BioResearch

Lonza

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

For use with plasmid DNA and/or siRNA

Note

The Primary Cell Optimization Protocol enables you to optimize 96-well Shuttle™ Conditions for a primary cell of your choice using our Primary Cell Optimization 96-well Nucleofector™ Kit. The optimization strategy is suitable both for optimizing Nucleofection™ of plasmid DNA, as well as, siRNA oligonucleotides. To view an up-to-date list of all primary cells for which either an Optimized Protocol or customer data exist, refer to our online Cell Database: **www.lonzabio.com/celldatabase**

Product Description

Recommended Kit(s)

Primary Cell Optimization 96-well Nucleofector™ Kit

Cat No.	**V4SP-9096**
Transfection volume	20 µl
Size [reaction]	5 x 32
P1 Primary Cell 96-well Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
P2 Primary Cell 96-well Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
P3 Primary Cell 96-well Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
P4 Primary Cell 96-well Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
P5 Primary Cell 96-well Nucleofector™ Solution	0.675 ml (0.525 ml + 22% overfill)
Supplement 1	4 x 0.15 ml (4 x 0.115 ml + 22% overfill)
Supplement 3 (for P5 Solution)	1 x 0.15 ml (1 x 0.115 ml + 22% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	2 x 50 µg
Nucleocuvette™ Plates	2

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 could be only used with conductive polymer Nucleocuvettes™, i.e. in the 4D-Nucleofector™ and in the 96-well Shuttle™ System. They are not compatible with the Nucleofector™ II/2b Device.

Optimization Guidelines

The initial optimization experiment is comprised of 160 reactions. Five different Primary Cell 96-well Nucleofector™ Solutions P1-P5 are tested in combination with 31 different Nucleofector™ Programs plus 1 control. The Nucleofection™ Condition with the highest efficiency and lowest mortality is selected for all subsequent experiments.

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 we will suggest additional programs to be tested in combination with the best Nucleofector™ Solution. This additional experiment requires purchase of the respective Primary Cell 96-well Nucleofector™ Kit.

Master Mixes

Prepare master mixes for each of the five Nucleofector™ Solutions tested. The volumes and cell numbers for each master mix 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).

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
P1 Primary Cell 96-well Nucleofector™ Solution	
P2 Primary Cell 96-well Nucleofector™ Solution	
P3 Primary Cell 96-well Nucleofector™ Solution	
P4 Primary Cell 96-well Nucleofector™ Solution	
P5 Primary Cell 96-well Nucleofector™ Solution	640 µl
pmaxGFP™ Vector:	12.8 µg

Experimental Setup

	P1 Primary Cell Nucleofector™ Solution				P2 Primary Cell Nucleofector™ Solution				P3 Primary Cell 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-13	96-CM-120	Control

	P4 Primary Cell Nucleofector™ Solution				P5 Primary Cell Nucleofector™ Solution							
	1	2	3	4	5	6	7	8				
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				
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				
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				
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				
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				
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				
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				
H	96-FF-138	96-DS-130	96-CM-120	Control	96-FF-138	96-DS-130	96-CM-120	Control				

Required Material

Note
Please make sure that the entire supplement is added to the Nucleofector™ Solution. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1 (see table 1). Please be aware that supplement 3 is dedicated to P5 Primary Cell Nucleofector™ Solution. For all other solutions, supplements are interchangeable.

- 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 primary cells we recommend the ReagentPack™ Subculture Reagents (Lonza; Cat. No. CC-5034) (if not recommended differently by cell supplier)or you may use trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA
- Culture medium: For commercially available primary cells 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 2.18)
- Prewarm appropriate volume of culture medium to 37 °C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Note
For commercially available primary cells 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 cells 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 cells 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 primary cells we recommend following the instructions of the supplier regarding detaching of cells.

2. Nucleofection™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to table 3.

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 primary cell optimization (for details see device and software manuals; please find all template parameter files, program definition file and latest software version for download on our website: **www.lonza.com/96w-software**)
2.3 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) 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 (see table 4)
2.5 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
2.6 Adherent cells: Harvest the cells by trypsinization (please see 1.5)
2.7 Count an aliquot of the cells and determine cell density
2.8 Centrifuge the required number of cells for each of the five aliquots (see "master mixes" in optimization guidelines) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend each cell pellet carefully in room temperature 96-well Nucleofector™ Solution (see "master mixes" in optimization guidelines)

2.10 Add required amount of substrates to each aliquot (max. 10 % of final sample volume)
2.11 Transfer 20 µl of each of the five aliquots into into the 96-well Nucleocuvette™ Plates, according to the experimental setup (see optimization guidelines)

Note
As leaving cells in 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 min at room temperature
2.17 Resuspend cells with pre-warmed medium (for recommended volume see table 5). 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 (for recommended volumes see table 5)

Optional:
If very high mortality is observed, a "recovery step" can be a useful option: Immediately after Nucleofection™, add indicated volume (see table 4) 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.

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. An 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 Team:
USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

Lonza Cologne AG
50829 Cologne, Germany

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Table 1: Volumes required for a single reaction

	20 µl Nucleocuvette™ Plate
Volume of 96-well Nucleofector™ Solution	16.4 µl
Volume of Supplement	3.6 µl

Table 2: Required amounts of cells and media for Nucleofection™

	20 µl Nucleocuvette™ Plate
Culture medium post Nucleofection™ per sample (for transfer and culture)	Suspension cells: 230 µl Adherent cells: 255 µl
Cell number per Nucleofection™ Sample	Suspension cells: 0.2–1 x 10^6 Adherent cells: 1–5 x 10^5 cells (Lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection™ Sample and recommended program

	20 µl Nucleocuvette™ Plate
Cells	Suspension cells: 0.2–1 x 10^6 cells Adherent cells: 1–5 x 10^5 cells
Substrate* pmaxGFP™ Vector	0.4 µg
P1/P2/P3/P4/P5 96-well Nucleofector™ Solution	20 µl
Program	See Optimization Guidelines

* Volume of substrate should comprise maximum 10% of total reaction volume

Table 4: Culture media volumes required for post Nucleofection™ Steps

	20 µl Nucleocuvette™ Plate*
96-well culture plate	Suspension cells: 150 µl Adherent cells: 175 µl
Culture medium to be added to the sample post Nucleofection™	80 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into 96-well culture plate

	20 µl Nucleocuvette™ Plate*
Culture medium to be added to the sample post Nucleofection™	80 µl
Volume of sample transferred to culture plate	Suspension cells: 50 µl Adherent cells: 25 µl

* Maximum cuvette volume 200 µl