BioResearch

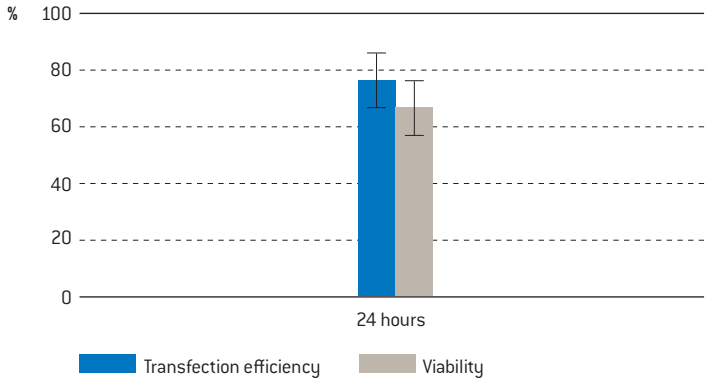
Lonza

Amaxa™ HT Nucleofector™ protocol for human umbilical vein endothelial cells (HUVEC)

Cell description

This protocol has been validated for Clonetics™ HUVEC (Lonza; Cat. No. CC-2519) or self isolated HUVEC; large flat adherent epitheloid cells with large nuclei; cells may grow in confluent monolayer.

Example for Nucleofection™ of HUVECs



Average transfection efficiency and viability of HUVEC 24 hours post Nucleofection™. HUVECs were transfected with program CA-167-DA and 0.4 µg of pmaxGFP™ vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability was determined as % PI negative cells compared to untreated.

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 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 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 96-well Shuttle™ device and in the 4D-Nucleofector™ system. They are not compatible with the Nucleofector™ II/2b device.

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
- Supplied 384-well Nucleocuvette™ plate(s)
- Supplied pmaxGFP™ vector, stock solution 1 µg/µl
- 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

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 culture plates or culture plates of your choice
- For trypsinization: Reagent Pack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) (Lonza, Cat. No. CC-5034)
- Culture medium: EGM™-2 BulletKit (Lonza; Cat. No. CC-3162)). We recommend storing 40 ml aliquots of the prepared medium at -80°C. Do not use medium stored at 4°C for more than two days, as this may lead to reduced cell viability and transfection efficiency
- Prewarm appropriate volume of culture medium to 37°C (95 µl per sample)
- Appropriate number of cells (1×10^5 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note
Transfection results may be donor dependent.

Cell culture recommendations

1.1 Seeding conditions: $5–6\times10^4$ cells per 25 cm^2 flask
1.2 Replace media 2–3 times per week; 2–3 ml media per 25 cm^2 flask
1.3 Cells should be passaged after reaching 80–90 % confluency
1.4 For Nucleofection™ cells should be preferably passaged 2 days before
1.5 Do not use cells after passage number 10 as this may result in substantially lower gene transfer efficiency and viability
1.6 Optimal confluency before Nucleofection™: 90 %

Trypsinization

1.7 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
1.8 For harvesting, incubate the cells ~1–3 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material). If necessary, prolong the incubation time for two more minutes at 37°C
1.9 Neutralize trypsinization reaction with TNS once the majority of the cells (>90 %) have been detached

2. Nucleofection™

One Nucleofection™ sample contains

- 1×10^5 cells
- 0.1–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM 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 please refer to the HT Nucleofector™ manuals)

2.3 Select the appropriate 384-well HT Nucleofector™ program **CA-167-DA**

Note

For self-isolated HUVEC we recommend testing the programs suggested in our basic protocol for primary mammalian endothelial cells in addition to CA-167-AA (DY-138-AA, EH-100-AA, EP-114-AA, FA-100-AA, FF-138-AA, FP-100-AA).

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 55 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 medium to 37°C (40µl per sample)

2.6 Prepare 0.1–1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Harvest the cells by trypsinization (please see 1.7–1.9)

2.8 Count an aliquot of the cells and determine cell density

2.9 Centrifuge the required number of cells (1×10^5 cells per sample) at 90×g for 10 minutes at room temperature. Remove supernatant completely

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 384-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 384-well plates: Transfer 5 µl of resuspended cells to 55 µl pre-warmed media

*** Note**

The indicated cell numbers and volumes have been found to produce optimal Nucleofection™ results in most cases. However, depending on your specific needs you may wish to test an extended range of cell numbers. 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 humidified 37°C/5 % CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours

Additional information

Up-to-date list of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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