BioResearch

Lonza

Amaxa™ HT Nucleofector™ optimization protocol for cell lines

For use with plasmid DNA and/or siRNA

The cell line optimization HT Nucleofector™ kit (V5SC-9001) enables you to optimize Nucleofection™ conditions for a cell line of your choice. This kit is suitable both for optimizing Nucleofection™ of plasmid DNA, as well as, siRNA oligonucleotides.

Overview

Step 1

The cell line of interest is transfected with the HT Nucleofector™ solutions SE, SF and SG in combination with 111 different Nucleofector™ programs plus negative control.

Step 2

The HT Nucleofector™ solution and program which result in highest transfection efficiency with lowest mortality are selected.

Step 3 (optional)

A further fine tuning of the Nucleofection™ conditions can be performed with the help of our scientific support team.

Product description

Cat. No.	V5SC-9001
Size (reactions)	1x384
SE Cell line HT Nucleofector™ solution	2.25 ml
SF Cell line HT Nucleofector™ solution	2.25 ml
SG Cell line HT Nucleofector™ solution	2.25 ml
Supplement	3x0.5 ml
pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0)	150 µg
Nucleocuvette™ plate(s)	1

Storage and stability

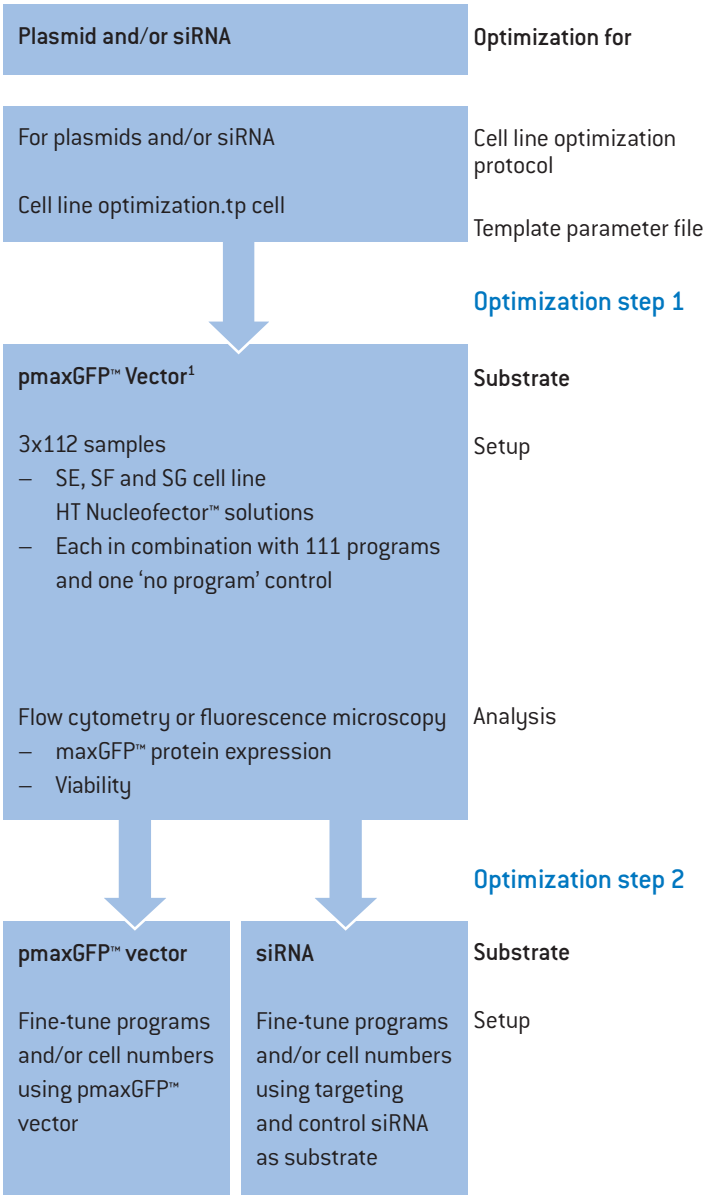
Store Nucleofector™ solution, supplement and pmaxGFP™ vector at 4°C. For long-term storage, pmaxGFP™ vector is ideally stored at -20°C. The expiry date is printed on the solution box. Once the Nucleofector™ supplement is added to the Nucleofector™ solution, it is stable for three months at 4°C.

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.

General considerations

Optimal Nucleofection™ conditions are substrate independent, meaning that siRNA oligonucleotides and plasmid DNA can be transfected using the same protocol. This has been confirmed by comparing conditions for plasmid DNA and labelled siRNA.



