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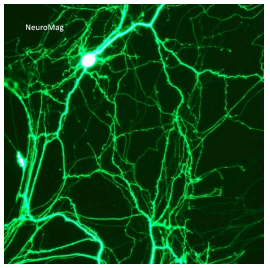
Transfection of Mature Primary Neurons (≥10 DIV) using Magnetofection™ with NeuroMag™ V.1



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Cedric Sapet[1]

[1]OZ Biosciences

Cédric Sapet
OZ Biosciences

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Protocol status: Working
We use this protocol and it's working

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Abstract

Post-mitotic neurons are highly sensitive to chemical and physical perturbation and conventional transfection methods show poor efficiency and high toxicity particularly in mature cultures where synaptic networks are functionally established. This protocol describe Magnetofection-mediated gene delivery into primary neurons cultured for 10-22 days in vitro (DIV) using NeuroMag reagent. By complexing magnetic nanoparticles with nucleic acids, magnetofection concentrates DNA onto cells within minutes achieving efficient transfection with high viability. This method is applicable to primary neurons (hippocampal, cortical, cerebellar, spinal neurons...) and is compatible with fluorescent imaging, electrophysiology as well as any biochemical analysis.

Attachments

PDF

NeuroMag_Protocol_OZ.
..
574KB

Image Attribution

OZBiosciences

Guidelines

■ This protocol represent standard protocol that should be used as a starting point. Optimal conditions (DNA amount and NeuroMag ratio) may vary depending on nucleic acid, cell type, size and condition of culture and DIV.
■ Once prepared complexes should be added to the cells within 1 hour.
■ For NeuroMag volumes <1 µL we recommend to prepare a dilution in H_2O only ; discard remaining dilution after use.
■ NeuroMag^{TM} is efficient for co-transfections: calculate NeuroMag ratio related to the total plasmid quantity.

Materials

■ Neuron Culture

Poly-L-lysine (Sigma, P-1520)

classic culture medium: MEM supplemented with 15% (vol/vol), Nu-serum, 2% (vol/vol) B27, 0.015 M HEPES (pH 7.2), 0.45% (wt/vol), D-glucose, 1 mM sodium pyruvate, 2 mM L-glutamine and 10 IU ml^{-1}, penicillin–streptomycin.

long term culture medium: MEM supplemented with 10% (vol/vol) Nu-serum, 15 mM HEPES (pH 7.2), 0.45% (wt/vol) D-glucose, 1 mM sodium pyruvate, 2 mM L-glutamine and 10 IU ml^{-1} penicillin–streptomycin

feeding medium: MEM supplemented with 15 mM, HEPES, 0.45% (wt/vol) D-glucose, 1 mM sodium pyruvate, 2 mM L-glutamine, and 2% (vol/vol) B27.

■ Transfection

NeuroMag^{TM} transfection reagent (OZ Biosciences, France) - **Ref NM50500**

Nucleic Acid construct

OptiMEM

Troubleshooting

Problem
Non-optimal neronal rowth
Solution
- check the cell density, pH of the culture medium (7.2) and % of CO2 - supplement cell culture with Nu-serum and B27

Problem
Astrocyte overgrowth
Solution
vary the concentration of Nu-serum

Problem
cells are very sensitive to transfection
Solution
medium can be changed right after the magnetofection procedure: keep the cells onto the magnetic plate and replace transfection medium with fresh pre-warm complete culture medium

Problem
low transfection efficiency
Solution
■ Vary dose of nucleic acid used, ratio of NeuroMag to nucleic acid, cell density, and incubation time. ■ Avoid any physical shock that could activate neurons

Safety warnings

■ Use medium without any supplement (growth factor, antibiotics, serum...) for complex preparation.
■ Magnetofection™ requires an appropriate magnetic field delivered by our **super magnetic plates**.

Before start

Bring NeuroMag and DNA suspension at room temperature before start

Glass coverslips preparation

1 Prepare a 1 mg/mL poly-L-lysine solution in water.

2 Prepare glass coverslips.

2.1 Place coverslips in 1% (vol/vol) nitric acid overnight. Overnight

2.2 Rinse coverslips vigorously.

2.3 Sonicate 3 times for 30 min in distilled water, 3 times in 70% (vol/vol) ehtanol and 2 times in 95 % (vol/vol) ethanol.

2.4 Sterilize at 160°c for 1h.

> Note
>
> Coverslips can be stored at room temperature under sterile conditions for few months.

