Amaxa® Basic Neuron SCN Nucleofector® Kit

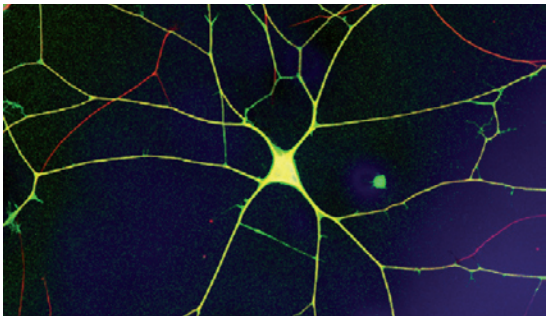
For Primary Mouse Dorsal Root Ganglion (DRG) Neurons (Small Cell Number)

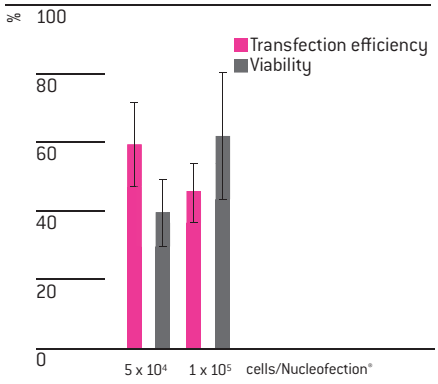
Primary dissociated mouse DRG neurons, isolated from embryonic mouse (E15.5), cultured as mixed glial cell culture or from adult mouse (P21 or older) as purified cells

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.**

SCN Nucleofection® of embryonic mouse DRG neurons

SCN Nucleofection® of freshly isolated embryonic mouse DRG neurons. 25.000 DRGs (E15.5) were transfected with 0.4 µg pmaxGFP® Vector(green) using SCN Basic Neuro Program 6 and seeded on a coated cover slip. 24 hours post Nucleofection®, cells were fixed and immunostained for beta-III-tubulin (red, neuronal marker). Figure shows a merge of both colours. Transfection efficiency in this experiment was around 60% as determined by fluorescence microscopy. Data by courtesy of B. Eickholt, MRC Centre for Developmental Neurobiology, King's College, London, United Kingdom.





Average transfection efficiencies and viabilities of embryonic mouse DRG neurons (n = 3). 5 x 10^4 and 1 x 10^5 cells were transfected with program SCN Basic Neuro Program 6 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 B. Eickholt, MRC Centre for Developmental Neurobiology, King's College, London, United Kingdom.

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 isolated by using endotoxin free plasmid kits
- 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
- For embryonic mouse DRGs (protocol A): per reaction and plating in 12 well plates 400 – 600 µl of DMEM [Lonza] containing GlutaMAX™ [Invitrogen] supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, and 20 ng/ml NGF [Promega].
- For adult, isolated mouse DRGs (protocol B): per reaction and plating in 12 well plates 400 – 600 µl of DMEM/F12 (high glucose, Lonza) containing GlutaMAX™, [Invitrogen], 10% FCS, and 100 ng/ml NGF [Promega].
- Appropriate number of cells (5×10^4 – 1×10^5 for embryonic DRGs or 1×10^4 – 2×10^4 for adult purified DRGs cells per Nucleofection®)

Note **Materials required for pre Nucleofection® are not listed.**

1. Pre Nucleofection®

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

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 [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)

A. Preparation of embryonic mouse DRGs (E15.5)

A 1.9 Separate heads from mouse embryos (E15.5 Do not use younger embryos)

A 1.10 Cut backbone on the dorsolateral areas above the dorsal horns on both sides from the embryo and transfer them into a Petri dish with ice-cold DMEM [Invitrogen]

A 1.11 Remove the spinal cord and the layer that covers the dorsal roots
A 1.12 Store dorsal root ganglions in at least 10 ml ice-cold DMEM in Falcon tubes
A 1.13 Remove dissection solution by decanting
A 1.14 Add 1.5 ml trypsin [Invitrogen] (1 mg/ml diluted in HBSS) and incubate for 10 minutes at 37°C
A 1.15 After trypsinization, spin down and resuspend cells in medium (as specified above)
A 1.16 Triturate about 20x with a fire-polished Pasteur pipette until all pieces of tissue are homogenously dispersed
A 1.17 Add 5 ml of culture medium (as specified above) and count cells
A 1.18 Continue at step 2.1 of the Nucleofection® Protocol

