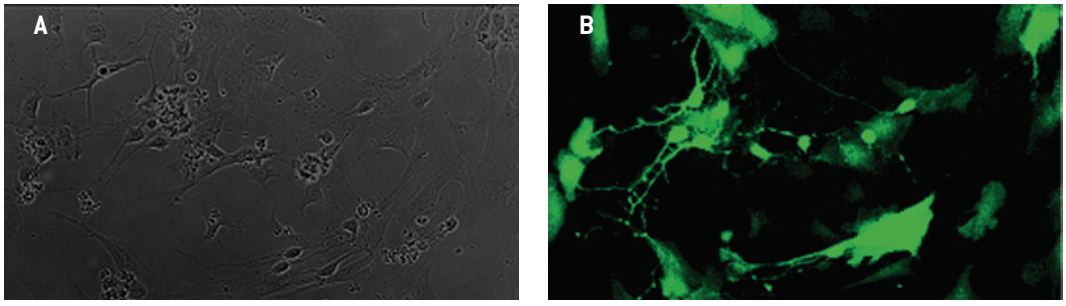
Amaxa® Mouse Neuron Nucleofector® Kit

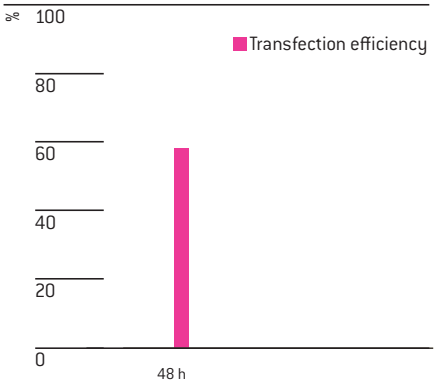
Primary Mouse Hippocampal and Cortical Neurons

Primary dissociated mouse hippocampal and cortical neurons, isolated from embryonic (E18) or neonatal (P1) mice and cultured as mixed glial cells.

Example for Nucleofection® of mouse hippocampal neurons



Primary dissociated hippocampal neurons of mixed glial cultures were transfected using the Mouse Neuron Nucleofector® Kit, program O-005 and a plasmid encoding enhanced green fluorescent protein eGFP. 48 hours post Nucleofection®, the cells were analyzed by light (A) and fluorescence microscopy (B). Photograph courtesy of A. Dityatev, Center for Molecular Neurobiology, Hamburg, Germany.



Transfection efficiency of primary mouse hippocampal neurons 48 hours post Nucleofection®. Cells were transfected with program O-005 and 3 µg of a plasmid encoding the enhanced green fluorescent protein eGFP.

Product Description

Cat.No.	**VPG-1001**
Size (Reactions)	25
Mouse Neuron Nucleofector® Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	10 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.**

- 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
- Prepared poly-L-lysine (PLL) (Sigma) coated cell culture plates or PLL and laminin [Invitrogen, Cat. No. 23017-015] coated glass coverslips (for cultivation over more than 3 days) [Marienfeld, 15 mm] (for microscopy or cultivation on feeder cells). As an alternative to PLL, poly-D-lysine (PDL) can be used as well
- Dissection solution (500 ml HBSS [Lonza; 10-508Q], 5 ml penicillin/streptomycin [Lonza; 17-602], 5 ml 1 M $MgCl_2$, 3.5 ml 1 M Hepes [pH 7.3], 5 ml 200 mM L-glutamine [Lonza; 17-605C], sterilized by filtration and pre-cooled on ice before use)
- Trypsin in calcium and magnesium-free HBSS [Lonza;17-160]
- Trypsin inhibition (0.8 mg/ml Trypsin inhibitor [Invitrogen; Cat.No. 17075-029] in dissection solution, sterilized by filtration)
- Equilibrate appropriate volume of culture medium I (DMEM [Lonza; BE12-604F/U1] supplemented with 10% fetal calf serum [FCS], 10 µg/ml gentamycin [optional, Lonza], 800 µl per reaction) to 37°C, 5% CO_2
- **Prepare culture medium II:** For embryonic neurons Neurobasal (Invitrogen) or for adult and postnatal neurons DMEM [Lonza; BE12-604F/U1], both supplemented with 100 µg/ml insulin [Invitrogen; Cat. No. 12585014], 100 µg/ml transferrin [Invitrogen; Cat. No. 11107018], 5% horse or fetal calf serum, 2% B27 supplement and 2 mM GlutaMAX™ I. After addition of GlutaMAX™, media should be refrigerated to avoid metabolisation to glutamate, which could be neurotoxic. Optionally 0,5 µg/ml gentamycin may be used. Optionally 5 µM ara C [EMD Calbiochem; Cat. No. 251010] may be used 24 hours after plating to inhibit the proliferation of glial cells, which are more abundant in preparations from postnatal brains
- Appropriate number of cells (4 – 5 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: 6 x 10^6 cells

1. Pre Nucleofection®

Note **This protocol only gives an outline for the isolation and culture of primary mouse hippocampal or cortical neurons. Please refer to more detailed protocols in the literature before starting the experiments. A selection of references is given at the end of this document.**

Preparation of coverslips

1.1 Put glass coverslips into a rack and submerge in 65% nitric acid for 18 – 36 hours. Wash coverslips in sterile, distilled and deionized water 3x for 5 minutes followed by 3x for 20 minutes

1.2 Place racks with coverslips in glass container and dry in an oven at 70°C

1.3 Cover glass containers with aluminium foil and sterilize in oven with dry heat at 220°C for 7 hours (do not autoclave!)

