Amaxa® Basic Neuron SCN Nucleofector® Kit

For Primary Neural Cells (Small Cell Number)

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.**

For more details please refer to **www.lonza.com/program-update** or please do not hesitate to contact our Scientific Support Team if you have any further questions.

Primary mammalian neurons, primary neurons freshly isolated embryonic (E18) or neonatal (P1-2) mammalian neural tissues.

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.

Optimization Guidelines

Please find some guidelines on neural cell culture for Nucleofection® and on the transfection procedure using our Basic SCN Nucleofector® Kit below. However, we recommend referring to more detailed culture protocols before you start the experiments. Having tested various neural cell types, high transfection efficiencies could be achieved using one of the programs indicated below.

More detailed protocols for small-cell-number Nucleofection® of specific neuron types (e.g. rat hippocampal neurons) are available at **www.lonza.com/cell-database**. In the event that you do not attain satisfying results with your neural cells of interest please contact our Scientific Support Team for further help with the optimization.

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] 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
- Equilibrate appropriate volume of **culture medium I** (e.g. DMEM [Lonza; BE12-604F/U1] supplemented with 10% fetal calf serum [FCS], 10 µg/ml gentamycin [optional], 80 µl per reaction) to 37°C, 5% CO_2
- **Prepare culture medium II:** For embyronic 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 (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 primary mammalian 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 (optional, if required e.g. for microscopy or cultivation with glial support cultures)

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 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 mixed neural cells

1.9 After fresh isolation and careful trituration, re-suspend cells in 1 – 3 ml of culture medium I and centrifuge for 5 min at 80xg
1.10 Remove supernatant and re-suspend the mixed neural cell population in 1 – 3 ml culture medium
1.11 Count the cells and determine cell density

2. Nucleofection®

One Nucleofection® Sample contains

Optimal number of 2 x 10^4 cells
0.1 – 0.6 µg 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 (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 **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® programs **SCN Basic Neuro Program 1 – 8** from the Cell Type list. The programs can be chosen from the Cell Type list only (see Nucleofector® Manual for details). Press the "X" button to start the program
2.9

Note **For optimal results these SCN Basic Neuro Programs should ALL be tested.**

2.10 Insert cuvette with cell/DNA suspension into Nucleofector® Cuvette Holder and apply program
2.11 Take the cuvette out of the holder once the program is finished
2.12 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

2.13 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
2.14 Place the cell suspension in incubator for 5 – 10 minutes (="Recovery Step")
2.15 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 fresh culture medium I 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. Depending on the gene, expression is often detectable after 6 – 8 hours and 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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