BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Basic Protocol for Mammalian Neural Cells

For 4D-Nucleofector™ Y Unit—Transfection in Adherence

Cell Description

Primary mammalian neurons; freshly isolated or cryopreserved cells from embryonic or neonatal mammalian neural tissues

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 AD1 Primary Cell 4D-Nucleofector™ Y kit [Cat. No. V4YP-1A24] in combination with 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.
You may check our cell data base for data on your cell type of interest (www.lonza.com/celldatabase).

Product Description

Recommended kit(s)—AD1 Primary Cell 4D-Nucleofector™ Y kit

Cat. No.	V4YP-1A24
Size (reactions)	24
AD1 4D-Nucleofector™ Y solution	2 x 4.5 ml
supplement	2 x 1 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	3 x 150 µg
Nunclon™ Δ Surface 24-well plate (Nunc)	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 of the Nucleofector™ solution 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 could be only used in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Optimization Guidelines

The initial optimization experiment should be performed with pmaxGFP™ Vector and is comprised of 8 reactions: 7 different Nucleofector™ programs are tested in parallel with a "no program" control. For this optimization experiment it is recommended to follow a standard time schedule:

Day -1: Coating of 24-well plates
Day 0: Preparation and seeding of neurons
Day x: Adherent Nucleofection™ (x = 2–6 DIV)
Day x+1: Analysis

	1	2	3	4	5	6
A	ED-158	No program				
B	EH-158	FF-158				
C	EH-166	FP-150				
D	ER-137	IF-100				

No program = Addition of Nucleofector™ Solution and pmaxGFP™ Vector, but no application of 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.

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 (volumes required for a single reaction: 287 µl Nucleofector™ solution and 63 µl supplement).

- 4D-Nucleofector™ System (4D-Nucleofector™ Core unit and 4D-Nucleofector™ Y Unit)
- supplemented 4D-Nucleofector™ Solution at room temperature
- 24-well dipping electrode array compatible plates: CELLSTAR™, 24W Plate (Greiner Bio-one, Cat. No. 662160) or Nunc Multidishes Nunclon™ (Nunc, Cat. No. 142475). Before using other types of 24-well plates for Nucleofection™, please ensure the 24-well plate is compatible with the dipping electrode array
- **Optional**: Cover slip circles suited for 24-well-plates, max. height 0.25 mm (e.g. Menzel Gläser, Cat. No. CB00120RA1, 12 mm diameter, No. 1 (thickness of 0.13–0.16 mm))
- Supplied pmaxGFP™ Vector
- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- **For Coating**: Use your established coating substance and procedure. If you do not have an established protocol you may contact our Scientific Support Team
- **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)
- **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).
- Prewarm appropriate volume of culture medium to 37°C, 5% CO_2 (1 ml per sample)
- Appropriate number of cells
  (1) **Freshly isolated neurons**: A plating density of 1.5 x 10^5 living cells per sample was found to be optimal for most neurons types in 24-well culture plates. Depending on cell type, application and culture duration other cell numbers might be appropriate. Transfection results may be influenced by cell numbers
  (2) **Cryopreserved neurons**: A plating density of 3 x 10^5 living cells per sample was found to be optimal for cryopreserved neurons in 24-well culture plates
  (3) **Glia cells**: When using the kit for growing neural cells (e.g. glial cells) different plating densities might be required depending on duration of culture prior and post Nucleofection™

1. Pre Nucleofection™

Coating of 24-well plates or cover slips

For coating use your established coating substance and procedure. If you need any recommendations for coating please contact our Scientific Support or check out or FAQ database at www.lonzabio.com/faq.

Isolation of primary neurons

When using freshly isolated neurons please refer to your established procedure or to protocols described in the literature. If you need any recommendations for coating please contact our Scientific Support or check out or FAQ database at www.lonzabio.com/faq.
When using cryopreserved neurons please refer to the cell-specific instructions accompanying the product about appropriate handling procedures.

