Amaxa™ Basic Nucleofector™ Kit

For Any Primary Mammalian Neurons

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

Note **Mammalian neurons cells display significant phenotypic variations due to the wide range of both species and body sites from which they may be sourced. You can determine the optimal Nucleofection™ Condition for your neurons using the Basic Nucleofector™ Kit for Primary Mammalian** Neurons **[Cat. No. VPI-1001]. Please find some guidelines on** neuron **culture for Nucleofection™ and on the transfection procedure using our Basic Nucleofection™ Kit below. However, we recommend referring to more detailed culture protocols before you start the experiments. Having tested various neuronal cell types, high transfection efficiencies could be achieved using one of the programs indicated below. In case you do not attain satisfying results with your neurons of interest please contact our Scientific Support Team for further help with the optimization. On our website (www.lonzabio.com) we provide a form you might use to enter the results achieved with the Basic Kit.**

Product Description

Cat. No.	**VPI-1003**
Size (reactions)	25
Basic 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.

Optimization Guidelines

The initial optimization experiment is comprised of 6 reactions: 5 different Nucleofector™ Programs are tested with 1 Nucleofector™ Solution plus 1 control (no program). The Nucleofector™ Program which turns out to be the most appropriate should be used for all subsequent transfections. A further fine tuning of the Nucleofection™ Condition can be performed with the help of our Scientific Support Team.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Program	A-33	C-13	G-13	O-03	O-05

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
- **For preparation of coverslips:** Poly-L-lysine [Sigma] solution (1 mg/ml, dissolved in borate buffer, sterilized by filtration) and incubate in a humidified 37°C/5% CO2 incubator overnight and laminin solution (10 µg/ml) [Invitrogen, Cat. No. 23017-015]
- **Culture medium (for embryonic neurons):** 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
- **Culture medium (for adult neurons):** PNBM™-A BulletKit® (Lonza; Cat. No. CC-4512; for adult neurons) including PNBM™ Basal Medium and PNGM™-A SingleQuot® Supplements (GA-1000, final concentration 0.1%; NSF-1, final concentration 2%; L-glutamine, final concentration 2 mM; additional components are included in the PNGM™-A SingleQuots® Kit to adjust for unique growth conditions required for optimal growth and survival of adult neurons). 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. 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)
- **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 (4–5 x 10^6 cells per sample)

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.**

Note **Preparation of glial support cultures (optional). If, after Nucleofection™, the experiment requires plating the transfected neurons on coverslips and subsequent culture for more than 3 days, it is recommended to establish a glial support culture. 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 rest of 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 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
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 5 ml of culture medium and centrifuge for 5 minutes 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

4–5 x 10^6 cells
1–3 µg plasmid DNA (in 1–5 µl H_2O or TE) or 2 µg pmaxGFP™ vector or 30–300 nM 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 and 5% CO_2

2.4 Centrifuge the required number of cells (4–5 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 Basic 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 (recommended for initial optimization), **1–2 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. Please try all 5 Nucleofector™ Programs initially to determine the most appropriate one for your neuron cell type for all subsequent experiments: **A-33, C-13, G-13, O-03** or **O-05**

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 an useful option: immediately after Nucleofection™, add 500 ul pre-equilibrated low $Ca2^+$ media such as RPMI and gently transfer it to 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 After 24–48 hours of incubation viability of cells can be evaluated by proportion of cells attached to the cover slips. Gene expression or down regulation, respectively, is often detectable after 6–8 hours. Gene expression can be observed up to 12–14 days after Nucleofection™

3.5 Replace half of the culture medium with fresh medium once a week

Additional Information

For an up-to-date list of all primary neurons successfully transfected with this Basic Nucleofector™ Kit, please refer to:
www.lonza.com/nucleofection-celldatabase

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. Banker G. and Goslin K. (1998) Culturing Nerve Cells. 2nd edition,Cambridge, MA: MIT Press, 666pp.
2. Gregory J. Brewer. Journal of Neuroscience Methods 1997; 71: 143-155.
3. Dityatev A *et al*. Neuron 2000; 26: 207-217.
4. Dityateva G *et al*. Neurosci Methods 2003; 130(1): 65-73
5. Chadborn NH *et al*. J Cell Sci 2006; 119(Pt 5): 951-7
6. Zeitelhofer M *et al*. Nature Protocols 2007; 7(2): 1692-1704

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DPI-1003_2011-06