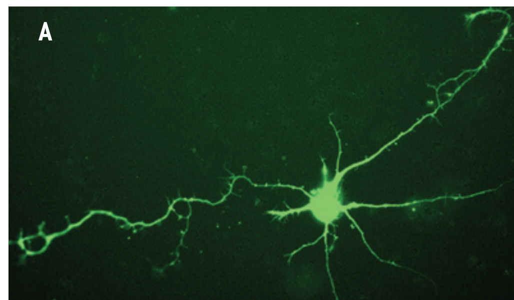
Lonza

Amaxa® Chicken Neuron Nucleofector® Kit

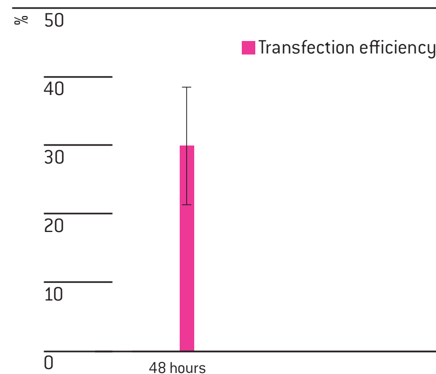
For Chicken Dorsal Root Ganglion (DRG) Neurons

Primary DRG neurons, freshly isolated from chicken embryos (E7)

Example for Nucleofection® of chicken DRG neurons



Primary dorsal root ganglion neurons were transfected using the Chicken Neuron Nucleofector® Kit, program O-003 and a plasmid encoding enhanced green fluorescent protein eGFP. 48 hours post Nucleofection®, the cells were analyzed by fluorescence microscopy. (Photograph courtesy of Dr. B. Eickholt, King's College, London, UK).



Transfection efficiency of chicken dorsal root ganglion neurons. Cells were transfected with program **O-003** and **5 µg** of a plasmid encoding the enhanced green fluorescent protein eGFP. **48 hours** post Nucleofection®, the cells were analyzed by fluorescence microscopy.

Product Description

Cat. No.	**VPG-1002**
Size (reactions)	25
Chicken 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:** Trypsin/EDTA-HBSS solution [Lonza;17-160]
- Culture medium: DMEM [Lonza; BE12-604F/U1], supplemented with 2 mM L-glutamine, [Lonza; 17-605C], 5 ml penicillin/streptomycin [Lonza; 17-602], 10% fetal calf serum [FCS] and 20 ng/ml NGF
- Appropriate number of cells (2×10^6 cells per sample)

1. Pre Nucleofection®

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

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 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)

Preparation of Chicken DRGs

1.9 Dissect out ganglia from chicken embryos (E7) and place them in ice-cold calcium- and magnesium-free HBSS
1.10 Carefully mince DRGs into small pieces
1.11 Transfer pieces into a tube containing Trypsin solution and incubate for 10 minutes with agitation in a water bath at 37°C until most cells have dissociated

1.12 Add culture medium and triturate the cell suspension with a firepolished Pasteur pipette until the solution is homogenous
1.13 Centrifuge for 5 minutes at 80xg
1.14 Remove supernatant and resuspend cells in culture medium
1.15 Count the cells and determine cell density

2. Nucleofection®

One Nucleofection® Sample contains

2 x 10^6 cells
2 - 10 µg plasmid DNA (in 1 - 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 - 300nM siRNA (3 - 30 pmol/sample)
100 µl Nucleofector® Solution

2.1 Please make sure that the whole 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 500 µl per Nucleofection® to 37°C and 5% CO_2
2.4 Centrifuge the required number of cells (2 x 10^6 cells per sample) at 80xg for 5 minutes at room temperature. Remove supernatant completely
2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample

Note **Avoid leaving the cells in Chicken 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 **2 - 10 µg DNA**, 2 µg pmaxGFP® Vector 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 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 Immediately add 500 µl of the pre-equilibrated culture medium 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 useful: immediately after Nucleofection®, add 100 - 300 µl pre-equilibrated low Ca^{2+} media such as RPMI to the cuvette (instead of the standard culture media) and gently transfer it to a 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 the protocol

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis
3.2 After 2 - 4 hours carefully replace medium with 750 µl fresh culture medium to remove cellular debris

Optional

3.3 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 media. 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:

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References

1. Dityateva G, et al Neurosci Methods 2003; 130(1): 65-73 (2003)
2. Chadborn NH, et al. J Cell Sci 2006; 119(Pt 5): 951-7 (2006)
3. Zeitelhofer M et al. Nature Protocols 2007; 7(2): 1692-1704 (2007)

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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

The Nucleofector® Technology, comprising Nucleofection® Process, Nucleofector® Device, Nucleofector® Solutions, Nucleofector® 96-well Shuttle® System and 96-well Nucleocuvette® plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne AG.

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