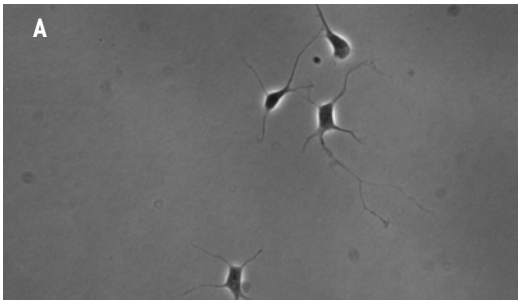
Amaxa® Basic Neuron SCN Nucleofector® Kit

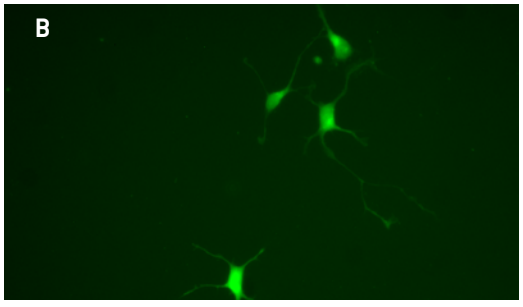
For Primary Rat Hippocampal Neurons (Small Cell Number)

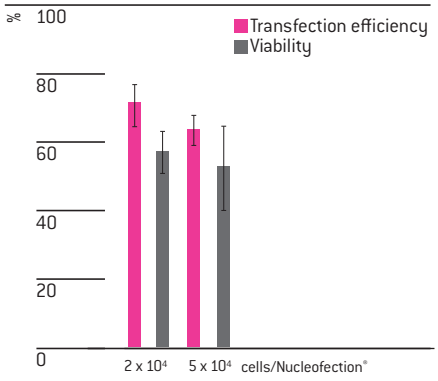
Primary dissociated rat hippocampal neurons, isolated from embryonic rats (E17) and cultured as mixed glial cells

Note **SCN Nucleofector® Kits are compatible with Nucleofector® II Devices of serial version "S" with software version S4 – 4 or higher only. Please make sure that your Nucleofector® II Device is serial version "S". Any other Nucleofector® Devices are not compatible with SCN Kits.**

Example for SCN Nucleofection® of embryonal rat hippocampal neurons







Freshly isolated rat hippocampal neurons (E17) were transfected with program SCN Basic Neuro Program 1 and 0.4 µg pmaxGFP® Vector (1 DIV*) and plated on coated coverslips. After 1 DIV neurons were fixed and analyzed by light and fluorescence microscopy. Transfection efficiency with optimized conditions ranged between 40 – 65%, depending on preparation. Neuron morphology (as well examined after 7 DIV*, data not shown) was unaltered compared to untransfected neurons. Data by courtesy of M.Kiebler,Department of Neuronal Cell Biology, Medical University of Vienna, Vienna, Austria.
* DIV = Days in-vitro

Average transfection efficiency and viability of rat embryonic hippocampal neurons (n = 3). 2 x 10^4 and 5 x 10^4 cells were transfected with program SCN Basic Neuro Program 1 and 0.4 µg pmaxGFP® Vector. 24 hours post Nucleofection®, the cells were fixed and analyzed by light and fluorescence microscopy. Data by courtesy of M.Kiebler, Department of Neuronal Cell Biology, Medical University of Vienna, Vienna, Austria.

