Lonza

Amaxa™ 96-well Shuttle™ Basic Protocol for Primary Mammalian Neurons – Transfection in Adherence

Cell Description

Primary mammalian neurons; freshly isolated or cryopreserved Clonetics™ Neural Cells from embryonic or neonatal 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. You can determine the optimal Adherent Nucleofection™ Condition for your neuron type using the Basic 96-well Nucleofector™ AD Kit for Primary Mammalian Neurons which contains P4 Nucleofector™ Solution and specialized Nucleocuvette™ Strips for adherent Nucleofection™. Having tested various neuron types, high transfection efficiencies can be achieved using one of the programs indicated below.

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

Product Description

Cat. No.	VIPI-1003
Size (reactions)	1×96
Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	2×50 µg
Nucleocuvette™ AD Plate(s)	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 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.

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.

Note

The outer side of the well bottom may show small plastic particle inclusions which can originate from production process or transport. These inclusions do not have any influence on cell viability or transfection efficiency. As the well bottom is very sensitive and prone to scratches, please handle the 96-well Nucleocuvette™ AD Plates carefully and avoid sliding them on the bench or in the incubator. Ideally, you would use the blister package or something appropriate as tray while handling the plates.

Optimization Guidelines

	Standard time schedule		Individual time schedule (optional)		
	1	2	3	4	5–12
A	CC[1]	CC[1]	CC[1]	CC[1]	—
B	96-CG-110	96-CG-110	96-CG-110	96-CG-110	—
C	96-CL-125	96-CL-125	96-CL-125	96-CL-125	—
D	96-DC-100	96-DC-100	96-DC-100	96-DC-100	—
E	96-CU-125	96-CU-125	96-CU-125	96-CU-125	—
F	96-DG-108	96-DG-108	96-DG-108	96-DG-108	—
G	96-DR-121	96-DR-121	96-DR-121	96-DR-121	—
H	No program[2]	No program[2]	No program[2]	No program[2]	—

[1] Cell culture control (completely untreated cells left in medium)
[2] Addition of Nucleofector™ Solution and pmaxGFP™ Vector, but no application of program

The initial optimization experiment should be performed with pmaxGFP™ Vector and is comprised of 16 reactions, using 1 Nucleocuvette™ Module: 6 different 96-well Nucleofector™ Programs are tested in duplicate with the 96-well Nucleofector™ Solution plus 2 controls (cell culture control and no program control). For this experiment it is recommended to follow a standard time schedule:

Day -1:	Coating of 96-well Nucleocuvette™ AD Plates
Day 0:	Preparation and seeding of neurons
Day 2:	Adherent Nucleofection™
Day 3:	Analysis

In parallel you may use a second Nucleocuvette™ Module with a second set of samples following your specific experimental time schedule concerning days of Nucleofection™ and analysis. Generally, any time point within the whole culturing period in 96-well Nucleocuvette™ AD Plates (see chapter 1, note 1) is suitable for Nucleofection™ and analysis. Nevertheless, transfection efficiency and viability might vary when using different time points for Nucleofection™ and analysis. The Nucleofector™ Program which turns out to be the most appropriate for your experimental setup 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.

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 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate(s)
- For sealing of non-used wells (optional): For preserving wells for lateruse, sterile sealing strips might be used, e.g. RotilaboR Sealing Strips (Roth; Cat. No. EN 79.1, sterile) or SealPlateR MiniStrips™ Sealing Films (Excel Scientific; Cat. No. SPS-2X8-50)
- Nucleocuvette™ compatible tips: Wells of a 96-well Nucleocuvette™ AD Plate are compatible with most standard pipette tips used for volumes up to 200 µl. Before using any special pipette tips, please ensure they reach the bottom of the Nucleocuvette™ wells without getting stuck
- 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
- Prepared poly-D-lysine (PDL, (e.g. P0899, Sigma-Aldrich, MW 70.000–150.000; 100µg/ml in PBS;sterilized by filtration)
- 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
- Prewarm appropriate volume of culture medium to 37°C, 5 % CO_2 (330 µl per sample)
- Appropriate number of cells (2×10^4 cells per sample ; maximal cell number: 3×10^4 cells; minimal cell number: 0.5×10^4 cells; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Coating of 96-well Nucleocuvette™ AD Plates

1.1 Add 50 µl of poly-D-lysine solution (100 µg/ml, dissolved in PBS, sterilized by filtration) to each well to cover the bottom surface. Alternatively, poly-L-lysine can be used. Other coating substances (polyornithin or laminin) might not be well suited for culturing neurons in Nucleocuvette™ AD Plates

1.2 Incubate in a humidified 37°C/5 % CO_2 incubator overnight

1.3 Wash plates 2× with sterile water and dry for approximately 2 hours with open lid under a sterile hood

Isolation of Primary Neurons

When using freshly isolated neurons please refer to your established procedure or to protocols described in the literature.

