

Amaxa™ 96-well Shuttle™ Automation Protocol for Neuro-2a

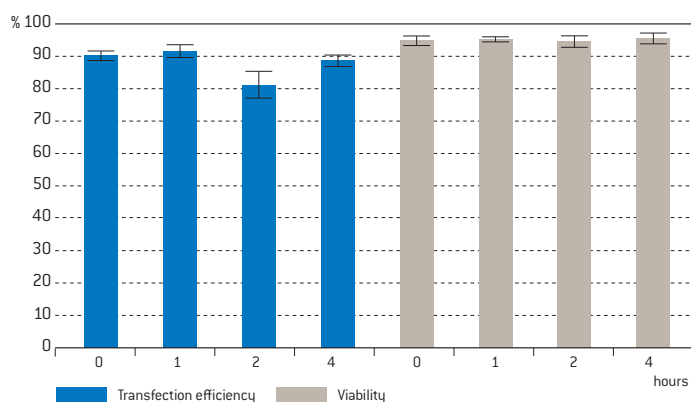
Cell Description

Neuro-2a; Mouse neuroblastoma; adherent neuronal and amoeboid stem cells.

Note

This optimized protocol is designed for the use in automated setups where a prolonged storage of cells (up to 4 hours) in Nucleofector™ Solution prior to the Nucleofection™ process is required. Longer incubation may lead to reduced transfection efficiency and viability.

Example for Nucleofection™ of Neuro-2a



Freshly isolated rat hippocampal neurons (P0) were transfected with program 96-CU-133 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 (c). 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

Cat. No.	VHCA-4003
Size (reactions)	customized
Kit components per 1 x 96 unit:	
*Volumes will be customized depending on the required overfill to cover void volumes etc.	
Cell Line 96-well Nucleofector™ Solution AC1	1.035 ml*
Cell Line 96-well Nucleofector™ Solution AC2	1.265 ml*
Supplement	0.23 ml*
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate	1

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. The mixture of Nucleofector™ Solutions AC1, AC2 and supplement should be prepared directly before the experiment.

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.

Required Material

Note

Please mix the entire content of Nucleofector™ Solutions AC1 and AC2 with supplement directly before the experiment. If the solutions are supposed to be used partially i.e. to split up the experiment, mix Nucleofector™ Solution AC1, AC2 and supplement in a ratio of 1.035 / 1.265 / 0.23 ml.

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented Nucleofector™ Solution equilibrated to 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
- Supplemented Nucleofector™ Solution AC equilibrated to room temperature
- Supplied Nucleocuvette™ Plates
- 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] or Matrix TallTips® [Matrix Technologies Corp., Cat. No. 7281]. Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- 96-well culture plates or culture plates of your choice
- For trypsinization: 0.5 mg/ml trypsin and 0.2mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- Appropriate volume of culture media at 37°C (255 µl per sample; MEM [Eagle] + 2mM L-glutamin, Earle's BSS 1.5g/l sodium bicarbonate, 0.1 mM non-essential amino acid, 1.0 mM sodium pyruvate [Invitrogen, Cat. No 31095], 10% FCS [Sigma, Cat. No. F7524], 1 % P/S)
- Appropriate number of cells (1 x 10⁵ cells per sample)

1. Pre Nucleofection™

Culturing of the cells

- 1.1 Do not use cells after passage 25 for Nucleofection™
- 1.2 Replace medium every 2–3 days
- 1.3 Passage cells 2–3 times a week/passage cells every second to third day
- 1.4 Seed out 2–3 x 10⁴ cells/cm²

- 1.5 Subculture 2–3 days before Nucleofection™ with a ratio of 1:5–1:6

Trypsinization

- 1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
- 1.7 For harvesting the cells incubate the cells at 37°C with e.g. 0.5 mg trypsin/0.2 mg EDTA in PBS
- 1.8 Inactivate trypsinization reaction with supplemented culture media or PBS/0.5% BSA

2. Nucleofection™

One Nucleofection™ Sample Contains

- 2 x 10⁵ cells
 - 0.4–1 µg plasmid DNA (in 1–2 µl H₂O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300nM siRNA (0.6–6 pmol/sample)
 - 20 µl Nucleofector™ Solution
- 2.1 Please make sure that the Nucleofector™ Solutions and supplement are mixed in the appropriate ratio
 - 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 the appropriate Nucleofector™ Program **96-DS-137**
 - 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 175 µl per well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
 - 2.5 Pre-warm an aliquot of culture medium to 37°C (80 µl per sample* see comments at the end of this chapter)
 - 2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30 to 300 nM siRNA (0.6–6 pmol/sample). Depending on cell type, the minimum effective siRNA concentration can range between 1 nM and 1 µM. To validate optimal conditions for down regulation we recommend performing a time course (mRNA: 12–72 hours, protein/phenotype: 24–96 hours) in addition
 - 2.7 Harvest the cells by trypsinization (please see 1.6–1.8)
 - 2.8 Count an aliquot of the trypsinized cells and determine cell density
 - 2.9 Centrifuge the required number of cells (1 x 10⁵ cells per sample) at 90xg for 10 minutes at room temperature
 - 2.10 Resuspend the cell pellet carefully in 20 µl room temperature 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. Make sure the sample covers the bottom of the well, if necessary gently tap the Nucleocuvette™ Plate. Avoid air bubbles while pipetting.

- 2.11 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well “A1” must be in upper left position
- 2.12 Start 96-well Nucleofection™ Process by either:
 - A pressing “Upload and start” in the 96-well Shuttle™ Software (please refer to Manual)
 - B or pressing “Upload” in the 96-well Shuttle™ Software and then the “Start” button at the 96-well Shuttle™ (please refer to Manual)
- 2.13 After retainer opening, carefully remove the 96-well Nucleocuvette™ Plate from the retainer
- 2.14 Resuspend cells with desired volume of pre-warmed medium (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 medium*
- 2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 50 µl of resuspended cells to 150 µl pre-warmed medium prepared in 96-well culture plates*

* Note

The indicated cell numbers and volumes have been found to produce optimal 96-well 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.

3. Post Nucleofection™

- 3.1 Incubate the cells in humidified 37°C/5% CO₂ incubator until analysis. Gene expression is often detectable after only 4–8 hours

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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