BioResearch

Lonza

Amaxa™ 96-well Shuttle™ Protocol for Primary Mammalian Neurons

Cell Description

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

Note

Mammalian neurons display significant phenotypic variations due to the wide range of both species and tissues from which they may be sourced. This basic protocol describes how to easily define optimal Nucleofection™ Conditions for different mammalian neural cells. We recommend to first test a set of pre-selected Nucleofector™ Programs together with the P3 Primary Cell 96-well Nucleofector™ Kit.

If you have questions regarding your neurons of interest, please contact our Scientific Support Team for further help with the optimization.

Product Description

Recommended Kits

P3 Primary Cell 96-well Nucleofector™ Kit

Cat. No.	V4SP-3096
Size (reactions)	1×96
P3 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-3960
Size (reactions)	10×96
P3 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5.0 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	10

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 expiry 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.

Note

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Optimization Guidelines

The initial optimization experiment is comprised of 16 reactions, using 1 Nucloecuvette™ Module: 7 different Nucleofector™ Programs are tested in duplicate 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 Nucleofection™ Conditions can be performed with the help of our Scientific Support Team.

	2.5 x 10^5 cells/sample											
	1	2	3	4	5	6	7	8	9	10	11	12
A	96-CA-138	96-CA-138										
B	96-CL-133	96-CL-133										
C	96-CU-110	96-CU-110										
D	96-DC-100	96-DC-100										
E	96-DC-104	96-DC-104										
F	96-DR-114	96-DR-114										
G	96-EM-110	96-EM-110										
H	negative control (no program)	negative control (no program)										

Required Material

Note

Please make sure that the entire supplement is added to the Nucleofector™ Solution.

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plates
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note

Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260 : A280 ratio should be at least 1.8
- Nucleocuvette™ compatible tips: epT.I.P.S.® [US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266], Matrix TallTips® [Matrix Technologies Corp., Cat. No. 7281] or LTS Tips [Rainin Instrument, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S]. Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- Solution for coating: 1 mg/ml poly-L-lysine [PLL; Sigma] dissolved in borate buffer, sterilized by filtration (as an alternative to PLL, poly-D-lysine can be used as well for cultivation over more than 3 days); 10 µg/ml laminin solution [Invitrogen, Cat. No. 23017-015]; PBS
- Poly-L-lysine (PLL) and laminin coated glass coverslips [Marienfeld; 15 mm] or PLL coated 96-well culture plates
- Dissection solution: 500 ml HBSS [Lonza; Cat. No. 10-508F], 5 ml penicillin/streptomycin [Lonza; Cat. No. 17-602E], 5 ml 1 M $MgCl_2$, 3.5 ml 1 M Hepes (pH 7.3) and 5 ml 200 mM L-glutamine; sterilized by filtration and pre-cooled on ice before use
- 1 mg/ml trypsin [Sigma; Cat. No. T4799] in calcium and magnesium-free HBSS [Lonza; Cat. No. 10-543F] or Trypsin/EDTA mixture [Lonza; Cat. No. 17-160; used as 1x solution]; HBSS [Lonza; Cat. No. 10-508F] for washing
- Culture medium (for embryonic neurons): PNGM™ BulletKit™ (Lonza; Cat. No. CC-4461) includingPNBM™ 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 of 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 2.15)
- Pre-equilibrate appropriate volume of culture medium to 37°C, 5% CO_2 (240 µl per sample)
- Appropriate number of cells (2.5 x 10^5 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.

Coating of 96-well culture plates

1.1 Add sufficient volume of poly-L-lysine solution to each well to cover the bottom surface
1.2 Incubate in a humidified 37°C/5% CO_2 incubator overnight
1.3 Wash 2 x with sterile water and dry
1.4 Fill each dish with culture medium and return to the incubator for at least 12 hours and up to 2 weeks

Preparation of coverslips (optional)

Put glass coverslips into a rack and boil in 100% ethanol (p.A.) for 5 minutes

1.5 Dry for 5 minutes under a laminar flow and autoclave
1.6 Place coverslips into an appropriate culture dish (e.g. one slide per well of 12-well plate)
1.7 Add 400 µl poly-L-lysine solution and incubate in a humidified 37°C/5% CO_2 incubator overnight
1.8 Wash 2 x with sterile water and dry
1.9 Incubate coverslips in 400 µl laminin solution in a humidified 37°C/5% CO_2 incubator over night
1.10 Wash 2 x 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 dissociated hippocampal or cortical neurons for Nucleofection™

