BioResearch

Lonza

Amaxa® 4D-Nucleofector® Basic Protocol for Human Stem Cells

For 4D-Nucleofector® X Unit – Transfection in suspension

Pluripotent cells, adherent

Note

This basic protocol describes how to easily define optimal Nucleofection® Conditions for different human stem cells (e.g. H1, H7, H14, HS306). We recommend to first test a set of pre-selected Nucleofector® Programs together with two of our Primary Cell 4D-Nucleofector® X Kits:

– P3 Primary Cell 4D-Nucleofector® X Kit
– P4 Primary Cell 4D-Nucleofector® X Kit

For subsequent experiments simply use the kit which yields the best results.

For highest convenience of the initial optimization step we recommend using the 16-well Nucleocuvette® Strip format. Determined conditions are transferable to the 100 µl single Nucleocuvette®. However, you may also directly perform the optimization with the 100 µl single Nucleocuvette® Format. If you have questions regarding your human stem cell of interest, please contact our Scientific Support Team for further help with the optimization.

Product Description

Recommended Kit(s) – P3 Primary Cell 4D-Nucleofector® X Kit or P4 Primary Cell 4D-Nucleofector® X Kit

	P3 Primary Cell 4D-Nucleofector® X Kit			P4 Primary Cell 4D-Nucleofector® X Kit		
Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032	V4XP-4012	V4XP-4024	V4XP-4032
Transfection volume	100 µl	100 µl	20 µl	100 µl	100 µl	20 µl
Size [reaction]	2 x 6	24	2 x 16	2 x 6	24	2 x 16
Nucleofector® Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg	30 µg	30 µg	30 µg	30 µg	-
pmaxGFP® Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	-	-	45 µg	-	-	45 µg
Single Nucleocuvette® (100 µl)	12	24	-	12	24	-
16-well Nucleocuvette® Strips (20 µl)	-	-	2	-	-	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

4D-Nucleofector® Solutions could be only 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 32 reactions, using 2 Nucleocuvette® Strips: 7 different Nucleofector® Programs are tested in duplicate with 2 4D-Nucleofector® X Solutions plus 1 control. The program and Nucleofector® Solution which turned out to be the most appropriate Nucleofection® Condition should be used for all subsequent transfections.

Nucleocuvette® Strip 1: P3 Solution

	1	2
A	CA-137	CA-137
B	CB-150	CB-150
C	CD-118	CD-118
D	CE-118	CE-118
E	CM-113	CM-113
F	DC-100	DC-100
G	DN-100	DN-100
H	Negative control (no program)	Negative control (no program)

Nucleocuvette® Strip 2: P4 Solution

	1	2
A	CA-137	CA-137
B	CB-150	CB-150
C	CD-118	CD-118
D	CE-118	CE-118
E	CM-113	CM-113
F	DC-100	DC-100
G	DN-100	DN-100
H	Negative control (no program)	Negative control (no program)

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)

- 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
- 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
- **Coated plates (for culture with feeder cells):** Prepare a culture plate coated with gelatine and inactivated feeder cells (one well per sample) 24 hours before Nucleofection®
- **Coated plates (for feeder-free culture):** Prepare culture plates coated with Matrigel™ [BD Biosciences, Cat. No. 354277]
- **For detaching cells:** Accutase solution [PAA Laboratories, Cat. No. L11-007] or 0.05 or 0.25% Trypsin/EDTA solution [Invitrogen, Cat. No. 253000-54 or 252000-56]
- **Culture medium (for culture with feeder cells):** DMEM:F-12 [Lonza, Cat. No. 12-719F] supplemented with 15 - 20% serum replacement [Invitrogen, Cat. No. 10828-028], 1 – 2% nonessential amino acids [Lonza, Cat. No. 13-114E], 1 – 4 mM L-glutamine [Lonza, Cat. No. 17-605C], 0.1 mM 2-Mercaptoethanol [Invitrogen, Cat. No. 21985-023] and 4 – 8 ng/ml fibroblast growth factor-2 [Milipore, Cat. No. GF003AF-MG]
- **Culture medium (for feeder-free culture):** mTesSR™ 1 medium [StemCell Technologies, Cat. No. 05850]
- Prewarm appropriate volume of culture medium to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection®

Note

Transfection results may vary due to different culture conditions prior and post Nucleofection®.

