BioResearch

Lonza

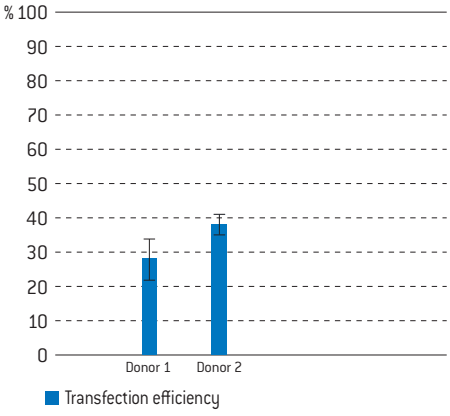
Amaxa™ 4D-Nucleofector™ Protocol for Human B Cells

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

Unstimulated CD19⁺ human B cells (small round lymphoblastoid cells) are a subpopulation of human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood treated with an anticoagulant or from leukocyte-rich buffy coat.

Note

This Protocol is not suitable for transfection of immortalized B cells (e.g. EBV immortalized LCLs). Please use the Cell Line Optimization 4D-Nucleofector™ Kit and the corresponding protocol instead.



Example for Nucleofection™ of primary human B cells

Transfection efficiency of freshly isolated human B cells 24 hours post Nucleofection™. 1 x 10^6 cells were transfected with program EO-117 using 0.4 µg pmaxGFP™ Vector in 20 µl Nucleovette™ Strips. Cells were analyzed 24 hours post Nucleofection™ using a FACSCalibur™ (Becton Dickinson). Cell viability (% PI negative B cells) is approximately 70% after 24 hours.

Product Description

Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032
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 (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 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, stock solution 1 µg/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 isolation:** PBS with 0.5% BSA (PBS/BSA); Ficoll-Paque™ Plus (GE Healthcare; Cat. No. 17-1440-03)
- **Culture medium:** RPMI 1640 (Lonza; Cat. No. 12-167F) supplemented with 10% autologous serum or 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, and 2 mM UltraGlutamine I (Lonza; Cat. No. 17-605E/U1)
- 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 be donor-dependent. This protocol is designed for unstimulated primary human B cells. No cultivation is required prior to Nucleofection™. It is preferable to use freshly isolated PBMC or fresh B cell enriched preparations (e.g. by magnetic separation) for Nucleofection™. For preparation, do not perform protocols using hypo-osmolar buffers. This may lead to high cell mortality after Nucleofection™.

Blood samples

1.1 Fresh human blood treated with an anticoagulant (e.g. heparin, citrate, ACD-A) or alternatively, leukocyte-enriched buffy coat not older than 8 hours. The samples should be diluted with 2–4 volumes of PBS containing 0.5% BSA (PBS/BSA)

Preparation of PBMC

1.2 Pipet 15 ml Ficoll-Paque™ Plus in a 50 ml conical tube
1.3 Overlay Ficoll- Paque™ Plus with 35 ml blood sample and centrifuge at 750xg for 20 minutes at 20°C in a swinging-bucket rotor without brake
1.4 Remove the upper layer leaving the mononuclear cell layer undisturbed at the interphase. Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube
1.5 Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully
1.6 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully
1.7 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 minutes at 4°C. Remove the supernatant carefully
1.8 Resuspend cell pellet in 5 ml PBS/BSA and count the cells
1.9 For freshly isloated cells no cultivation is required prior to Nucleofection™. For crypopreserved cells we recommend incubating the thawed cells for 1–2 hours at 37°C in culture medium before Nucleofection™

Note
Purified PBMC may be stored at 4°C overnight in PBS/BSA, but this can cause a significant loss of transfection efficiency

2. Nucleofection™

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 4)
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 90xg 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:

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

References

1. Shi GX et al, J Immunol. 2002;169(5):2507-15.
2. Tolnay M et al, J Immunol. 2002;169(11):6236-43.

Lonza Cologne GmbH
50829 Cologne, Germany

D4XP-3001_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)	2 ml	230 µl
Cell number per Nucleofection™ Sample	1–5 x 10^6 (Lower or higher cell numbers may influence transfection results)	1 x 10^6 (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		1–5 x 10^6	1 x 10^6
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	1–5 µg	0.2–1 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		EO-117	EO-117

* 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*
6-well culture plate	1.5 ml	-
96-well culture plate	-	150 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)	50 µl

* Maximum cuvette volume 200 µl