 12 – 14	200	2
60mm dish	10	10 – 20 – 30 - 35	300	4

Table 2: Recommended DNA amount, NeuroMag volume and transfection conditions

3. Transfection

a. 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 15-20 min.

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).

NOTE: 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.

Protocol | Co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. 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.

<u>Option for co-transfection</u>

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 change can be also performed between the two transfections.

1. siRNA/NeuroMag complexes preparation

The siRNA and NeuroMag solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (see table 3) of culture medium without serum and antibiotics.

b. *NeuroMag preparation*. Add 0.5 to 5 µL of NeuroMag in an empty microtube (refer toTable 4).

c. Add the siRNA solution onto the NeuroMag reagent.
Mix gently by carefully pipetting up and down and incubate the mixture for 15 min at room temperature.
Do not vortex or centrifuge!

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50 µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
1 nM	0.2	2.7	0.5	6.75	2	27
5 nM	1	13.5	2.5	33.75	10	135
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 3: Suggested dilution procedure and amount of siRNA to test

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	25 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Amount of NeuroMag (µl)			
Final siRNA concentration			
1 nM	0.5	1	1
5 nM	0.5	1	2
10 nM	0.5	1	2
20nM	1	1	3
≥ 50 nM	1	2	5

Table 4: Recommended amount of NeuroMag per nM of siRNA used

2. Transfection

a. Add the complexes to the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
b. Place the cell culture plate on the magnetic plate during 30 min.
c. Remove the magnetic plate

d. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis.

NOTE: Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection.

Optimization Protocol

Although high transfection efficiencies can be achieved with the quick protocol, some optimizations 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

NOTE: Neurons are very "shock sensitive": avoid any physical shock that could activate the neurons and impair the transfection efficiency.

1. General considerations

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.

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 5).

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.15 – 1	200 µL
24 well	0.2 – 2	500 µL
6 well	1 – 8	2 mL
60 mm dish	2 – 16	4 mL

Table 5: Suggested range of DNA amounts for optimization

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.

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.

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 h 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.

2. Optimization in 24 well plate

Key parameters 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…).
- ✓ NeuroMag nanoparticles must be stored at -20°C.

NOTE: 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 (see Figure 1).

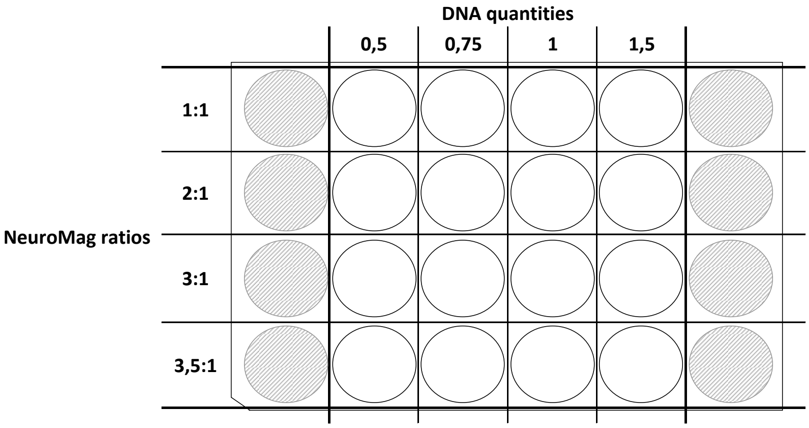


Figure 1: Recommended transfection conditions in 24-well plate

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

- **Cell preparation**

Culture cells as usual, we recommend following culture conditions described by Buerli *et al*[1], 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
A. 0.5 µg/well : Dilute 2.5 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
B. 0.75 µg/well: Dilute 3.8 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
C. 1.0 µg/well : Dilute 5.0 µg DNA in 500 µL of **DMEM alone (or OptiMEM)**.
D. 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 min 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.

PUBLICATIONS

[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 Gourrierec 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.

Additional products

- **Glial-Mag** for glial cells transfection
- **BrainFectIN** for *in vivo* transfection in central nervous system
- **pVectOZ Transfection plasmids (CAT, GFP, LacZ, Luciferase, SEAP)** - Positive controls and optimization of all transfection experiments

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