Lonza

Amaxa™ 4D-Nucleofector™ Basic Protocol for Primary Mammalian Neurons

For 4D-Nucleofector™ X Unit—Transfection in adherence

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 **Basic4D-Nucleofector™ X AD Kit for Primary Mammalian Neurons** which contains P4 Nucleofector™ Solution and specialized Nucleocuvette™ Strips for adherent Nucleofection™.

This protocol provides guidelines on neuron culture in 16-well Nucleocuvette™ AD Strips and on the transfection procedure using our Basic Neuron 4D-Nucleofector™ X AD Kit.
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

Recommended Kit(s)—Basic Neuron 4D-Nucleofector™ X Kit

Cat. No.	V4XP-1A32
Transfection volume	20 µl
Size [reaction]	2 x 16
Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
Supplement	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Single Nucleocuevette® (100 µl)	-
16-well Nucleocuvette™ Strips (20 µl)	2

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

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

Note

The outer side of the Nucleocuvette™ 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 16-well Nucleocuvette™ AD Strips carefully and avoid sliding them on the bench or in the incubator. Ideally, you would use the 16-well Nucleocuvette™ Rack provided with the 4D-Nucleofector™ System.

Optimization Guidelines

The initial optimization experiment should be performed with pmaxGFP™ Vector and is comprised of 16 reactions, using 1 16-well Nucleocuvette™ AD Strip: 6 different 4D-Nucleofector™ Programs are tested in duplicate with the 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 16-well Nucleocuvette™ AD Strip 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 16-well Nucleocuvette™ AD Strips (see chapter "Pre Nucleofection™", 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.

Nucleocuvette™ Strip 1: Standard time schedule

	1	2
A	CC	CC
B	CG-110	CG-110
C	CL-125	CL-125
D	DC-100	DC-100
E	CU-125	CU-125
F	DG-108	DG-108
G	DR-121	DR-121
H	No program	No program

CC = cell culture control

Nucleocuvette™ Strip 2: Individual time schedule (optional)

	1	2
A	CC	CC
B	CG-110	CG-110
C	CL-125	CL-125
D	DC-100	DC-100
E	CU-125	CU-125
F	DG-108	DG-108
G	DR-121	DR-121
H	No program	No program

Required Material

Note

Please make sure that the entire supplement is added to the Nucleofector™ Solution. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 20 µl 16-well Nucleocuvette™ AD Strips
- 16-well Nucleocuvette™ Rack provided with the 4D-Nucleofector System
- **For sealing of non-used wells (optional):** For preserving wells for later use, sterile sealing strips might be used, e.g. Rotilabo® Sealing Strips (Roth; Cat. No. EN 79.1, sterile) or SealPlate® MiniStrips™ Sealing Films (Excel Scientific; Cat. No. SPS-2X8-50)
- **Compatible tips for 20 µl Nucleocuvette™ AD Strips:** Wells of a 16-well Nucleocuvette™ AD Strip 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

For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- 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; 100 µg/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 (see table 2)
– Appropriate number of cells/sample (see table 2)

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 Strips
1.2 Incubate in a humidified 37°C/5% CO_2 incubator overnight
1.3 Wash strips 2x with sterile water and dry for approximately 2 hours with open lid under a sterile hood

Isolation of primary neurons

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

Plating and culturing of cells in 16-well Nucleocuvette™ AD Strips

Notes:

1. Generally, the 16-well Nucleocuvette™ AD Strips 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.
2. A plating density of 2 x 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 x 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 postNucleofection™
3. It is known for any culture plate working with small liquid volumes 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 and may see any edge effects using 16-well Nucleocuvette™ Strips, we recommend to incubate cells for 30 minutes at RT directly after plating before placing them into the incubator. Furthermore, for culturing you may replace the individual lid of each 16-well Nucleocuvette™ Strip by a 96-well lid that covers the whole 16-well Nucleocuvette™ Rack holding up to four 16-well Nucleocuvette™ Strips.

1.5 Centrifuge the required number of cells (e.g. 2 x 10^4 cells per sample) at 80xg for 10 minutes at room temperature
1.6 Resuspend the cell pellet carefully in the appropriate amount of pre-warmed cell culture medium (200 µl per sample)
1.7 Plate the desired amount of samples into the wells of the 16-well Nucleocuvette™ AD Strip and place the strips into the 16-well Nucleocuvette™ Rack for culturing
1.8 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™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

Notes

1. 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™.
2. Please perform all pipetting steps very carefully to avoid disturbing neuron adherence.
3. Avoid leaving neurons without any liquid coverage (medium or Nucleofector™ Solution) for longer times. If handling several Nucleocuvette™ Strips, 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.
4. 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 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
2.4 Pre-warm an aliquot of culture medium to 37°C (see table 4)
2.5 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA in room temperature 96-well Nucleofector™ Solution per sample (see table 3). Prepare mastermixes according to the number of samples

Note

As leaving cells in 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 16-well Nucleocuvette™ Strips

2.8 Place Nucleocuvette™ Strip with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Strip
2.9 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.10 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.11 Carefully add pre-warmed medium to each well (for recommended volumes see table 5). 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

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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D4XP-9A01_2011-03

Table 1: Volumes required for a single reaction

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

Table 2: Required amounts of cells and media for Nucleofection™

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	not applicable	330 µl
Cell number per Nucleofection™ Sample	not applicable	2 x 10^4 cells per sample Maximal cell number: 3 x 10^4 cells; minimal cell number: 0.5 x 10^4 cells; lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection™ Sample and recommended program

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		not applicable	0.5–3 x 10^4 adherent cells
Substrate*	**pmaxGFP™ Vector**	not applicable	1 µg
or	**plasmid DNA (in H_2O or TE)**	not applicable	0.1–5 µg
or	**siRNA**	not applicable	30–300nM siRNA (0.6–6 pmol/sample)
P4 Primary Cell 4D-Nucleofector™ X Solution		not applicable	20 µl
Program		not applicable	CG-110 or CL-125 or DC-100 or CU-125 or DG-108 or DR-121

* Volume of substrate should comprise maximum 10% of total reaction volume

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	not applicable	330 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	not applicable	180 µl
Volume of sample transferred to culture plate	not applicable	not applicable

* Maximum cuvette volume 270 µl