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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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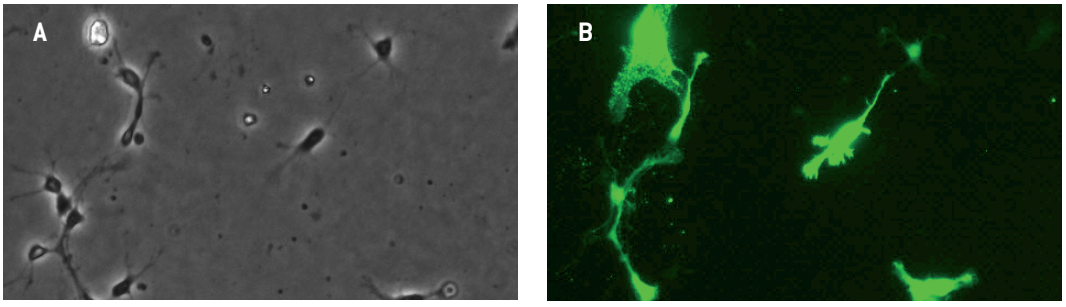
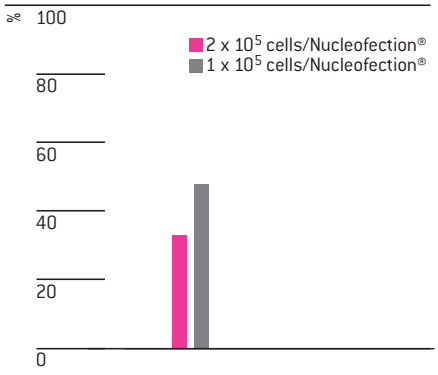
Amaxa® Basic Neuron SCN Nucleofector® Kit

For Primary Mouse Hippocampal Neurons (Small-Cell-Number)

Embryonic mouse hippocampal neurons transfected using the Nucleofector® Technology



Approximately 100.000 freshly isolated embryonic mouse hippocampal neurons (C57BL/6 E17-18) were transfected using the Basic Neuron SCN Nucleofector® Kit, SCN Basic Neuro Program 1 and 0.4 µg of pmaxGFP® Vector. Transfected cells were seeded on a coated coverslip. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence (B) microscopy. Data courteously provided by Dr. B. Knöll, Dept. Molecular Biology, University of Tübingen, Germany.



Average transfection efficiencies of freshly isolated embryonic mouse hippocampal neurons (C57BL/6 E17-18). Cells were transfected using the SCN Basic Neuro Program 1 and 0.4 µg of pmaxGFP® Vector. Cells were analyzed by brightfield and fluorescence microscopy 24 hours post Nucleofection®.

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 SCN 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 Basic Neuron SCN 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, Sigma) 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 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], 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) [Lonza], 10 µg/ml gentamycin [Lonza, optional]) 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 (1×10^5 – 2×10^5 cells per sample; a lower or higher cell number may lead to a major increase in cell mortality)

1. Pre Nucleofection®

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

Note **Preparation of glial support cultures can be a useful option if a low seeding density is required or 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 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)
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 overnight
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 neurons for Nucleofection®

1.9 Separate heads from mouse embryos (E17-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
1.12 Store hippocampi in 50 ml Falcon™ Tubes (BD Biosciences) containing at least 10 ml ice-cold dissection solution
1.13 Centrifuge hippocampi at 80xg for 5 min and remove dissection solution
1.14 Add 1.5 ml Trypsin/EDTA-HBSS and incubate for 10 – 20 min at 37°C
1.15 Replace Trypsin/EDTA-HBSS with trypsin inhibitor and incubate for 5 min at room temperature
1.16 Centrifuge hippocampi at 80xg for 5 min and rinse hippocampal cells with 3 ml equilibrated culture medium I. Repeat this washing 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 min 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

Recommended number of 1 x 10^5 cells
0.1 – 0.6 plasmid DNA (in 1 – 2 µl H_2O or TE) or 0.4 µg pmaxGFP® Vector or 30-300nM siRNA (0.6 – 6 pmol/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 (1 x 10^5 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**, 0.4 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (0.6 – 6 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 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 a useful option: immediately after Nucleofection®, add 80 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 an incubator for 5 – 10 minutes (="Recovery Step")
2.14 Transfer the sample to the prepared culture dish containing the coated coverslip

3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 After 2 – 4 hours, remove cellular debris by carefully replacing 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, the viability of cells can be evaluated. Cells attached to the coverslip are most likely viable. Gene expression is often detectable after 6 – 8 hours and can be observed up to 12 – 14 days after Nucleofection® , depending on the gene of interest.
3.5 After 3 days, transfer coverslips to glial support culture (optional)
3.6 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.amaxa.com/citations

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References

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