BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Optimization Protocol for Primary Cells

For 4D-Nucleofector™ X Unit—Transfection in suspension

For use with plasmid DNA and/or siRNA

Note

The Primary Cell Optimization Protocol enables you to optimize 4D-Nucleofection™ Conditions for a primary cell of your choice using our **Primary Cell Optimization 4D-Nucleofector X Kit.** For highest convenience the initial optimization step is performed in the 16-well Nucleocuvette™ Strip format. Determined conditions are transferable to the 100 µl single Nucleocuvette™.

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 on-line Cell Database: www.lonzabio.com/celldatabase

Product Description

Recommended Kit(s)—Primary Cell Optimization 4D-Nucleofector™ X Kit

Cat. No.	V4XP-9096
Transfection volume	20 µl
Size [reaction]	6 x 16
P1 4D-Nucleofector™ X Solution	0.675 ml (0.525 ml + 22 % overfill)
P2 4D-Nucleofector™ X Solution	0.675 ml (0.525 ml + 22 % overfill)
P3 4D-Nucleofector™ X Solution	0.675 ml (0.525 ml + 22 % overfill)
P4 4D-Nucleofector™ X Solution	0.675 ml (0.525 ml + 22 % overfill)
P5 4D-Nucleofector™ X Solution	0.675 ml (0.525 ml + 22 % overfill)
Supplement 1	4 x 0.15 ml (3 x 0.115 ml + 22 % overfill)
Supplement 3 (for P5 Solution)	1 x 0.15 ml (3 x 0.115 ml + 22 % overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
16-well Nucleocuvette™ Strips (20 µl)	6

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

4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. They are not compatible with the Nucleofector™ II/2b Device.

Optimization Guidelines

The initial optimization experiment is comprised of 80 reactions. Five different Primary Cell 4D-Nucleofector™ Solutions P1-P5 are tested in combination with 15 different Nucleofector™ Programs plus 1 control. The Nucleofection™ Condition with the highest efficiency and lowest mortality is selected for all subsequent experiments and can be also used in the 100 µl single Nucleocuvette™.
Optional: A subsequent experiment using the residual 16-well Nucleocuvette™ Strip can be performed to fine tune Nucleofection™ Results. A further set of programs is tested based on the three best Nucleofector™ Programs and the best Nucleofector™ Solution from the initial experiment. For further program suggestions, please submit your complete results to our Scientific Support Team.

Experimental setup

Nucleocuvette™ Strip 1: P1 Solution			Nucleocuvette™ Strip 2: P2 Solution			Nucleocuvette™ Strip 3: P3 Solution			Nucleocuvette™ Strip 4: P4 Solution			Nucleocuvette™ Strip 5: P5 Solution		
	1	2		1	2		1	2		1	2		1	2
A	CA-137	DS-150	A	CA-137	DS-150	A	CA-137	DS-150	A	CA-137	DS-150	A	CA-137	DS-150
B	CM-138	DS-120	B	CM-138	DS-120	B	CM-138	DS-120	B	CM-138	DS-120	B	CM-138	DS-120
C	CM-137	EH-100	C	CM-137	EH-100	C	CM-137	EH-100	C	CM-137	EH-100	C	CM-137	EH-100
D	CM-150	EO-100	D	CM-150	EO-100	D	CM-150	EO-100	D	CM-150	EO-100	D	CM-150	EO-100
E	DN-100	EN-138	E	DN-100	EN-138	E	DN-100	EN-138	E	DN-100	EN-138	E	DN-100	EN-138
F	DS-138	EN-150	F	DS-138	EN-150	F	DS-138	EN-150	F	DS-138	EN-150	F	DS-138	EN-150
G	DS-137	EW-113	G	DS-137	EW-113	G	DS-137	EW-113	G	DS-137	EW-113	G	DS-137	EW-113
H	DS-130	Control	H	DS-130	Control	H	DS-130	Control	H	DS-130	Control	H	DS-130	Control

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 16 samples:	
Suspension cells:	3.2 x 10^6 to 1.6 x 10^7
Adherent cells:	1.6 x 10^6 to 0.8 x 10^7
P1 Nucleofector™ Solution or P2 Nucleofector™ Solution or P3 Nucleofector™ Solution P4 Nucleofector™ Solution P5 Nucleofector™ Solution	320 µl
pmaxGFP™ Vector:	6.4 µg

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.

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips:** 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 Instruments, 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
- **Supplied pmaxGFP™ Vector, stock solution 1µg/µl**

Note

For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- Cell culture plates of your choice
- **For detaching adherent cells:** For commercially available primary cells we recommend 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 2.20)

- 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 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 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 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)

2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)

2.4 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

2.5 Pre-warm an aliquot of culture medium to 37 °C (see table 4)

2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)

2.7 Adherent cells: Harvest the cells by trypsinization (please see 1.5)

2.8 Count an aliquot of the cells and determine cell density

2.9 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

2.10 Resuspend each cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see "master mixes" in optimization guidelines)

2.11 Add required amount of substrates to each aliquot (max. 10 % of final sample volume)

2.12 Transfer 20 µl of each of the five aliquots into the wells of the five 16-well Nucleocuvette™ Strips, 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.

2.13 Gently tap the Nucleocuvette™ Strips to make sure the sample covers the bottom of the cuvette

2.14 Place Nucleocuvette™ Strip with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Strip

2.15 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)

2.16 After run completion, carefully remove the Nucleocuvette™ Strip from the retainer

2.17 Incubate Nucleocuvette™ Strip 10 min at room temperature

2.18 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times

2.19 Plate desired amount of cells in 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. A usual analysis time is 24 hours post Nucleofection™

Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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D4XP-9001_2011-03

Table 1: Volumes required for a single reaction

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

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

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	Not applicable	Suspension cells: 230 µl Adherent cells: 255 µl
Cell number per Nucleofection™ Sample	Not applicable	Suspension cells: 0.2 –1 x 10^6 Adherent cells: 1–5 x 10^5 (Lower or higher cell numbers may influence transfection results)

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

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		Not applicable	Suspension cells: 0.2–1 x 10^6 cells Adherent cells: 1–5 x 10^5 cells
Substrate*	**pmaxGFP™ Vector**	Not applicable	0.4 µg
P1/P2/P3/P4/P5 4D-Nucleofector™ X Solution		Not applicablel	20 µl
Program		Not applicable	See Optimization Guidelines

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

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
96-well culture plate	-	Suspension cells: 150 µl Adherent cells: 175 µl
Culture medium to be added to the sample post Nucleofection™	Not applicable	80 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	Not applicable	80 µl
Volume of sample transferred to culture plate	Not applicable	Suspension cells: 50 µl Adherent cells: 25 µl

* Maximum cuvette volume 200 µl