Important considerations - Single cell suspension:

1. We recommend transfecting the cells in single cell suspension. Nucleofection® of clumps leads to lower transfection efficiency and less reproducibility (for details see reference 1)
2. If single cell suspension passage is not established, please do some pre-experiments by testing Accutase (reference 2 and 3) and Trypsin (reference 4) for detachment. Cultivate the cells afterwards and analyze which method led to highest viability and lowest differentiation

3. The use of apoptosis inhibitors like ROCK inhibitor (reference 5) and neurotrophins (reference 6) have been reported to increase viability of hES cells. Depending on hESC culture conditions it might be advantageous to use ROCK inhibitor or neurotrophins to obtain higher viabilities

Cell culture recommendations

1.1 Replace media every day

1.2 Cells should be passaged 1 – 2 times per week with a sub-cultivation ratio of 1 : 3 to 1 : 10. You may use collagenase, dispase or another enzyme for this purpose

Detachment of stem cells

A. Harvest of stem cells cultured on feeder cells

– There are three possibilities to remove feeder cells from your stem cell culture prior to Nucleofection®:

– If your stem cells are usually cultured on feeder cells, passage them once to Matrigel™ coated plates to remove the feeder cells (described in reference 2). Then proceed to step 1.3 B

– Cultivate the cells on feeder cells until the day of the experiment. Detach the stem cells with Collagenase. Dissociate the clumps with Accutase into a single cell suspension

– Cultivate the cells on feeder cells until the day of the experiment. Detach all cells with Accutase. Incubate the cells on an uncoated cell culture flask for 1 hour in a humidified 37°C/5% CO_2 incubator. The feeder cells will attach and the stem cells will stay in suspension. Harvest the cells in suspension

B. Harvest of feeder-free stem cell cultures

– Prior to Nucleofection® detach the hES cells from the Matrigel™ plates by incubation with Accutase for 5 minutes at 37°C. Dissociate the cells into a single cell suspension by pipetting the suspension carefully up and down 4 – 6 times.

– Add medium to stop Accutase

2. Nucleofection®

For Nucleofection® Sample contents and recommended Nucleofector® Program, please refer to Table 3.

Note

Human stem cells are quite sensitive to environmental conditions. Therefore please ensure you proceed with the Nucleofection® steps as fast as possible.

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) coated with BD Matrigel™ or gelatine and feeder cells 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 Harvest the cells (please see 1.3)

2.8 Count an aliquot of the cells and determine cell density

2.9 Centrifuge the required number of cells (see table 3) at 115xg for 3 minutes at room temperature. Remove supernatant completely

2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector® Solution (see table 3)

2.11 Prepare mastermixes by dividing cell suspension according to number of substrates

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

2.13 Transfer mastermixes into the Nucleocuvette® Vessels

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.

2.14 Gently tap the Nucleocuvette® Vessels to make sure the sample covers the bottom of the cuvette

2.15 Place Nucleocuvette® Vessel with closed lid into the retainer of the 4D-Nucleofector® X Unit. Check for proper orientation of the Nucleocuvette® Vessel

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

2.17 After run completion, carefully remove the Nucleocuvette® Vessel from the retainer

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. When working with the 100 µl Nucleocuvette® use the supplied pipettes and avoid repeated aspiration of the sample

2.19 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

2.20 If post Nucleofection® Cell Culture is done in BD Matrigel™ (BD Biosciences) coated plates, centrifuge the culture plates loaded with cells at this point to guarantee proper attachment of the cells (70xg, 3 minutes, room temperature)

Notes

1. The Matrigel™ plates used for culturing of human stem cells should be fresh. Storage of these plates for more than 7 days leads to reduced attachment of the cells post Nucleofection®.
2. The plating density post Nucleofection® is a critical aspect for the viability of human stem cells. Our experience is that higher densities lead to better viability of the cells. Therefore we recommend plating human stem cells at densities from 4 x 10^5 to 6.5 x 10^5 cells per cm^2.
3. The indicated plating cell numbers and volumes produce optimal Nucleofection® Results in most cases. However, you may wish to test an extended range of cell numbers depending on your specific needs.

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable after only 4 – 8 hours. To validate optimal conditions for down regulation we recommend performing a time course experiment (please see 2.6)

3.2 As cells were plated at high density post Nucleofection®, a passage step 48 hours post Nucleofection® using collagenase or dispase might be necessary

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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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)	1 ml	200 µl
Cell number per Nucleofection® Sample	8×10^5 (Lower or higher cell numbers may influence transfection results)	$2 \times 10^5 - 4 \times 10^5$ (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		8×10^5	2×10^5
Substrate*	**pmaxGFP® Vector**	2 µg	0.4 µg – 0.8 µg
or	**plasmid DNA (in H_2O or TE)**	1 – 5 µg	0.4 – 1 µg
or	**siRNA**	30 – 300nM siRNA (3 – 30 pmol/sample)	30 – 300nM siRNA (0.6 – 6 pmol/sample)
P3 or P4 4D-Nucleofector® X Solution		100 µl	20 µl
Program		CA-137 or CB-150 or CD-118 or CE-118 or CM-113 or DC-100 or DN-100	CA-137 or CB-150 or CD-118 or CE-118 or CM-113 or DC-100 or DN-100

* 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*
24-well culture plate	500 µl	-
96-well culture plate	-	120 µl
Culture medium to be added to the sample post Nucleofection®	500 µl	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®	500 µl	80 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	80 µl

* Maximum cuvette volume 200 µl