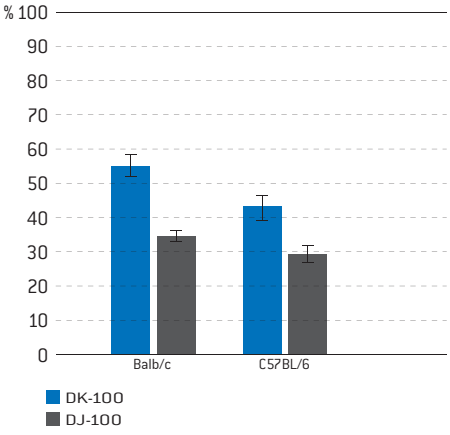
BioResearch

Lonza

Amaxa® 4D-Nucleofector® Protocol for Immature Mouse Dendritic Cells

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

Progenitor cells derived from bone marrow; non-adherent or loosely adherent cells of irregular shape with typical protrusions ("dendrites") of variable shape and length



Example for Nucleofection® of immature mouse dendritic cells

Average transfection efficiency of immature mouse dendritic cells 24 hours post Nucleofection®. 5 x 10^4 immature mouse dendritic cells were transfected with program DK-100 (high efficiency) or DJ-100 (high viability) and 0.4 µg of pmaxGFP® Vector in 20 µl Nucleocuvette® Strips. 24 hours post Nucleofection® cells were analyzed on a FACSCalibur™ [Becton Dickinson]. Cell viability was approximately 74% for DK-100 and 98% for DJ-100 after 24 hours (% PI-negative compared to untreated).

Product Description

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

Cat. No.	V4XP-4012	V4XP-4024	V4XP-4032
Transfection volume	100 µl	100 µl	20 µl
Size [reaction]	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)
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)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg	30 µg	-
pmaxGFP® Vector (0.2 µg/µl in 10 mM Tris pH 8.0)	-	-	45 µg
Single Nucleocuvette® (100 µl)	12	24	-
16-well Nucleocuvette® Strips (20 µl)	-	-	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 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.

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

Note

As contamination of the DNA with low molecular weight compounds may reduce cell viability, we strongly recommend performing an additional purification step after using a plasmid purification kit. Therefore precipitate the purified DNA twice using 20% PEG/2.5 M NaCl (final concentration).

- Cell culture plates of your choice
- **Culture medium I:** RPMI 1640 (Lonza; Cat. No. 12-167F) supplemented with 10% calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml Penicilin, 2 mM glutamine and 2000 U/ml GM-CSF (BD Pharmingen; Cat. No.:554586)
- **Culture medium II:** Culture medium I without GM-CSF
- Prewarm appropriate volume of culture medium I to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection®

Preparation of cells and cell culture

Preparation of bones

1.1 Carefully remove the femurs and tibia of freshly prepared mouse hind legs using forceps and scissors. Cut off both ends of the bones

Isolation of dendritic cells

1.2 Use a 27G needle mounted to a 5 ml syringe to gently flush the bone marrow into a petri dish. Use 2 – 3 ml culture medium II per bone

1.3 Count the viable cells

1.4 Spin down cells at 300 xg for 10 minutes at RT and discard the supernatant

1.5 Resuspend the cell pellet in culture medium I to reach a cell density of 1 x 10^6 cells/ml

1.6 Transfer the cells into 24-well plates (1 ml/well) and incubate them in a 37°C incubator with a 5% CO_2 atmosphere

Note

To yield a high number of functional dendritic cells it is necessary to maintain a sufficient level of GM-CSF. Fresh culture medium I (containing GM-CSF) should be added every second day.

1.7 On day 2, carefully remove 700 µl culture medium I from each well and replace it by fresh culture medium I, to maintain an appropriate GM-CSF concentration

1.8 On day 3, remove and discard the culture medium I completely. Wash the cells carefully with 500 µl per well using culture medium II to remove residual non adherent cells

1.9 Add 1 ml fresh culture medium I per well

1.10 Incubate the cells at 37°C in an incubator with 5% CO_2 atmosphere

1.11 On day 6, harvest the dendritic cells by collecting non adherent cells and loosely adherent cells. To release loosely adherent cells, wash off the cells thoroughly by pipetting with culture medium I. Discard the adherent cells

1.12 Continue with Nucleofection® as described in chapter 2

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 Count an aliquot of the cells and determine cell density

2.8 Centrifuge the required number of cells (see table 3) at 300xg for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector® Solution (see table 3)
2.10 Prepare mastermixes by dividing cell suspension according to number of substrates
2.11 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.12 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.

2.13 Gently tap the Nucleocuvette® Vessels to make sure the sample covers the bottom of the cuvette
2.14 Place Nucleocuvette® Vessel with closed lid into the retainer of the 4D-Nucleofector® X Unit. Check for proper orientation of the Nucleocuvette® Vessel
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® Vessel from the retainer
2.17 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
2.18 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

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

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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References

1. Inaba K. et al, (1992) J. Exp. Med. 176: 1693-1702

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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)	0.8 ml	190 µl
Cell number per Nucleofection® Sample	2.5 x 10^5 cells (Lower or higher cell numbers may influence transfection results)	5 x 10^4 cells (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		2.5 x 10^5	5 x 10^4
Substrate*	**pmaxGFP® Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	2 µg	0.4 – 0.8 µg
or	**siRNA**	30 – 300nM siRNA (3 – 30 pmol/sample)	30 – 300nM siRNA (0.6 – 6 pmol/sample)
P4 Primary Cell 4D-Nucleofector® X Solution		100 µl	20 µl
Program		DK-100 (for high efficiency) DJ-100 (for high viability)	DK-100 (for high efficiency) DJ-100 (for high viability)

* 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*
48-well culture plate	400 µl culture medium I	-
96-well culture plate	-	110 µl culture medium I
Culture medium to be added to the sample post Nucleofection®	400 µl culture medium I	80 µl culture medium I

* 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®	400 µl culture medium I	80 µl culture medium I
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	90 µl

* Maximum cuvette volume 200 µl