BioResearch

Lonza

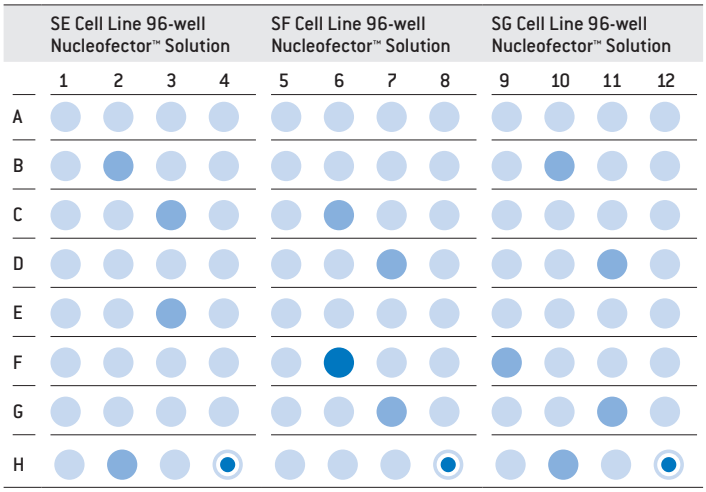
Amaxa™ 96-well Shuttle™ Optimization Protocol for Cell Lines

For Use with Plasmid DNA and/or siRNA

The Cell Line Optimization 96-well Nucleofector™ Kit (V4SC-9096) enables you to optimize 96-well 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. For optimization of Nucleofection™ Conditions for siRNA only, please download the 'Cell Line Optimization Protocol – For siRNA Only (www.lonzabio.com/protocols). To view an up-to-date list of all cell lines for which either an Optimized Protocol or customer data exist, refer to our on-line Cell Database:
www.lonzabio.com/celldatabase

Overview

Step 1



The cell line of interest is transfected with the 96-well Nucleofector™ Solutions SE, SF and SG in combination with 31 different Nucleofector™ Programs plus a negative control. Control wells: Encircled blue dots.

Step 2

The 96-well Nucleofector™ Solution and Program which result in highest transfection efficiency with lowest mortality are selected. Optimal Nucleofection™ Condition: dark blue dot, suboptimal conditions: medium blue dots; inappropriate conditions: light blue dots.

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.	V4SC-9096
Size (reactions)	1×96
SE Cell Line 96-well Nucleofector™ Solution	0.675 ml
SF Cell Line 96-well Nucleofector™ Solution	0.675 ml
SG Cell Line 96-well Nucleofector™ Solution	0.675 ml
Supplement	3×0.150 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 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 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

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in 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. Nevertheless, if you are interested in siRNA only we offer an alternative optimization strategy.

Plasmid and/or siRNA	siRNA only	Optimization for
For Plasmids and/or siRNA	For siRNA only	Cell Line Optimization Protocol
Cell Line Optimization.tp Cell	Cell Line Optimization_siRNA.tp	Template parameter file
		Optimization Step 1
pmaxGFP™ Vector[1]	**siRNA**	**Substrate**
3×32 samples – SE, SF and SG Cell Line 96-well Nucleofector™ Solutions – Each in combination with 31 programs and one 'no program' control	3×16×2 samples[2] – SE, SF and SG Cell Line 96-well Nucleofector™ Solutions – Each in combination with 15 programs and one 'no program' control – With targeting siRNA and negative control siRNA[3]	Setup
Flow cytometry or fluorescence microscopy – maxGFP™ Protein Expression – Viability	– mRNA (or protein) knockdown – Viability	Analysis
		Optimization Step 2
pmaxGFP™ Vector / **siRNA**	**siRNA**	**Substrate**
Fine-tune programs and/or cell numbers using pmaxGFP™ Vector / Fine-tune programs and/or cell numbers using targeting and control siRNA as substrate	Fine-tune programs and/or cell numbers using targeting and control siRNA as substrate.	Setup

1. 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 photobleaching of the fluorophore. Analysis 4–6 hours post nucleofection™ recommended.
2. As compared to DNA, siRNA gets transfected more easily and has negligible influence on viability. Therefore, fewer conditions need to be tested to achieve optimal siRNA transfection.
3. For reduction of the sample number that needs to be analyzed, one can co-transfect pmaxGFP™ and pre-select 3. samples with high plasmid transfection efficiencies.

Optimization Guidelines

Step 1

The first experiment is comprised of 96 reactions. Three different Cell Line 96-well Nucleofector™ Solutions SE, SF and SG are tested in combination with 31 different Nucleofector™ Programs plus 1 control. The 96-well 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 96-well Nucleofector™ Kit 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 96-well Nucleofector™ Kit.