1.4 Place coverslips into an appropriate culture dish (e.g. one slide per well of 12-well plate)
1.5 Add 400 µl poly-L-lysine solution (1 mg/ml, dissolved in borate buffer, sterilized by filtration) and incubate in a humidified 37°C/5% CO_2 incubator overnight
1.6 Wash 2x with sterile water and dry
1.7 Incubate coverslips in 400 µl laminin solution (10 µg/ml) in a humidified 37°C/5% CO_2 incubator over night
1.8 Wash 2x with sterile PBS
For more details please refer to Zeitelhofer M et al. 2007 (see reference list at the end of this document)

Preparation of dissociated hippocampal or cortical neurons for Nucleofection®

1.9 Separate heads from mouse embryos (E18) or early postnatal mice
1.10 Dissect brains from the skull and transfer them into a Petri dish with pre-cooled dissection solution
1.11 Cut brains along midline and extract hippocampi or cortices
1.12 Store hippocampi or cortices in at least 10 ml dissection solution in Falcon tubes on ice
1.13 Centrifuge hippocampi or cortices at 80xg for 5 minutes and remove dissection solution
1.14 Add 1.5 ml Trypsin/EDTA-HBSS and incubate for 20 – 30 minutes at 37°C
1.15 Replace Trypsin/EDTA-HBSS with Trypsin inhibitor and incubate for 5 minutes at room temperature
1.16 Centrifuge hippocampi or cortices at 80xg for 5 minutes and rinse hippocampal cells with 3 ml culture medium I. Repeat this 2x
1.17 Add 1.5 ml fresh culture medium I
1.18 Triturate 30x with a fire-polished Pasteur pipette until the suspension is homogenous
1.19 Add 5 ml of culture medium I and centrifuge for 5 minutes at 80xg
1.20 Remove supernatant and resuspend the mixed glial cells in 1 – 3 ml culture medium I
1.21 Count the cells and determine cell density

2. Nucleofection®

One Nucleofection® Sample contains

4 – 5 x 10^6 cells
1 – 3 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM 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 coated coverslips in 12-well plates by filling appropriate number of wells with 300 µl culture medium II and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Equilibrate additional volume of 500 µl per Nucleofection® to 37°C and 5% CO_2
2.4 Centrifuge the required number of cells (4 – 5 x 10^6 cells per sample) at 80xg for 5 minutes at room temperature
2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample

Note **Avoid leaving the cells in Mouse Neuron Nucleofector® Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability.**

2.6 Combine 100 µl of cell suspension with **1 – 3 µg DNA**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates
2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles)
2.8 Select appropriate Nucleofector® Program **O-005**
2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.10 Take the cuvette out of the holder once the program is finished
2.11 Add 500 µl of the pre-equilibrated culture medium I to the cuvette and gently transfer the sample into the prepared culture dish with the coated coverslip. Use the supplied pipettes and avoid repeated aspiration of the sample

Optional

2.12 If very high mortality is observed, a recovery step can be useful: immediately after Nucleofection®, add 100 – 300 µl pre-equilibrated low Ca^{2+} media such as RPMI to the cuvette (instead of the standard culture media) and gently transfer it to a reaction tube
2.13 Place the cell suspension in incubator for 5 – 10 minutes (="Recovery Step")
2.14 Transfer the sample into the prepared culture dish with the coated coverslip and continue at 3.1 of protocol

3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 After 2 – 4 hours carefully replace medium with 750 µl fresh culture medium II to remove cellular debris
3.3 After 24 hours replace medium with fresh culture medium II
3.4 After 24 – 48 hours of incubation viability of cells can be evaluated by proportion of cells attached to the cover slips. Gene expression or down regulation, respectively, is often detectable after 6 – 8 hours Gene expression can be observed up to 12 – 14 days after Nucleofection®
3.5 Replace half of the culture medium II with fresh medium once a week

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

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2. Dityatev A et al; Neuron 2000; 26: 207-217
3. Dityateva G et al; Neurosci Methods 2003; 130(1): 65-73
4. Zeitelhofer M et al; Nature Protocols 2007; 7(2): 1692-1704

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