B. Preparation of purified adult mouse DRGs (P21 or older)

B 1.8 Dissect the heads of mice
B 1.9 Cut backbone on the dorsolateral areas above the dorsal horn on both sides
B 1.10 Remove spinal cord and pull out dorsal roots
B 1.11 Cut off the roots with a sterile blade
B 1.12 Transfer DRGs into PBS/BSA (0.1% BSA)
B 1.13 If DRGs are not at the bottom of the tube, spin for 2 minutes at 50 – 65xg and remove buffer with a pipette
B 1.14 Add 1 ml Liberase [Blendzyme 1, Roche, Cat. No. 11988409001] to the solution for 40 minutes and incubate at 37°C
B 1.15 Remove the Liber ase and add 1 ml of fresh Liberase to the DRGs and incubate again for 40 minutes
B 1.16 Remove the Liberase and wash the DRGs two times with PBS
B 1.17 Incubate the DRGs for 30 minutes with 5 ml of trypsin (0.05%) [Invitrogen, Cat. No. 15090]
B 1.18 Remove the trypsin and wash one time carefully with 1 ml of PBS
B 1.19 Remove PBS and add 1.5 ml of Media B and titruate the DRGs about 10x with a fire polished Pasteur pipette until you get a turbid solution
B 1.20 Overlay 7 ml of medium containing 3.5% (w/v) BSA with the 1.5 ml of titruated cells
B 1.21 Centrifuge for 20 minutes at 14xg in a swing-out rotor
B 1.22 Remove supernatant and resuspend cells in 3 – 5 ml of culture medium (as specified above)
B 1.23 Centrifuge for another 10 minutes at 20xg
B 1.24 Remove the medium and resuspend cells in 5 ml of culture medium (as specified above) and count the cells
B 1.25 Continue at step 2.1 of the Nucleofection® Protocol

2. Nucleofection®

One Nucleofection® Sample contains

5 x 10^4 cells for embryonic DRGs or 2 x 10^4 for adult purified DRGs (optimal cell numbers)
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 (3 – 30 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 and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator

2.3 Equilibrate additional volume of 100 – 300 µl per Nucleofection® (adjust volume according to desired seeding density) to 37°C and 5% CO_2
2.4 Count the cells and determine cell density
2.5 Centrifuge the required numbers of cells (per sample 5 x 10^4 – 1 x 10^5 mixed cells for embryonic DRGs or 1 x 10^4 – 2 x 10^4 purified cells for adult purified DRGs cells) at 80xg for 10 minutes at room temperature
2.6 Resuspend the cells carefully in 20 µl room temperature 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.**

2.7 Combine 20 µl of cell suspension with **0.1 – 0.6 µg DNA**, 0.4 µg pmaxGFP® Vector or appropriate amount of siRNA or other substrates
2.8 Transfer cell/DNA suspension into certified SCN cuvette (sample must cover the bottom of the cuvette without air bubbles)
2.9 Select SCN Basic Neuro Program 6 from the Cell Type list
2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.11 Take the cuvette out of the holder once the program is finished
2.12 Immediately add ~100 – 300 µl of the pre-equilibrated culture media 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 100 – 300 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 cover slip and continue at 3.1 of protocol

3. Post Nucleofection®

3.1 Incubate cells in a humidified 37°C/5% CO_2 incubator
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®

Optional

3.3 If very high mortality is observed after transfection, medium changes 2 – 4 hours and 24 hours after transfection can be useful options: gently remove the media and add new, pre-equilibrated culture media (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. Eickholt BJ *et al.* PLoS ONE: Sep 11; 2007; 2(9):e869.
2. Zeitelhofer M *et al.* Nature Protocols 2007 ; 7(2): 1692-1704
3. Krauss M *et al.* (2003) J Cell Biol 162(1):113-24.
4. Zhou FQ et al. Neuron 2004; 42(6): 897-912
5. Banker G. and Goslin K. Culturing Nerve Cells. 1998; Cambridge, MA: MIT Press, 666pp.

Lonza Cologne AG
50829 Cologne, Germany

DSPG-1001_2009-06