3 Coat coverslips with poly-L-lysine diluted 1:100 and incubate overnight. Overnight

4 Rinse 3 times in PBS

> Note
>
> Rigourous procedure of washing and coating are crucial for neuron culturing. Residual poly-L-lysine is toxic for neurons.

Preparation of the cells of interest

5 Dissect and collect the tissues of interest in accordance with institutional and national guidelines for animal experimentation.

6 Dissociate neurons using papain or trypsin at 37°C and stop the action by washing twice with DMEM.

7 Dilute dissociated neurons at the desired concentration and plate coverslips for 1h at 5% CO_2, 37°C.
Transfer coverslip in 12-well plate and add 2 mL culture medium.

8 Grow the cells under standard culture conditions (5% CO_2, 37°C) until transfection experiment.

Long-term culture neurons

9 For long term transfection a high cellular density is necessary: as a starting point, 800.000 cells per 35 mm dish can be used.

> Note
>
> culture medium should be replaced gradually with feeding medium containing B27.

10 Until DIV 4, use classic culture medium (MEM supplemented with 15% (vol/vol), Nu-serum, 2% (vol/vol) B27, 0.015 M HEPES (pH 7.2), 0.45% (wt/vol), D-glucose, 1 mM sodium pyruvate, 2 mM L-glutamine and 10 IU ml^{-1}, penicillin–streptomycin).

11 Start feeding neurons at DIV 5 (MEM supplemented with 15 mM, HEPES, 0.45% (wt/vol) D-glucose, 1 mM sodium pyruvate, 2 mM L-glutamine, and 2% (vol/vol) B27).

12 Change 50% of the medium every 3 days.

13 Replace 50% of the culture medium with fresh medium 24H before Magnetofection.

Magnetofection™ with NeuroMag™ 40m

14 **Complexes preparation**

14.1 Vortex NeuroMag and place the appropriate amount in a microtube (standard procedure: 3.5µL per µg DNA).

Refer to table 1 below to optimize NeuroMag volume depending on the cell format.

14.2 Prepare nucleic acid suspension by diluted DNA into OPTIMEM without any supplement.
Refer to table 1 below for DNA quantity depending on the cell vessel format.

14.3

	Format	DNA (µg)	Dilution vol (µL)	NeuroMag (µL)	Transfection vol (mL)
	96-well	0.5	50	0.5, 1, 1.5, 1.75	0.2
	24-well	1	100	1, 2, 3, 3.5	0.5
	6-well	4	200	4, 8, 12, 14	2
	60 mm dish	10	300	10, 20, 30, 35	4

Table 1: recommended DNA and NeuroMag quantities

14.4 Add DNA solution to NeuroMag.
Mix by vigourous pipetting.

14.5 Incubate at room temperature for 20 min. 20m

15 Transfection

Note

For long-term cultures (>10 DIV) replace 50% of culture medium 24H before transfection.

15.1 Add complexes of NeuroMag/DNA onto cells drop by drop.
Gently rock the plate to ensure uniform distribution.

15.2 Place the cells onto magnetic plate.

15.3 Incubate under standard culture conditions for 20 min. 20m

15.4 Remove the magnetic plate.

15.5 Cultivate the cells under standard culture conditions until evaluation of the transgene expression (24 H to 7 days).

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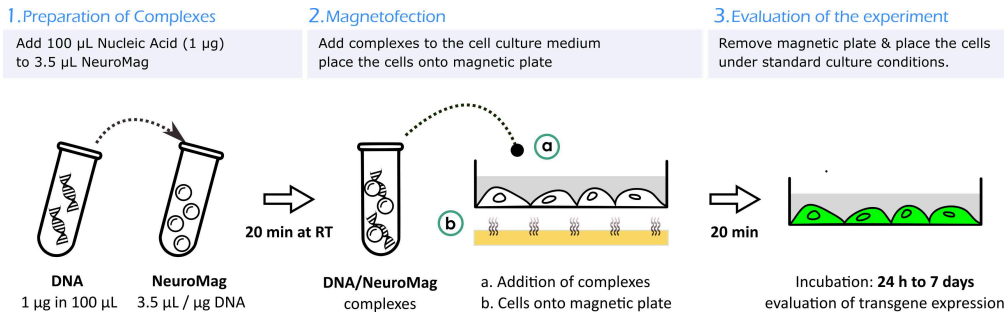

Representative protocol for transfection with 1 µg DNA and NeuroMag at ratio 3.5:1

Protocol references

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