Experimental Setup

Plate Allocation

	SE Cell Line 96-well Nucleofector™ Solution				SF Cell Line 96-well Nucleofector™ Solution				SG Cell Line 96-well Nucleofector™ Solution			
	1	2	3	4	5	6	7	8	9	10	11	12
A	96-FF-100	96-FP-100	96-DS-137	96-CM-150	96-FF-100	96-FP-100	96-DS-137	96-CM-150	96-FF-100	96-FP-100	96-DS-137	96-CM-150
B	96-FF-104	96-EH-100	96-DS-138	96-CM-130	96-FF-104	96-EH-100	96-DS-138	96-CM-130	96-FF-104	96-EH-100	96-DS-138	96-CM-130
C	96-FF-113	96-DS-100	96-DN-100	96-CM-137	96-FF-113	96-DS-100	96-DN-100	96-CM-137	96-FF-113	96-DS-100	96-DN-100	96-CM-137
D	96-FF-120	96-DS-104	96-DC-100	96-CM-138	96-FF-120	96-DS-104	96-DC-100	96-CM-138	96-FF-120	96-DS-104	96-DC-100	96-CM-138
E	96-FF-150	96-DS-113	96-CM-100	96-CA-150	96-FF-150	96-DS-113	96-CM-100	96-CA-150	96-FF-150	96-DS-113	96-CM-100	96-CA-150
F	96-FF-130	96-DS-120	96-CM-104	96-CA-137	96-FF-130	96-DS-120	96-CM-104	96-CA-137	96-FF-130	96-DS-120	96-CM-104	96-CA-137
G	96-FF-137	96-DS-150	96-CM-113	96-CA-138	96-FF-137	96-DS-150	96-CM-113	96-CA-138	96-FF-137	96-DS-150	96-CM-113	96-CA-138
H	96-FF-138	96-DS-130	96-CM-120	Control	96-FF-138	96-DS-130	96-CM-120	Control	96-FF-138	96-DS-130	96-CM-120	Control

Master Mixes

	SE Cell Line 96-well Nucleofector™ Solution				SF Cell Line 96-well Nucleofector™ Solution				SG Cell Line 96-well Nucleofector™ Solution			
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	For 32 samples: – Suspension cells: 6.4×10^6 to 3.2×10^7 – Adherent cells: 3.2×10^6 to 1.6×10^7 – Nucleofector™ Solution SE: 640 µl – pmaxGFP™ Vector: 12.8 µg				For 32 samples: – Suspension cells: 6.4×10^6 to 3.2×10^7 – Adherent cells: 3.2×10^6 to 1.6×10^7 – Nucleofector™ Solution SF: 640 µl – pmaxGFP™ Vector: 12.8 µg				For 32 samples: – Suspension cells: 6.4×10^6 to 3.2×10^7 – Adherent cells: 3.2×10^6 to 1.6×10^7 – Nucleofector™ Solution SG: 640 µl – pmaxGFP™ Vector: 12.8 µg			
D												
E												
F												
G												
H												

Required Material

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

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied 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
- Nucleocuvette™ 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)
- Prewarm appropriate volume of culture medium to 37°C (230 µl per sample for suspension cells; 255 µl per sample for adherent cells)
- Appropriate number of cells (0.2 –1×10^6 per sample for suspension cells; 1–5×10^5 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 supplierregarding 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

- 0.2–1×10^6 cells (suspension cells) or 1–5×10^5 cells (adherent cells)
- 0.4 µg pmaxGFP™ Vector
- 20 µl SE, SF and SG Cell Line 96-well 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 Nucleofector™ 96-well Shuttle™ Software, verify device connection and generate Parameter file from predefined template for cell line optimization (for details see device and software 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: 175 µl per well*
  - Suspension cells: 150 µl per well*
    (*see note at the end of this chapter)

2.4 Pre-warm an aliquot of culture medium to 37°C (80 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 32 samples (one aliquot for each 96-well Nucleofector™ Solution and both types of siRNA, see optimization guidelines):
- Suspension cells: 6.4×10^6–3.2×10^7 cells*
- Adherent cells: 3.2×10^6–1.6×10^7 cells*

2.8 Centrifuge the required number of cells at 90×g for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend each cell pellet in 640 µl room temperature 96-well Nucleofector™ Solution to a final concentration of:
- Suspension cells: 2×10^5–1×10^6 cells/20 µl
- Adherent cells: 1×10^5–5×10^5 cells/20 µl

2.10 Mix each cell suspension with 12.8 µg pmaxGFP™ Vector
2.11 Transfer 20 µl of each of the 3 aliquots into 32 wells of the 96-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 96-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 96-well 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 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
2.13 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position
2.14 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)
2.15 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer
2.16 Incubate Nucleocuvette™ Plate 10 minutes at room temperature
2.17 Resuspend cells with 80 µl* (recommendation for 96-well plates) or desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times
2.18 Plate desired amount of cells in 96-well culture plates or culture system of your choice. Recommendation for 96-well plates:
- Suspension cells: Transfer 50 µl* of resuspended cells to 150 µl pre-warmed medium prepared in 96-well culture plates
- Adherent cells: Transfer 25 µl* of resuspended cells to 175 µl pre-warmed medium prepared in 96-well culture plates

Note
If very high mortality is observed, a "recovery step" can be a useful option: Immediately after Nucleofection™, add 80 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 96-well 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

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