Plating and culturing of cells in 24-well plates (with or without cover slips)

1.1 Centrifuge the required number of cells (see "Required material") at 80xg for 10 minutes at room temperature.
1.2 Resuspend the cell pellet carefully in an appropriate amount of pre-warmed cell culture medium (1 ml per sample)
1.3 Plate the desired amount of cells in the wells of the 24-well plate.
1.4 Incubate the cells in humidified 37°C/5% CO_2 incubator until Nucleofection™. Replace 50% of the media with fresh, pre-warmed media every 3 to 4 days

2. Nucleofection™

One Nucleofection™ sample contains

- 1.5 x 10^5 fresh cells or 3 x 10^5 cryopreserved cells
- 8.75–35 µg plasmid DNA (in max. 35 µl H_2O or TE) or 17.5 µg pmaxGFP™ Vector or 30 nM–300 nM siRNA (10.5–105 pmol/sample)
- 350 µl AD1 4D-Nucleofector™ Y solution

Important notes:

(1) Nucleofection™ can be performed at any time during the culturing period
(2) Nucleofection™ performance may depend on neuron species and type, isolation or thawing procedures, culturing and handling conditions and time point of Nucleofection™.
(3) If cells were incubated for more than 3 days without changing media, it is recommended to wash cells twice with medium prior to Nucleofection™
(4) Please perform all pipetting steps very carefully to avoid disturbing neuron adherence

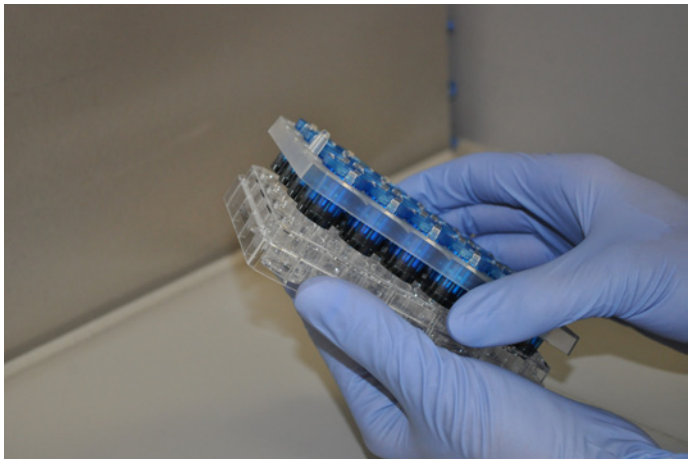
(5) Avoid leaving neurons without any liquid coverage (medium or Nucleofector™ solution). To keep a small liquid film on the cells it is recommended to aspirate medium or solution individually from each well by using a pipette. Usage of a vacuum pump is not recommended. Medium and solution removal and addition (see 2.6 and 2.12) should be performed carefully at the edge of the well

(6) Avoid air bubbles while pipetting

(7) Prevention of air bubbles underneath the 24-well Dipping Electrodes is important for the success of the Nucleofection™ Process. To reduce the accumulation of air bubbles underneath the electrodes during insertion of the 24-well dipping electrode array into the plate, hold the 24-well plate in a 60-75° angle and insert the dipping electrode array parallel to surface of the 24-well plate (see figure below)

(8) If transfected neurons are analyzed by fluorescence microscopy it could be recommended to use higher DNA amounts as fluorescence in neurons could be weak

(9) Re-use of dipping electrode array is not recommended and may lead to lower transfection efficiencies



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: Please try all 7 recommended Nucleofector™ programs (ED-158, EH-158, EH-166, ER-137, FF-158, FP-150, IF-100) initially to determine the optimal one for your specific neuron type

2.4 Pre-warm an aliquot of culture medium to 37°C (1 ml per sample)

2.5 Prepare 8.75–35 µg plasmid DNA (in max. 35 µl H_2O or TE) or 17.5 µg pmaxGFP™ Vector or 30 nM–300 nM siRNA ($10.5–10^5$ pmol/sample) in 350 µl room temperature Nucleofector™ solution per sample

2.6 Carefully remove media and immediately transfer 350 µl of substrate-solution mix into each well of the 24-well plate containing the cells (prepare each well individually, see note 5)

2.7 Insert the 24-well dipping electrode array into the 24-well Plate. Make sure that the dipping electrode array is inserted in the right orientation.

2.8 Place 24-well plate with inserted dipping electrode array into the retainer of the 4D-Nucleofector™ Y Unit. Well "A1" must be in upper left position

2.9 Start Nucleofection™ Process by pressing "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 24-well plate from the retainer

2.11 Carefully withdraw the 24-well dipping electrode array from 24-well plate without spilling liquid from one well to another and discard the array.

2.12 Carefully remove Nucleofector™ solution by pipetting and immediately add 1 ml of pre-warmed medium to each well (handle each well individually, see note 5)

3. Post Nucleofection™

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

3.2 It is recommended to keep the cells untouched for at least 24 hours. 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 Continue neuron culture according to your established protocols.

Additional Information

Up-to-date list of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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