1.11 Separate heads from rat embryos (E17-18) or early postnatal rats (P0-2)
1.12 Dissect brains from the skull and transfer them into a Petri dish with pre-cooled dissection solution
1.13 Cut brains along midline and extract hippocampi or cortices
1.14 Store hippocampi or cortices in at least 10 ml dissection solution in Falcon tubes on ice
1.15 Centrifuge at 80xg for 5 minutes and carefully remove supernatant (alternatively, if experienced, remove dissection solution by very careful decanting)
1.16 Add 1.5 ml trypsin solution and incubate for 10–20 minutes at 37°C
1.17 After trypsinization, centrifuge at 80xg for 5 minutes and carefully remove supernatant (alternatively, if experienced, remove dissection solution by very careful decanting)
1.18 Wash two times with HBSS
1.19 After the second wash, add 1.5 ml of culture medium, prewarmed to 37°C
1.20 riturate about 20–30 x with a fire-polished Pasteur pipette until all pieces of tissue are homogenously dispersed
1.21 Triturate a second time for exactly 1 minute with a fire-polished Pasteur pipette
1.22 Add 5 ml of culture medium and count cells
1.23 Continue at step 2.1 of the Nucleofection™ Protocol

2. Nucleofection™

One Nucleofection™ Sample Contains

– 2.5 x 10^5 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 P3 Primary Cell 96-well Nucleofector™ Solutions

Note

The volumes and cell numbers indicated below refer to the exact numbers required for a 96-well Nucleofection™ Experiment. Please include sufficient extra volume and cells when setting up your pipetting scheme to account for possible losses during pipetting or small discrepencies in pipetted volumes (which may arise from inexactly calibrated pipettes or loose pipette tips).

Note

Ideally, establish plating densities in 96-well culture plates before performing a 96-well Nucleofection™ Experiment. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded. When transfecting lower cell numbers you will likely need to transfer a larger volume of resuspended cells. As a guideline, number of transferred cells should be in the range of 4 x 10^4 to 8 x 10^4 cells per 96-well (125–250 cells/mm^2). As transfected neural cells can quickly form aggregates it is important to transfer cells to culture plate as quickly as possible after addition of medium.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual "Nucleofector™ 96-well Shuttle™ System")
2.3 Select appropriate Nucleofector™ Program. Please try all 7 Nucleofector™ Programs (96-CA-138, 96-CL-133, 96-CU-110, 96-DC-100, 96-DR-104, 96-DR-114 and 96-EM-110) initially to determine the optimal one for your specific neuron type
2.4 Prepare culture dishes with PLL/laminin-coated cover slips or PLL-coated 96-well culture plates by filling appropriate number of dishes/wells with desired volume of culture medium (recommendation 160 µl per well for 96-well plates; see notes above) and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator

2.5 Prepare 0.1–0.6 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30 nM–300 nM siRNA (0.6–6 pmol/sample)
2.6 Centrifuge the required number of cells (2.5 x 10^5 cells per sample) at 80xg for 10 minutes at room temperature
2.7 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

– Prepare mastermixes by dividing cell suspension according to number of substrates
– Add required amount of substrates to each aliquot (max. 2 µl per sample)
– Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

– Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
– Add 2 µl substrates (maximum) to each well
– Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

2.8 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
2.9 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well "A1" must be in upper left position
2.10 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)
2.11 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer
2.12 Optional: Incubate the 96-well Nucleocuvette™ Plate 10 minutes at room temperature
2.13 Resuspend cells with desired volume of pre-warmed media (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 80 µl* of pre-warmed media
2.14 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 40 µl of resuspended cells to 160 µl* pre-warmed media prepared in 96-well culture plates*

Optional (in case of very high mortality)

2.15 If very high mortality is observed, a recovery step can be useful: immediately after Nucleofection™, add 100–300 µl pre-equilibrated recovery medium to the cuvette (instead of the standard culture media) and gently transfer it to a reaction tube
2.16 Place the cell suspension in incubator for 5–10 minutes (="Recovery Step")
2.17 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 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. 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 with fresh medium once a week

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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D4SP-9012_2011-02