BioResearch

Lonza

Amaxa™ HT Nucleofector™ basic protocol for primary mammalian endothelial cells

Cell description

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 HT Nucleofector™ kit.

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 kits

P5 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-5002
Size (reactions)	2×384
P5 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-5010
Size (reactions)	10×384
P5 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well Nucleocuvette™ plate(s)	10

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 expiry 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

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, the well Shuttle™ device and in the 4D-Nucleofector™ system. They are not compatible with the Nucleofector™ II/2b device.

Optimization guidelines

The initial optimization experiment is comprised of 16 reactions: 7 different Nucleofector™ programs are tested in duplicate plus 1 control. The program, which turned out to be the most appropriate Nucleofection™ condition, should be used for all subsequent transfections.
The P5 primary cell HT Nucleofector™ solution has been tested successfully for the following cell types provided by Lonza:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17–24
A	CA-167-AA	DY-138-AA	EH-100-AA	EP-114-AA	FA-100-AA	FF-138-AA	FP-100-AA	control	CA-167-AA	DY-138-AA	EH-100-AA	EP-114-AA	FA-100-AA	FF-138-AA	FP-100-AA	control	
B																	
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Cell type	Lonza Cat. No.	Optimal program	Transfection efficiency	Viability*
HAEC (human aortic endothelial cells)	CC-2535	EH-100-AA	73 %	70 %
HMVEC-L (human lung microvascular endothelial cells)	CC-2527	FP-100-AA	79 %	48 %

*Determined by ViaLight™ Plus Kit, Lonza

Required material

Note
Please make sure that the entire supplement is added to the Nucleofector™ solution.

- HT Nucleofector™ system
- Supplemented HT Nucleofector™ solution at room temperature prior to Nucleofection™
- Supplied 384-well Nucleocuvette™ plates
- 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use compatible tips: 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 Instrument, 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
- 384-well culture plates or culture plates of your choice
- For trypsinization: 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]
- Pre-warm appropriate volume of culture medium to 37°C (122 µl per sample)
- Appropriate number of cells (5×10^4–1×10^5 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note
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). 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 trypsinization reagent (please see required material)
1.7 Neutralize trypsinization reaction with TNS once the majority of the cells (>90 %) have been detached

2. Nucleofection™

One Nucleofection™ sample contains

- 5×10^4–1×10^5 cells
- 0.4 µg pmaxGFP™ vector or 0.4 µg plasmid DNA (in 1–2 µl H_2O or TE) or 30–300nM siRNA (0.6–6 pmol/sample)
- 20 µl P5 primary cell HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution
2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to the HT Nucleofector™ manuals)

2.3 Select appropriate HT Nucleofector™ program. Please try all 7 Nucleofector™ programs (**CA-167-AA, DY-138-AA, EH-100-AA, EP-114-AA, FA-100-AA, FF-138-AA and FP-100-AA**) initially to determine the most appropriate Nucleofection™ condition for your specific endothelial cell type

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 82 µl (see note at the end of this chapter) for one well of a 384-well plate and preincubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Pre-warm an aliquot of culture media to 37°C (40 µl per sample)

2.6 Prepare 0.4 µg pmaxGFP™ vector (recommended for initial optimization) or 0.4 µg plasmid DNA. For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample). Depending on cell type, the minimum effective siRNA concentration can range between 1 nM and 1 µM. To validate optimal conditions for down-regulation we recommend performing a time course (mRNA: 12–72 hours, protein/phenotype: 24–96 hours) in addition

2.7 Harvest the cells by trypsinization (please see 1.5–1.7)

2.8 Count an aliquot of the trypsinized cells and determine cell density

2.9 Centrifuge the required number of cells (5×10^4–1×10^5 cells per sample) at 90×g for 10 minutes at room temperature

2.10 Resuspend the cell pellet carefully in 20 µl room temperature HT Nucleofector™ solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

– Prepare mastermixes by dividing cell suspension according to number of substrates
– Add required amount of substrates to each aliquot (max. 2 µl per sample)
– Transfer 20 µl of mastermixes into the wells of the 384-well Nucleocuvette™ plates

B: Multiple substrates (e.g. library transfection)

– Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
– Add 2 µl substrates (maximum) to each well
– Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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.

2.11 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.12 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position

2.13 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)

2.14 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

2.15 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 18 µl of resuspended cells to 82 µl pre-warmed media

*** Note**

The indicated cell numbers and volumes produce optimal Nucleofection™ results in most cases. However, you may wish to test an extended range of cell numbers depending on your specific needs. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

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. To validate optimal conditions for down regulation we recommend performing a time course (please see 2.6)

Additional information

Up-to-date list of all Nucleofector™ references

www.lonza.com/nucleofection-citations

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