BioResearch

Lonza

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

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

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

Note

Mammalian endothelial cells display significant phenotypic variations due to the wide range of both species and tissues from which they may be sourced.

This basic protocol describes how to easily define optimal Nucleofection™ Conditions for different mammalian endothelial cells. We recommend to first test a set of pre-selected Nucleofector™ Programs together with the **P5 Primary Cell 4D-Nucleofector™ X Kit.**

For highest convenience of the initial optimization step we recommend using the 16-well Nucleocuvette™ Strip format. Determined conditions are transferable to the 100 µl single Nucleocuvette™. However, you may also directly perform the optimization with the 100 µl single Nucleocuvette™ Format.

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)—P5 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-5012	V4XP-5024	V4XP-5032
Transfection volume	100 µl	100 µl	20 µl
Size [reaction]	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µl)	12	24	-
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.

Optimization Guidelines

The initial optimization experiment is comprised of 16 reactions, using 7 different Nucleofector™ Programs plus 1 control tested in duplicate. The program which turned out to be the most appropriate Nucleofection™ Condition should be used for all subsequent transfections. A further fine tuning of the Nucleofection™ Condition can be performed with the help of our Scientific Support Team.

Nucleocuvette™ Strip 1: P5 Solution

	1	2
A	CA-167	CA-167
B	DY-138	DY-138
C	EH-100	EH-100
D	EP-114	EP-114
E	FA-100	FA-100
F	FF-138	FF-138
G	FP-100	FP-100
H	Negative control (no program)	Negative control (no program)

The P5 Primary Cell 4D-Nucleofector™ Solution has been tested successfully for the following Clonetics™ Primary Cells provided by Lonza:

Cell type	Lonza Cat. No.	Optimal Program	Transfection Efficiency	Viability*
HAEC (Human Aortic Endothelial Cells)	CC-2535	EH-100	73%	70%
HMVEC-L (Human Lung Microvascular Endothelial Cells)	CC-2527	FP-100	79%	48%

*Determined by ViaLight™ Plus Kit, Lonza

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 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips:** epT.I.P.S. (US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266), Matrix TallTips® (Matrix Technologies Corp., Cat. No. 7281) or LTS Tips (Rainin Instruments, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S). Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- **Supplied pmaxGFP™ Vector, stock solution 1µg/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
- Cell culture plates of your choice
- **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; Cat. No. CC-2535] and HUVEC [Lonza; Cat. No. CC-2517] or EGM™-2 MV BulletKit™ [Lonza; Cat. No. CC-3202] for HMVEC-L [Lonza; Cat. No. CC-2527]
- Prewarm appropriate volume of culture medium to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Notes

1. Transfection results may be source-dependent.
2. Transfection results may vary due to different culture conditions prior and post Nucleofection™. Especially endothelial cells show a dramatic decrease of transfection efficiency, if cells were grown confluent during subculture procedure (contact inhibition).
3. Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.

Cell Culture recommendations

1.1 Replace medium every 2–3 days
1.2 Cells should be passaged after reaching 70–80% confluency
1.3 Do not use cells after passage 9 for Nucleofection™ as this may lead to reduced viabilities and transfection efficiencies
1.4 Cells should be passaged 2–5 days before Nucleofection™ depending on growth rate of cells

Trypsinization

Note

Please follow your established procedure or the supplier's recommendations (e.g. for Human Aortic Endothelial Cells [Lonza; Cat. No. CC-2535] follow procedure described below).

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

2. Nucleofection™

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

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 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.5 Pre-warm an aliquot of culture medium to 37°C (see table 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
2.7 Harvest the cells by trypsinization (please see 1.5–1.7)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see table 3) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
2.11 Prepare mastermixes by dividing cell suspension according to number of substrates
2.12 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.13 Transfer mastermixes into the Nucleocuvette™ Vessels

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.

2.14 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.15 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.16 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.17 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.18 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
2.19 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

3. Post Nucleofection™

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours, but ideally, cells should be left undisturbed for 24 hours

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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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)	3 ml	150 µl
Cell number per Nucleofection™ Sample	4–5 x 10^5 cells (Minimal cell number: 2 x 10^5 cells, a lower cell number may lead to a major increase in cell mortality; maximal cell number: 7 x 10^5)	5 x 10^4–1 x 10^5 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		4–5 x 10^5	5 x 10^4–1 x 10^5
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	1–3 µg	0.4 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P5 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		CA-167 or DY-138 or EH-100 or EP-114 or FA-100 or FF-138 or FP-100	CA-167 or DY-138 or EH-100 or EP-114 or FA-100 or FF-138 or FP-100

* 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*
6 cm culture dish	2.5 ml	-
96-well culture plate	-	70 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	80 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™	500 µl	80 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	30 µl (if 1 x 10^5 cells were transfected). If lower cell numbers were used, please adjust the transferred volume as such that approx. 3 x 10^4 cells are plated.

* Maximum cuvette volume 200 µl