Plating and Culturing of Cells in 96-well Nucleocuvette™ AD Plates

Notes

- Generally, the 96-well Nucleocuvette™ AD Plates are suited for long-term culture and Nucleofection™ of neurons for at least 14 days. However, individual culture duration depends on neuron species and type, isolation or thawing procedures, culturing and handling conditions. With longer culture durations well-to-well variations of Nucleofection™ Results may develop.
- A plating density of 2×10^4 cells per sample was found to be optimal for various neurons in Nucleocuvette™ Wells. If necessary, lower cell numbers can be used depending on cell type, application and culture duration. As a guideline, the number of seeded cells should be in the range of 0.5 to 3×10^4 cells per well (750–4500 cells/mm^2). When using the kit for proliferating neural cells (e.g. glial cells) different plating densities might be required depending on duration of culture prior and post Nucleofection™
- It is known for any 96-well culture plate that edge effects (e.g. reduced cell numbers) may occur in the outer wells during prolonged culturing in many incubators. There are special incubators available which reduce such effects. If you do not work with such an incubator, we strongly recommend testing for potential edge well effects and eventually leaving the outer wells filled with H_2O only.

1.4 Centrifuge the required number of cells (e.g. 2×10^4 cells per sample) at 80×g for 10 minutes at room temperature.

1.5 Resuspend the cell pellet carefully in the appropriate amount of pre-warmed cell culture medium (200 µl per sample)

1.6 Plate the desired amount of samples into the wells of the 96-well Nucleocuvette™ AD Plate

1.7 Incubate the cells in humidified 37°C/5 % CO_2 incubator until Nucleofection™. If Nucleofection™ is not performed within 72 hours post plating replace 50 % of the media with fresh, pre-warmed media every 3 to 4 days

2. Nucleofection™

One Nucleofection™ Sample Contains

- $0.5–3\times10^4$ adherent cells
- 0.1 –5 µg plasmid DNA (in 1–2 µl H_2O or TE) or 1 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl Nucleofector™ Solution

Notes

- If cells were incubated for more than 4 days without changing media before Nucleofection™, it is recommended to wash cells very carefully twice with medium prior Nucleofection™
- Please perform all pipetting steps very carefully to avoid disturbing neuron adherence
- Avoid leaving neurons without any liquid coverage (medium or Nucleofector™ Solution) for longer times. If handling samples of a whole 96-well plate, it is recommended to perform the liquid exchange steps (see 2.6–2.7 and 2.12) column by column using a 8-channel pipette
- If transfected cells are analyzed by fluorescence microscopy it may be of benefit to use higher DNA amounts (see above) as fluorescence in neurons could be weak

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 device and software manuals)

2.3 Select the appropriate 96-well Nucleofector™ Program. Please try all 6 Nucleofector™ Programs (**96-CG-110, 96-CL-125, 96-DC-100, 96-CU-125, 96-DG-108 and 96-DR-121**) initially to determine the optimal one for your specific neuron type

2.4 Pre-warm an aliquot of culture medium to 37°C (330 µl per sample)

2.5 Prepare 0.1–5 µg plasmid DNA or 1 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample) in 20 µl room temperature 96-well Nucleofector™ Solution per sample. Prepare mastermixes according to the number of samples

Note

It is advisable to pre-dispense mastermix into a sterile round-bottom 96-well plate. Use a multichannel 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.

2.6 Carefully remove media from each well

2.7 Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

2.8 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.9 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)

2.10 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer.

2.11 Carefully add 180 µl pre-warmed medium to each well. To improve viability post Nucleofection™ it is recommended to carefully remove immediately 150 µl medium and replace it by 150 µl fresh prewarmed medium. (maximum cuvette volume 270 µl)

3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5 % CO_2 incubator

3.2 Optional: In case you have a lot of debris in your culture you may replace 50 % medium by fresh medium 4 hours post Nucleofection™

3.3 Replace half of the culture medium with pre-warmed fresh medium every 2–3 days

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

Technical Assistance and Scientific Support

USA/Canada
Tel 800 521 0390 (toll-free)
Fax 301 845 8338
scientific.support@lonza.com

Europe and Rest of World
Tel +49 221 99199 400
Fax +49 221 99199 499
scientific.support.eu@lonza.com

www.lonza.com

Lonza Cologne AG–50829 Cologne, Germany