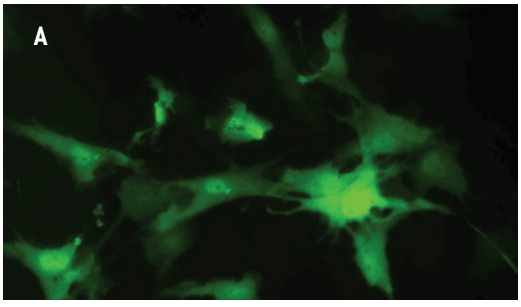
Amaxa™ Nucleofector™ Protocol for Mouse Astrocytes

For primary mouse astrocytes

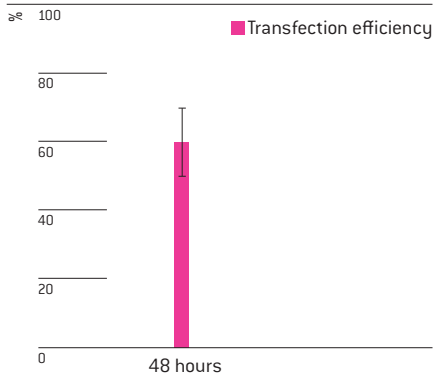
isolated from cortex, midbrain or striatum of mouse embryos (E12-16); fibroblastoid cells

Note **Cells must be cultured for *7* – 10 days until confluent.**

Example for Nucleofection™ of primary mouse astrocytes



Primary mouse astrocytes isolated from mouse embryos (E14) were transfected using program T-020 and a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 2 days post Nucleofection™ using fluorescence microscopy. Photograph courtesy of Dr. S. Franken, Dr. M. Eckhardt, Prof. V. Gieselmann, Institute for Physiological Chemistry, University of Bonn.



Average transfection efficiency of primary mouse astrocytes 48 hours post Nucleofection™. Cells were transfected with program T-020 and 5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cell viability: 60–70%

Product Description

Recommended Kit: Basic Nucleofector™ Kit for Primary Mammalian Glial Cells

Cat. No.	**VPI-1006**
Size (reactions)	25
Nucleofector™ Solution	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP™ Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
Storage and stability	Store Nucleofector™ Solution, Supplement and pmaxGFP™ Vector at 4°C. For long-term storage, pmaxGFP™ Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector™ Supplement is added to the Nucleofector™ Solution it is stable for three months at 4°C.

Required Material

Note **Please make sure that the entire supplement is added to the Nucleofector™ Solution. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofector™ Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector™ Device
- Supplemented Nucleofector™ Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP™ Vector
- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 60 mm dishes pre-coated with poly-L-ornithine (1.5 µl/ml) or culture system of your choice
- **For detaching cells**: 1 mg/ml trypsin/EDTA in calcium and magnesium-free HBSS [Lonza, Cat. No. 10-547F] and supplemented culture media
- **Culture medium**: DMEM containing 1 g/L glucose [Lonza; Cat. No. 12-707F] supplemented with 4 mM L-glutamine [Lonza; Cat. No. 17-605E], 110 mg/l sodium pyruvate, 10 mM HEPES (pH 7.4), 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS)
- **Isolation medium**: Culture medium without serum
- Prewarm appropriate volume of culture medium to 37°C (5.5 ml per sample)
- Appropriate number of cells (2 x 10^6 cells per sample; minimal cell number: 1 x 10^6 cells, a lower cell number may lead to a major increase in cell mortality; maximum cell number: 4 x 10^6

1. Pre Nucleofection™

Isolation and cultivation of cells

Note **This protocol only gives an outline for the isolation and culture of primary mouse astrocytes. Please refer to more detailed protocols in the literature (see Additional Information) before starting the experiments.**

1.1 Dissect striata from mouse embryos (E12-16)
1.2 Dissociate striata mechanically in isolation medium
1.3 Plate cells in culture medium on 60 mm poly-L-ornithine coated dishes
1.4 Culture the cells for 7 – 10 days until they reach confluency
1.5 Wash briefly with PBS
1.6 Detach cells with Trypsin/EDTA-HBSS solution for 5 minutes at 37°C
1.7 Neutralize trypsin by adding culture medium
1.8 Count the cells
1.9 Spin down the required number of cells for 10 minutes at 80xg
1.10 Remove the supernatant completely
1.11 Directly continue with Nucleofection™ (see chapter 2)

2. Nucleofection™

One Nucleofection™ Sample contains

2 x 10^6 cells
2-5 µg plasmid DNA (in 1–5 µl H_2O or TE) or 2 µg pmaxGFP™ Vector or 30–300nM siRNA (3–30 pmol/sample)
100 µl Nucleofector™ Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Prepare 60 mm dishes pre-coated with poly-L-ornithine (1.5 µl/ml) by filling with 5 ml of supplemented culture media per sample and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Resuspend the cell pellet carefully in 100 µl room-temperature Nucleofector™ Solution per sample

Note **Avoid leaving the cells in Nucleofector™ Solution for extended periods of time (longer than 20 minutes), as this may reduce cell viability and gene transfer efficiency.**

2.4 Combine 100 µl of cell suspension with **2 – 5 µg DNA**, 2 µg pmaxGFP™ Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates
2.5 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.6 Select the appropriate Nucleofector™ Program **T-020** (**T-20** for Nucleofector™ I Device)
2.7 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program by pressing the X-button
2.8 Take the cuvette out of the holder once the program is finished
2.9 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 60 mm dishes (final volume 5.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 Change medium every other day
3.3 Gene expression or down regulation, respectively, is often detectable after only 6 – 8 hours. After 24 – 48 hours of incubation viability of cells can be evaluated by proportion of cells attached to the dish. Regulation, respectively, is often detectable after only 4 – 8 hours

Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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References

1. Etienne-Manneville et al. [1999] J. Immunol. 163: 668-674

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