BioResearch

Lonza

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

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

Cell Description

Large, flat adherent cells derived from mammalian endothelial cell tissues from various flat organs

Mammalian endothelial cells 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 endothelial cell type using the **AD1 Primary Cell 4D-Nucleofector™ Y Kit** [Cat. No. V4YP-1A24] in combination with one of the programs indicated below. If you have questions regarding your endothelial cells of interest, please contact our Scientific Support Team for further help with the optimization.

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 used only 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, using 1/3 of a 24-well culture plate: 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 0: Preparation and seeding of cells
Day 1: Adherent Nucleofection™
Day 2: Analysis

	1	2	3	4	5	6
A	CA-215	EW-166				
B	CV-142	FB-100				
C	DC-142	FB-166				
D	EG-142	no program				

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.

The AD1 Primary Cell 4D-Nucleofector™ Y Kit has been tested successfully for the following Clonetics™ Primary Cells provided by Lonza. For up-to-date information about other endothelial cell types, please refer to our cell database www.lonza.com/celldatabase.

Cell type	Lonza Cat No.	Optimal Program	Transfection Efficiency	Viability*
HUVEC (Human Umbilical Vein Endothelial Cells)	C2519A	CA-215	40%	76%

* Determined by FACS (cell number relative to non-pulsed control)

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**: Supplied Nunclon™ Δ Surface 24-well plate (Nunc). **Alternatively**: CELLSTAR™, 24W Plate [Greiner Bio-one, Cat. No. 662160]. Before using other types of 24-well plates for Nucleofection™, please contact Lonza Scientific Support to check if your 24-well plate is compatible with the Dipping Electrode Array.
- **Optional**: Cover slip circles suited for 24-well-plates, max. height 0.25mm [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 detaching cells**: Please use trypsin as recommended by the cell supplier e.g. ReagentPack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HEPES-BSS) and Trypsin Neutralizing Solution (TNS) [Lonza; Cat. No. CC-5034]
- **Culture medium**: Please use media as recommended by the cell supplier e.g. EGM™-2 BulletKit™ [Lonza; Cat. No. CC-3162] for HAEC [Lonza; e.g. Cat. No. CC-2535] and HUVEC [Lonza; e.g. Cat. No. CC-2517] or EGM™-2 MV BulletKit™ [Lonza; Cat. No. CC-3202] for HMVEC-L [Lonza; e.g. Cat. No. CC-2527]
- Prewarm appropriate volume of culture medium to 37°C, 5% CO_2 (1 ml per sample)
- **Appropriate number of cells:** A plating density of 2.5–5.0 x 10^4 living cells per sample was found to be optimal for different primary endothelial cell 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.

1. Pre Nucleofection™

Notes

(1) Transfection results may be source-dependent.
(2) Transfection results may vary due to different culture conditions prior to and post Nucleofection™. If cells were grown to confluency during sub-culturing allowing contact inhibition prior to Nucleofection™, a dramatic decrease in transfection efficiency would be expected.
(3) Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.
(4) General cell culture conditions in 24-well plates should be established before performing a first Nucleofection™ Experiment.

Coating of 24-well plates or cover clips

If any coating is required use your established coating substance and procedure.

Cell culture recommendations

1.1 Replace medium every 2–3 days
1.2 Cells should be passaged after reaching 70–80% confluency
1.3 Cells should be passaged every 2–5 days depending on growth rate of cells
1.4 Do not use cells after passage 9 for Nucleofection™ as this may lead to reduced viabilities and transfection efficiencies
1.5 For adherent Nucleofection™ a cell density of 50%-80% on the day of transfection turned out to be appropriate in most cases

Trypsinization

Note

Please follow your established procedure or the supplier's recommendations, e.g. for Human Umbelical Vein Endothelial Cells [Lonza; e.g. Cat. No. C2519] follow procedure described below.

1.6 Remove media from the cultured cells and wash cells once with HEPES-BSS
1.7 For harvesting, incubate the cells ~5 minutes at 37°C with the recommended volume of the indicated trypsinization reagent (please see required material)
1.8 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

Transfer into 24-well culture plates

Note

Please make sure you are using the 24-well plate provided with the kit or another Dipping Electrode Array compatible plate (see "Required Material").

1.9 Centrifuge the required number of cells (see "Required Material") at 80xg for 10 minutes at room temperature.
1.10 Resuspend the cell pellet carefully in an appropriate amount of pre-warmed cell culture medium (1 ml per sample)
1.11 Plate the desired amount of cells in the wells of the 24-well plate.
1.12 Incubate the cells in humidified 37°C/5% CO_2 incubator until Nucleofection™.

2. Nucleofection™

One Nucleofection™ Sample contains

– 2.5–5.0 x 10^4 cells seeded 1 day prior to Nucleofection™.
– 8.75–35 µg plasmid DNA (in max. 35 µl H_2O or TE) or 16 µg pmaxGFP™ Vector or 30 nM–300 nM siRNA (10.5–10^5 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 endothelial cell type and species, isolation or thawing procedures, culturing and handling conditions and time point of Nucleofection™.
(3) If cells were incubated for more than 4 days without changing media before Nucleofection™, it is recommended to wash cells twice with medium prior to Nucleofection™
(4) Please perform all pipetting steps very carefully to avoid disturbing cell adherence
(5) It is crucial that the cells are not permitted to dry out. In order to keep a small liquid film on the cells it is recommended to pipette off the medium or solution individually from each well. 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) 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 (CA-215, CV-142, DC-142, EG-142, EW-166, FB-100 and FB-166) initially to determine the optimal one for your specific endothelial cell 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 16 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 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 Optional: If there is a lot of cell debris in the culture replace 50% of the medium with fresh medium 4 hours post Nucleofection™
3.3 Continue endothelial cell culture according to your established protocols.

Additional Information

For an up-to-date list of all Nucleofection™ References, please refer to:

www.lonza.com/nucleofection-citations

Technical Assistance and Scientific Support Team:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: Scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: Scientific.support.eu@lonza.com

Lonza Cologne GmbH–50829 Cologne, Germany

D4YP–9A02_2012–03