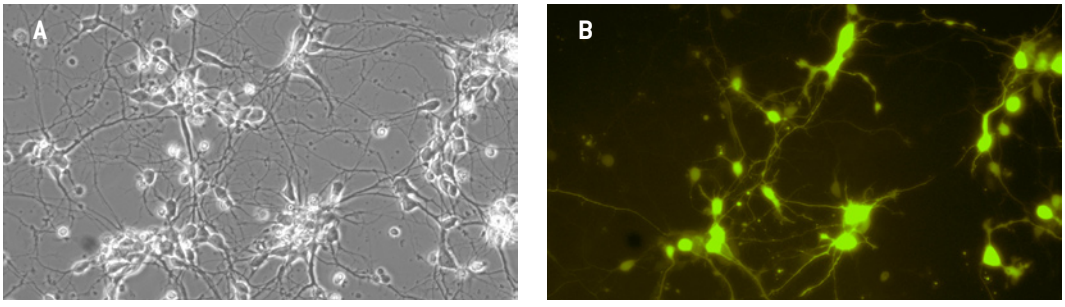
Amaxa® Rat Neuron Nucleofector® Kit

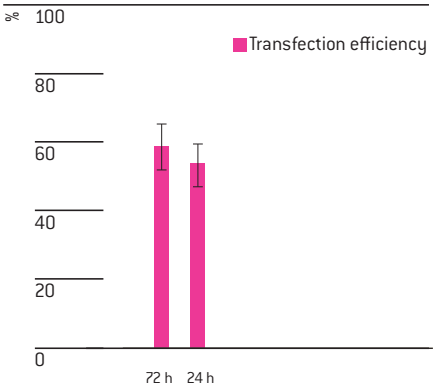
For Primary Rat Hippocampal or Cortical Neurons

Primary dissociated rat hippocampal or cortical neurons, isolated from embryonic (E18) or neonatal rats (P1-2) and cultured as mixed glial cells

Example for Nucleofection® of primary rat cortical neurons



Primary dissociated cortical neurons of mixed glial cultures were transfected using the Rat Neuron Nucleofector® Kit, program O-003 and a plasmid encoding enhanced yellow fluorescent protein eYFP. 48 hours post Nucleofection® the cells were analyzed by light (A) and fluorescence microscopy (B). Photograph courtesy of J. Köhler, R. Klein, MPI for Neurobiology, Munich, Germany.



Average transfection efficiency of primary rat cortical and hippocampal neurons 72/24 hours post Nucleofection®. Cells were transfected with program **O-003** and 3 µg of a plasmid encoding the enhanced yellow fluorescent protein eYFP. Courtesy of J. Köhler, R. Klein, MPI for Neurobiology, Munich, Germany.

Product Description

Cat. No.	**VPG-1003**
Size (reactions)	25
Rat 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 [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 1M $MgCl_2$, 3.5ml 1M Hepes (pH 7.3), 5 ml 200 mML-glutamine [Lonza; 17-605C], sterilized by filtration and pre-cooled on ice before use
- Trypsin/EDTA-HBSS solution [Lonza;17-160]
- Equilibrate appropriate volume of culture medium I (DMEM [Lonza; BE12-604F/U1] supplemented with 10% fetal calf serum [FCS], 10 µg/ml gentamycin [optional], 800 µl per reaction) to 37°C/5% CO_2
- **Prepare culture medium II:** For embryonic neurons: Neurobasal medium [Invitrogen; Cat. No. 21103-049] supplemented with 2% B27 supplement [Invitrogen; Cat. No. 17504-044] and 2 mM GlutaMAX™ I [Invitrogen; Cat. No. 35050-061]; 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 is 1 x 10^6 cells; a lower cell number may lead to a major increase in cell mortality. Maximum cell number is 6 x 10^6 cells

1. Pre Nucleofection®

Note **This protocol only gives an outline for the isolation and culture of primary rat 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.**

Note **Preparation of glial support cultures (optional). If, after Nucleofection®, the experiment requires plating the transfected neurons on coverslips and subsequent culture for more than 3 days, it is recommended to establish a glial support culture. To establish a glial support culture, begin approximately 12 days before the Nucleofection®. Follow the procedure detailed in Zeitelhofer M et al., 2007 to isolate the glial cells before proceeding with the rest of the protocol outlined below.**

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 min followed by 3x for 20 min

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 [BD Falcon])
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 rat embryos (E17-18) or early postnatal rats (P0-2)
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. Cut cortices in small pieces
1.13 Centrifuge at 80xg for 5 minutes and carefully remove supernatant (alternatively, if experienced, remove dissection solution by very careful decanting)
1.14 Add 1.5 ml Trypsin/EDTA-HBSS and incubate for 10 – 20 minutes at 37°C
1.15 After Trypsinization, centrifuge at 80xg for 5 minutes and carefully remove supernatant (alternatively, if experienced, remove dissection solution by very careful decanting)
1.16 Wash two times with HBSS
1.17 After the second wash, add 1.5 ml of culture medium I, prewarmed to 37°C
1.18 Triturate about 20 – 30x with a fire-polished Pasteur pipette until all pieces of tissue are homogenously dispersed
1.19 Triturate a second time for exactly 1 minutes with a fire-polished Pasteur pipette
1.20 Add 5 ml of culture medium I and count cells
1.21 Continue at step 2.1 of the Nucleofection® Protocol

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 culture medium I 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 Rat 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** 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 the appropriate Nucleofector® Program **O-003** or **G-013**
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 immediately 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 an useful option: immediately after Nucleofection®, add 500 µl pre-equilibrated low Ca^{2+} media such as RPMI and gently transfer it to the 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 and can be observed up to 12 – 14 days after Nucleofection®
3.5 After 3 days transfer coverslips to glial support culture
3.6 Replace half of the culture medium II with fresh medium once a week (optional)

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:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

References

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Lonza Cologne AG
50829 Cologne, Germany

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