BioResearch

Lonza

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

For siRNA only

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 DNA Nucleofection™ Conditions, please refer to the "Cell Line Optimization Protocol – For Plasmids and / or siRNA" of the Cell Line 96-well Nucleofector™ Kit (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

	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												
D												
E												
F												
G												
H												

The cell line of interest is transfected with targeting siRNA (A1-D12) or negative control siRNA (E1-H12) using the SE, SF, SG Cell Line 96-well Nucleofector™ Solutions in combination with 15 different Nucleofector™ Programs plus a negative transfection control.

Step 2

The 96-well Nucleofector™ Solution and Program which result in highest knock-down efficiencies 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.	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

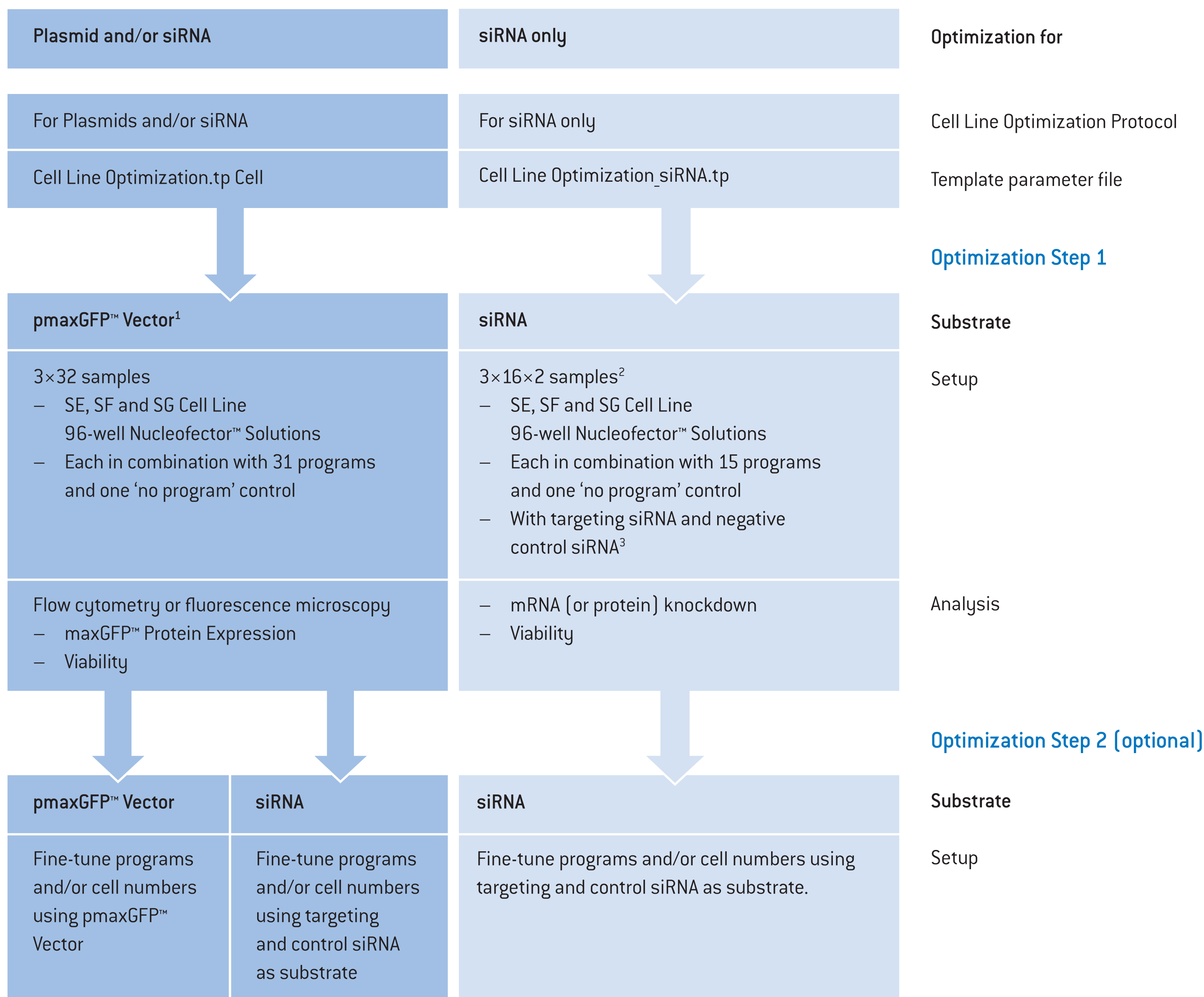
Store Nucleofector™ Solution, Supplement and pmaxGFP™ Vector at 4°C. For long-term storage, pmaxGFP™ Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector™ Supplement is added to the Nucleofector™ Solution, it is stable for three months at 4°C.

Note

96-well Nucleofector™ Solutions and standard Nucleofector™ Solutions are not compatible.

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.



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™ Vector and pre-select samples with high plasmid transfection efficiencies.

Optimization Guidelines

Step 1

Perform a first optimization with targeting siRNA and control siRNA. Targeting siRNA and control siRNA are separately transfected, each with three different Cell Line 96-well Nucleofector™ Solutions SE, SF and SG in combination with 15 different Nucleofector™ Programs plus 1 non-treated control. Viability and knock-down of the target mRNA are evaluated at suitable time points, using the control siRNA samples as reference. The 96-well Nucleofector™ Solution and Program with the highest knock-down efficiency and lowest mortality are selected. For further transfections of siRNA, order the respective Cell Line 96-well Nucleofector™ Kit and use it in combination with the selected program (step 2 below). Optional (for reduction of samples to be analyzed): Perform a first optimization with targeting siRNA and control siRNA, as described above, but with co-transfection of pmaxGFP™ Vector. Select samples with strong GFP expression for analysis of target gene knock-down.