Nucleofection™ conditions optimized with pmaxGFP™ vector are also optimal for siRNA. Alternatively to pmaxGFP™. vector, you could use fluorescently labelled siRNA. However, microscopic evaluation of fluorescently labelled siRNA is often hampered by rapid photo-bleaching of the fluorophore. Analysis 4–6 hours post Nucleofection™ recommended.

Optimization guidelines

Step 1

The first experiment is comprised of 336 reactions. Three different cell line HT Nucleofector™ solutions SE, SF and SG are tested in combination with 111 different Nucleofector™ programs plus 1 control for each solution. The HT Nucleofector™ solution and program with the highest efficiency and lowest mortality are selected. For further transfections of plasmid DNA or siRNA, order the respective Cell line HT Nucleofector™ kit (SE, SF or SG) and use it in combination with the selected program

Step 2 (optional)

To maximize Nucleofection™ efficiency, a second experiment based on the best results obtained can be performed. You can test an extended range of cell numbers or DNA amounts per sample or further programs. For this purpose submit your complete results to our scientific support team and within one workday we will suggest additional programs to be tested in combination with the best Nucleofector™ solution. This additional experiment requires purchase of the respective cell line HT Nucleofector™ kit.

Experimental setup

Plate allocation

Cell line optimization

Solution SE								Solution SF	Solution SG
no pulse	DS-130-AA	CA-113-AA	ER-100-AA	CA-139-AA	DS-106-AA	DS-198-AA	empty		
CA-138-AA	DS-150-AA	CA-132-AA	FA-100-AA	CA-158-AA	DS-112-AA	DT-100-AA	empty		
CA-137-AA	DS-120-AA	CA-167-AA	DG-135-AA	CA-189-AA	DS-118-AA	DT-113-AA	empty		
CA-150-AA	DS-113-AA	CL-138-AA	DG-130-AA	CA-201-AA	DS-123-AA	DT-120-AA	empty		
CM-138-AA	DS-104-AA	CM-167-AA	DG-120-AA	CL-135-AA	DS-128-AA	DT-130-AA	empty		
CM-137-AA	DS-100-AA	CU-137-AA	DG-137-AA	CL-137-AA	DS-132-AA	DT-137-AA	empty		
CM-130-AA	EH-100-AA	CL-120-AA	DG-113-AA	CM-132-AA	DS-134-AA	DT-138-AA	empty		
CM-150-AA	FP-100-AA	CL-150-AA	EN-100-AA	CM-134-AA	DS-135-AA	DT-150-AA	empty	...	...
CM-120-AA	FF-138-AA	CM-156-AA	EO-100-AA	CM-135-AA	DS-139-AA	DU-100-AA	empty		
CM-113-AA	FF-137-AA	CU-150-AA	EN-138-AA	CM-158-AA	DS-141-AA	DZ-113-AA	empty		
CM-104-AA	FF-130-AA	DG-150-AA	EN-150-AA	CM-189-AA	DS-142-AA	DZ-120-AA	empty		
CM-100-AA	FF-150-AA	CY-100-AA	EW-113-AA	CM-198-AA	DS-155-AA	DZ-135-AA	empty		
DC-100-AA	FF-120-AA	DB-100-AA	EH-116-AA	CU-138-AA	DS-156-AA	DZ-137-AA	empty		
DN-100-AA	FF-113-AA	DH-100-AA	EH-109-AA	CV-100-AA	DS-158-AA	DZ-150-AA	empty		
DS-138-AA	FF-104-AA	DI-100-AA	EH-156-AA	DG-138-AA	DS-167-AA	ED-100-AA	empty		
DS-137-AA	FF-100-AA	DP-100-AA	EH-118-AA	DR-100-AA	DS-189-AA	EH-104-AA	empty		

Master mixes

SE Cell line HT Nucleofector™ solution									SF Cell line HT Nucleofector™ solution								SG Cell line HT Nucleofector™ solution							
	1	2	3	4	5	6	7	8		9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24
A									A									A							
B									B									B							
C									C									C							
...									...									...							
...									...									...							
N									N									N							
O									O									O							
P									P									P							

For 112 samples:
- Suspension cells: $2.3x10^7$ to $1.3x10^8$
- Adherent cells: $1.3x10^6$ to $5.6x10^7$
- HT Nucleofector™ solution SE: 2.24 ml
- pmaxGFP™ vector: 44.8 µg

