BioResearch

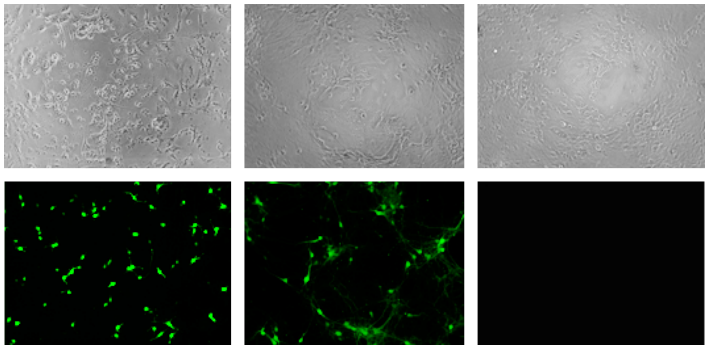
Lonza

Amaxa™ HT Nucleofector™ protocol for rat neurons

Cell description

This protocol is designed for primary dissociated rat hippocampal or cortical neurons, isolated from embryonic (E17-18) or neonatal rats (P0-2) and cultured as mixed glial.

Nucleofection™ of neonatal rat hippocampal neurons



Freshly isolated rat hippocampal neurons (P0) were transfected with program EM-110-AA or CU-133-AA and 0.4 µg pmaxGFP™ vector (a, 1 DIV*) or pSyn-GFP (b, 7 DIV) and cultured in 96-well plates. After 1 or 7 DIV neurons were fixed and analyzed by light and fluorescence microscopy and compared to untransfected neurons. Transfection efficiency with optimized conditions ranged between 30–50 %. Neuron morphology (examined after 7 DIV) was unaltered compared to untransfected neurons. *DIV: days in vitro. Data by courtesy of M.Kiebler, Department of Neuronal Cell Biology, Medical University of Vienna, Vienna, Austria.

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2x384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-3010
Size (reactions)	10x384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well 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

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

Required material

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

- HT Nucleofector™ System
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 384-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)
- Trypsin solution: 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. 17-161E); HBSS (Lonza; Cat. No. 10-508F) for washing
- 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): Low calcium medium, e.g. RPMI (Lonza; Cat. No. 12-167F)
- Pre-equilibrate appropriate volume of culture medium to 37°C, 5 % CO_2 (64 µl per sample)
- Appropriate number of cells ($2.5x10^5$ cells per sample). Lower (down to $5x10^4$) or higher (up to $5x10^5$) cell numbers can be used with slightly reduced transfection efficiency and viability. At even lower cell numbers viability is strongly decreased

1. Pre Nucleofection™

Note
This protocol only gives an outline for the isolation and culture of primary rat hippocampal and cortical 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 384-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 2x 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)

1.5 Put glass coverslips into a rack and boil in 100 % ethanol (p.A.) for 5 minutes
1.6 Dry for 5 minutes under a laminar flow and autoclave
1.7 Place coverslips into an appropriate culture dish (e.g. one slide per well of 12-well plate)
1.8 Add 400 µl poly-L-lysine solution and incubate in a humidified 37°C/5 % CO_2 incubator overnight
1.9 Wash 2x with sterile water and dry
1.10 Incubate coverslips in 400 µl laminin solution in a humidified 37°C/5 % CO_2 incubator over night
1.11 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 dissociated hippocampal or cortical neurons for Nucleofection™

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

2. Nucleofection™

One Nucleofection™ sample contains

– $2.5x10^5$ cells (optimal cell number, recommended for initial experiment)
– 0.4–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
– 20 µl P3 primary cell HT Nucleofector™ solution

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-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 HT 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.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution
2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details please refer to the HT Nucleofector™ manuals)
2.3 Select the appropriate HT Nucleofector™ program **CU-133-AA** for high viability or **EM-110-AA** for high expression level
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 176 µl* per well for 96-well plates;* see note above) and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator
2.5 Prepare 0.4–0.1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector. For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample)
2.6 Centrifuge the required number of cells ($2.5x10^5$ cells per sample) at 80xg for 10 minutes at room temperature. Remove supernatant completely
2.7 Resuspend the cell pellet carefully in 20 µl room temperature HT 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 384-well Nucleocuvette™ plates

B: Multiple substrates (e.g. library transfection)

– Pipette 20 µl of cell suspension into each well of a sterile U or V-bottom 384-well microtiter plate
– Add 2 µl substrates (maximum) to each well
– Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

*** Note**

The indicated cell numbers and volumes have been found to produce optimal Nucleofection™ results in most cases, however, depending on your specific needs you may wish to test an extended range of cell numbers. cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

2.8 Briefly shake the 384-well Nucleocuvette™ plate with an apprpriate microtiter plate shaker to make sure the sample covers the bottom and sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
2.9 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position
2.10 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details please refer to the HT Nucleofector™ manuals)
2.11 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

2.12 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 384-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.13 Plate desired amount of cells in culture system of your choice. Recommendation for 384-well plates: Transfer 20 µl of resuspended cells to 40 µl pre-warmed media

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): Replace half of the medium with fresh culture medium after 2–4 hours

3.3 Optionally (in case of much debris): 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 After 3 days transfer coverslips to glial support culture

3.6 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

Technical assistance and scientific support

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