Step 2 (optional)

To maximize siRNA Nucleofection™ Efficiency, a second experiment based on the best results obtained in step 1 can be performed. You can test further programs or titrate optimal cell numbers and siRNA amounts per sample (with up to 24 different conditions in duplicates). Minimizing the amount of siRNAs will also minimize potential off-target effects. For receiving further program suggestions, submit your complete results to our Scientific Support Team, and within one workday we will suggest additional conditions to be tested. This additional experiment requires purchase of the respective Cell Line 96-well Nucleofector™ Kit SE, SF or SG.

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-113	96-DS-113	96-DS-137	96-CM-137	A	96-FF-113	96-DS-113	96-DS-137	96-CM-137	A	96-FF-113	96-DS-113	96-DS-137	96-CM-137	Samples with targeting siRNA
B	96-FF-150	96-DS-120	96-DS-138	96-CM-138	B	96-FF-150	96-DS-120	96-DS-138	96-CM-138	B	96-FF-150	96-DS-120	96-DS-138	96-CM-138	
C	96-FF-137	96-DS-150	96-DN-100	96-CA-137	C	96-FF-137	96-DS-150	96-DN-100	96-CA-137	C	96-FF-137	96-DS-150	96-DN-100	96-CA-137	
D	96-DS-100	96-DS-130	96-CM-150	Control	D	96-DS-100	96-DS-130	96-CM-150	Control	D	96-DS-100	96-DS-130	96-CM-150	Control	
E	96-FF-113	96-DS-113	96-DS-137	96-CM-137	E	96-FF-113	96-DS-113	96-DS-137	96-CM-137	E	96-FF-113	96-DS-113	96-DS-137	96-CM-137	Samples with control siRNA
F	96-FF-150	96-DS-120	96-DS-138	96-CM-138	F	96-FF-150	96-DS-120	96-DS-138	96-CM-138	F	96-FF-150	96-DS-120	96-DS-138	96-CM-138	
G	96-FF-137	96-DS-150	96-DN-100	96-CA-137	G	96-FF-137	96-DS-150	96-DN-100	96-CA-137	G	96-FF-137	96-DS-150	96-DN-100	96-CA-137	
H	96-DS-100	96-DS-130	96-CM-150	Control	H	96-DS-100	96-DS-130	96-CM-150	Control	H	96-DS-100	96-DS-130	96-CM-150	Control	

Master Mixes

	SE Cell Line 96-well Nucleofector™ Solution (columns 1–4)		SF Cell Line 96-well Nucleofector™ Solution (columns 5–8)		SG Cell Line 96-well Nucleofector™ Solution (columns 9–12)	
A B C D	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SE: 320 µl – Targeting siRNA: 80 pmol	A B C D	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SF: 320 µl – Targeting siRNA: 80 pmol	A B C D	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SG: 320 µl – Targeting siRNA: 80 pmol	Samples with targeting siRNA
E F G H	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SE: 320 µl – Targeting siRNA: 80 pmol	E F G H	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SF: 320 µl – Targeting siRNA: 80 pmol	E F G H	**For 16 samples:** – Suspension cells: 3.2×10^6 to 1.6×10^7 – Adherent cells: 1.6×10^6 to 8×10^6 – Nucleofector™ Solution SG: 320 µl – Targeting siRNA: 80 pmol	Samples with control siRNA

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 (optional, for co-transfection)
- Targeting siRNA: We strongly recommend establishing siRNA Nucleofection™ by using a siRNA which has previously proven to show effective RNAi of its target. Such siRNAs are readily available from a number of commercial suppliers. Suitable targets for this optimization protocol are housekeeping genes such as vimentin, GAPDH, cyclophilin B or known siRNA molecules effective in your particular functional readout assay. In an optimal case, the level of target mRNA or protein can be analyzed in 96-well format
- Control siRNA: Always include samples with control siRNA (e.g. non-targeting siRNA) for the complete set of tested Nucleofection™ Conditions (see experimental set-up). Target mRNA or protein levels should always be analyzed relative to control samples with the same solution and program. This will avoid any possible influences of the Nucleofection™ Process on the evaluation of knock-down efficiencies
- 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 2.17)
- 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 $\times 10^6$ per sample for suspension cells; 1–5 $\times 10^5$ 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 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

- 0.2–1 $\times 10^6$ cells (suspension cells) or 1–5 $\times 10^5$ cells (adherent cells)
- 5 pmol siRNA (=250 nM)*; optional: add 0.4 µg pmaxGFP™ Vector for co-transfection
- 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 siRNA (for details see device and software manuals)

*Higher or lower siRNA amounts may be more suitable for your specific cells and target. The optimal siRNA amount can be determined in a second experiment (see optimization guidelines)

2.3 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 150 µl per well* (for suspension cells) or 175 µl per well* (for adherent cells) (*see note at the end of this chapter) and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

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 six aliquots of cell suspension, each with the number of cells required for 16 samples (one aliquot for each 96-well Nucleofector™ Solution and both types of siRNA, see optimization guidelines):
- Suspension cells: 3.2×10^6–1.6×10^7 cells*
- Adherent cells: 1.6×10^6–0.8×10^7 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 320 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 Add 80 pmol (final concentration: 250 nM) of targeting siRNA or control siRNA each to the corresponding aliquots

2.11 Transfer 20 µl of each of the 6 aliquots into 16 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 in96-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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