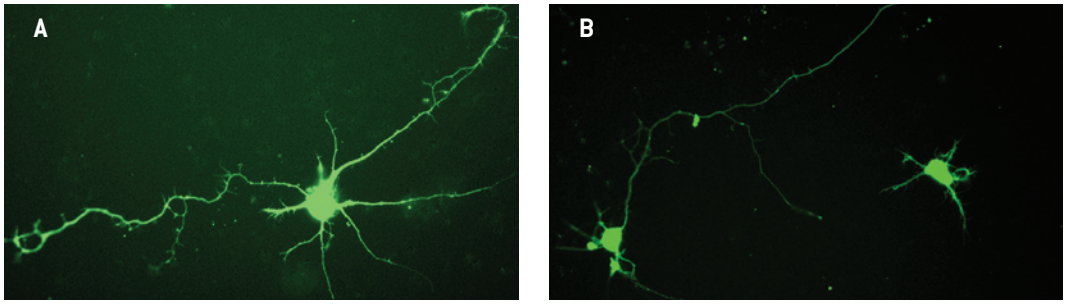
Amaxa® Chicken Neuron Nucleofector® Kit

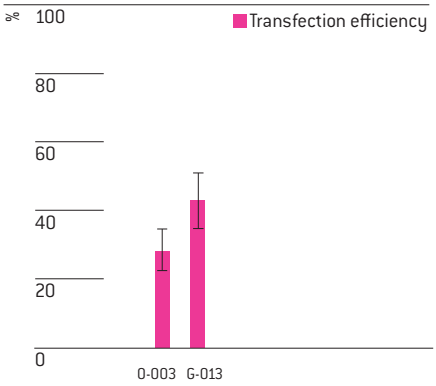
For Primary Chicken Hippocampal Neurons

Primary dissociated chicken hippocampal neurons, prepared from chicken embryos (E7) as mixed glial cultures.

Example for Nucleofection® of chicken hippocampal neurons



Primary dissociated hippocampal neurons of mixed glial cultures were transfected using the Chicken Neuron Nucleofector® Kit, program G-013 and a plasmid encoding enhanced green fluorescent protein eGFP. 5 days post Nucleofection®, the cells were analyzed by fluorescence microscopy.



Transfection efficiency of primary chicken hippocampal neurons. Cells were transfected with program 0-003 or G-013 and 3 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. 24 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 (500 ml D-PBS [Invitrogen; Cat. No. 14190-094])
- Trypsin/EDTA-HBSS solution [Lonza;17-160; used as 1x solution]
- **Culture medium:** PNGM™ BulletKit® (Lonza; Cat. No. CC-4461) including PNBM™ Basal Medium and PNGM™ SingleQuot® Supplements (GA-1000, final concentration 0.1%; NSF-1, final concentration 2%; L-glutamine, final concentration 2 mM). The medium is supposed to be serum free. On occasion 5% horse serum or FCS serum can be used transiently during plating of the cells
- **Recovery medium (optional):** In case high mortality you may use a low calcium medium, e.g. RPMI [Lonza; cat. No. 12-167F], for the transfer from the cuvette into the culture plate (see note after 2.11)
- Pre-equilibrate appropriate volume of culture medium to 37°C, 5% CO_2 (800 µl per sample)
- Appropriate number of cells (3 – 6 x 10^6 cells per sample)

1. Pre Nucleofection®

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

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)

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 hippocampal chicken neurons for Nucleofection®

1.9 Breed 20 – 40 fresh chicken eggs at 37°C for exactly 7 days
1.10 Wipe each egg with 70% ethanol and open it . Continue under a laminar flow with the following steps
1.11 Collect the embryos in a Petri dish with fresh pre-warmed PBS and discard the rest of the egg
1.12 Separate heads from chicken embryos
1.13 Dissect brains from the skull and transfer them into a new Petri dish with pre-warmed PBS
1.14 Carefully remove the outer thin membrane of the brains, separate the hippocampi and cut them into small pieces with a scalpel
1.15 Transfer the hippocampi in PBS into a 15 ml Falcon tube
1.16 Add 25 µl 0.5% Trypsin/0.2% EDTA solution to 5 ml of the cell suspension and resuspend with a fire-polished Pasteur pipette
1.17 Incubate for 15 minutes in a 37°C waterbath and mix cell suspension 2 – 3 times by shaking the tube
1.18 Add 10 ml culture medium
1.19 Centrifuge cell suspension at 80xg for 10 minutes, remove supernatant and rinse with 10 – 12 ml supplemented culture medium. Repeat this step
1.20 Triturate 30 times with three fire-polished Pasteur pipettes, starting with the largest diameters and progressively getting smaller until the suspension is homogenous
1.21 Rinse the mixed glial cells through a 70 µm nylon cell strainer [BD Falcon Cat. No. 352350]
1.22 Count the cells and determine cell density

Note **You will get between 1 – 3 x 10^8 mixed hippocampal cells out of 25 – 30 chicken embryo brains.**

2. Nucleofection®

One Nucleofection® Sample contains

4 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 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 500 µl per Nucleofection® to 37°C/5% CO_2
2.4 Centrifuge the required number of cells (4 x 10^6 cells per sample) at 80xg for 5 minutes at room temperature
2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
2.6 Combine 100 µl of cell suspension with **2 – 10 µg DNA** or **30-300 nM** siRNA (3 – 30 pmol per sample) or other substrates

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.**

2.7 Transfer cell/DNA suspenison into certified cuvette (sample must cover the bottom of the cuvette without air bubbles

2.8 Select appropriate Nucleofector® Program **0-003** (for high cell survival) or G-013 (for high transfection efficiency)
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 Add 500 µl of the pre-equilibrated culture medium to the cuvette and **gently** transfer the sample immediately 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 protocol

3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis
3.2 Optionally (in case of much debris): Carefully replace half of the medium with fresh culture medium after 2 – 4 hours
3.3 Optionally (in case of much debris): Carefully replace the medium completely with fresh culture medium after 24 hours
3.4 Replace culture medium every 3 days with fresh culture medium
3.5 After 24 – 48 hours of incubation viability of cells can be evaluated by proportion of cells attached to the culture wells. Gene expression or down regulation, respectively, is often detectable after 6 – 8 hours. Gene expression may be observed up to 12 – 14 days after Nucleofection®

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.amaxa.com/citations

For more technical assistance, contact our Scientific Support Team:

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References

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2. Dityatev A et al; Neuron 2000; 26: 207-217
3. Dityateva G et al; Neurosci Methods 2003; 130(1): 65-73
4. Zeitelhofer M et al; Nature Protocols 2007; 7(2): 1692-1704

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