Product Description

Cat. No.	**VSPI-1003**
Size (reactions)	25
Basic Neuron SCN Nucleofector® Solution	0.45 ml
SCN Supplement	0.1 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® II Device, serial version "S"
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified SCN 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) 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 1 M $MgCl_2$, 3.5 ml 1 M Hepes (pH 7.3), 5 ml 200 mM L-glutamine [Lonza; 17-605C], 5 ml penicillin/ streptomycin [Lonza; 17-602], 5 ml 1M $MgCl_2$, 3.5 ml 1 MHepes (pH 7.3), 5 ml 200 mM L-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% horse serum (HS) [Lonza; 14-403E], 1 mM sodium pyruvate [Sigma, P2256-5G] and 2 mM L-glutamine , stable [Lonza; BE17-605E/U1].) to 37°C, 5% CO_2
- Prepare **culture medium II** (MEM [Lonza; 12-125Q], supplemented with 26 mM $NaHCO_3$ [Riedel Haen, 31437], 1 mM sodium pyruvate [Sigma, P2256-5G], 2 mM L-glutamine, stable [Lonza; BE17-605E/U1], 33 mM D-glucose [Merck, 1.08337.1000] and 2% B27 supplement [Invitrogen, 17504044, dissolved in ddH_2O]. Optionally 5 µM ara C [EMD Calbiochem; Cat. No. 251010] may be used 24 hours after plating the neurons to inhibit the proliferation of glial cells, which are more abundant in preparations from postnatal brains).
- Appropriate number of cells (2×10^4 – 1×10^5 cells per sample . A lower cell number may lead to a major increase in cell mortality.)

1. Pre Nucleofection®

Note **This protocol only gives an outline for the isolation and culture of hippocampal 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) A glial support culture can be a useful option if a low seeding density is required or long-term cultivation over more than 3 days needs to be supported.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.**

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) [Invitrogen, Cat. No. 23017-015] in a humidified 37°C/5% CO_2 incubator over night
1.8 Wash 2x with sterile PBS

Preparation of dissociated hippocampal 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
1.12 Store hippocampi in at least 10 ml dissection solution in Falcon tubes on ice
1.13 Centrifuge at 80xg for 5 min 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 min at 37°C
1.15 After trypsinization, centrifuge at 80xg for 5 min 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 min 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

Optimal number of 2 x 10^4 cells
0.1 – 0.6 µg plasmid DNA (in 0.2 – 1 µl H_2O or TE) or 0.4 µg pmaxGFP® Vector or 30 – 300 nM siRNA (0.6 – 6 pmol per sample)
20 µl Basic Neuron SCN 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 volume of 80 µl culture medium I per Nucleofection® to 37°C and 5% CO_2
2.4 Centrifuge the required number of cells (2 x 10^4 cells per sample) at 80xg for 10 minutes at room temperature. Remove supernatant completely
2.5 Resuspend the cell pellet carefully in 20 µl room temperature Basic Neuron SCN Nucleofector® Solution per sample

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

2.6 Combine 20 µl of cell suspension with **0.1 – 0.6 µg DNA** or appropriate amount of siRNA or other substrates
2.7 Transfer cell/DNA suspension into certified SCN cuvette (sample must cover the bottom of the cuvette without air bubbles)
2.8 Select the appropriate Nucleofector® Program **SCN Basic Neuro Program 1** from the **Cell Type list**. The program can be chosen from the Cell Type list only (see Nucleofector® Manual for details). Press the "X" button to start the program.
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 Immediately add 80 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 an useful option: immediately after Nucleofection, add 80 µl pre-equilibrated low Ca^{2+} media such as RPMI and gently transfer it to 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 cells in a humidified 37°C/5% CO_2 incubator until analysis
3.2 After 24 – 48 hours of incubation viability of cells can be evaluated by proportion of cells attached to the cover slips or culture dish. Depending on the gene, expression is often detectable after 6 – 8 hours and can be observed up to 12 – 14 days after Nucleofection®
3.3 After 3 days transfer coverslips to glial support culture

Optional

3.4 If very high mortality is observed after transfection, medium changes at 2 – 4 hours and 24 hours after transfection can be useful options: gently remove the media and add new, pre-equilibrated culture medium II (as specified on page 2 of this protocol). Take care not to release living cells

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

1. Gregory J. Brewer Isolation and culture of adult rat hippocampal neurons. Journal of Neuroscience Methods 1997; 71: 143-155.
2. Banker G. and Goslin K. Culturing Nerve Cells. 2nd edition, Cambridge, MA: MIT Press, 1998; 666pp.
3. Krauss M *et al*., J Cell Biol. 2003; 162(1):113-24.
4. Kaech S and Banker G. Nature Protocols 2006;1(5): 2406-2415
5. Zeitelhofer M *et al* Nature Protocols 2007: 7(2): 1692-1704

Lonza Cologne AG
50829 Cologne, Germany

DSPG-1003_2009-06