Required material

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

- HT Nucleofector™ system
- Supplemented HT Nucleofector™ solutions at room temperature
- Supplied 384-well Nucleocuvette™ plate(s)
- 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
- 96-well culture plates or culture plates of your choice
- 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
- For detaching adherent cells: For commercially available cell lines use e.g. 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5 % BSA (if not recommended differently by cell supplier)
- Culture medium: For commercially available cell lines we recommend following the instructions of the supplier regarding culture medium and supplements
- Recovery medium (optional for adherent cells): For cells grown in high-calcium medium, such as Dulbecco's Modified Eagle Medium (DMEM), you may use a low calcium medium, like RPMI, for the transfer from the cuvette into the plate (see chapter 2, note after 2.16)
- Pre-warm appropriate volume of culture medium to 37°C (210 µl per sample for suspension cells; 225 µl per sample for adherent cells)
- Appropriate number of cells ($0.2–1x10^6$ per sample for suspension cells; $1–5x10^5$ cells per sample for adherent cells; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note
For commercially available cell lines we recommend following the instructions of the supplier regarding media renewal, passaging and seeding conditions. Best Nucleofection™ results will be obtained with standardized cell culture conditions.

Cell culture recommendations for adherent cells

1.1 Subculture 1–2 days before Nucleofection™
1.2 Optimal confluency for Nucleofection™: 70–85 %. Higher cell densities may cause lower Nucleofection™ efficiencies

Cell culture recommendations for suspension cells

1.3 Subculture 1–2 days before Nucleofection™
1.4 Optimal density for Nucleofection™: cells must be in their logarithmic growth phase

Trypsinization (for adherent cells only)

1.5 For commercially available cell lines we recommend following the instructions of the supplier regarding detaching of cells. You may e.g. use trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5 % BSA

2. Nucleofection™

One Nucleofection™ sample contains

- $0.2–1x10^6$ cells (suspension cells) or $1–5x10^5$ cells (adherent cells)
- 0.4 µg pmaxGFP™ vector
- 20 µl SE, SF and SG Cell line HT Nucleofector™ solutions

Note
The volumes and cell numbers indicated below refer to the exact numbers required for the experiment. Please include sufficient extra volume and cells when setting up your pipetting scheme to account for possible losses during pipetting or small discrepancies in pipetted volumes (which may arise from inexactly calibrated pipettes or loose pipette tips).

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 generate parameter file from predefined template for cell line optimization (for details refer to the HT Nucleofector™ manuals)
2.3 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator
  - Adherent cells: 185 µl per well*
  - Suspension cells: 170 µl per well*

  (*see note at the end of this chapter)

2.4 Pre-warm an aliquot of culture medium to 37°C (40 µl per sample)
2.5 Optional (adherent cells): Harvest the cells by trypsinization (please see 1.5)
2.6 Count an aliquot of the cells and determine cell density
2.7 Prepare three aliquots of cell suspension, each with the number of cells required for 112 samples (one aliquot for each well Nucleofector™ solution and both types of siRNA, see optimization guidelines)
2.8 Centrifuge the required number of cells at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend each cell pellet in room temperature HT Nucleofector™ solution to a final concentration of:
- Suspension cells: $2x10^5$–$1x10^6$ cells/20 µl
- Adherent cells: $1x10^5$–$5x10^5$ cells/20 µl

2.10 Mix each cell suspension with the approriate amount of pmaxGFP™ vector
2.11 Transfer 20 µl of each of the 3 aliquots into 112 wells of the 384-well Nucleocuvette™ plate, according to the experimental setup (see optimization guidelines)

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 multi-channel or single-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.

2.12 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
2.13 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
2.14 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)
2.15 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel
2.16 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*
2.17 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 15 µl for adherent cells respectively 30 µl for suspension cells of resuspended cells to 185 µl for adherent cells respectively 170 µl for suspension cells pre-warmed media

Note
If very high mortality is observed, a "recovery step" can be a useful option: Immediately after Nucleofection™, add 40 µl pre-equilibrated low-calcium media such as RPMI and gently transfer it to the reaction tube. Place the cell suspension in an incubator for 5–10 minutes. Then transfer the sample to the prepared culture dish with culture medium.

*** 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. A usual analysis time is 24 hours post Nucleofection™

Additional information

Up-to-date list of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

USA/Canada
Tel 800 521 0390 (toll-free)
Fax 301 845 8338
scientific.support@lonza.com

Europe and Rest of World
Tel +49 221 99199 400
Fax +49 221 99199 499
scientific.support.eu@lonza.com

www.lonza.com

Lonza Cologne GmbH – 50829 Cologne, Germany

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