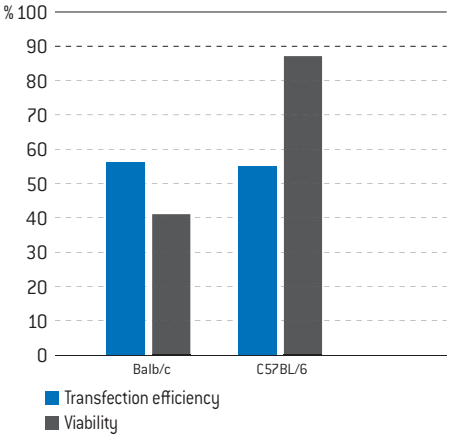
BioResearch

Lonza

Amaxa™ 4D-Nucleofector™ Protocol for stimulated Mouse B Cells

For 4D-Nucleofector™ X Unit

Cells derived from mouse spleen (mice strain BALB/c and C57BL/6). Small round cells, suspension



Note

This Kit is NOT suited for unstimulated B cells.

Example for Nucleofection™ of stimulated mouse B cells

Average transfection efficiency and viability of mouse B cells 6 x 10^5 of stimulated cells were transfected with program DI-100 using 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 analyzed by using the CellTiter Glo® assay [Promega] 24 hours post Nucleofection™.

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 (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 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.

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 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
- **Culture medium I:** RPMI1640 (Lonza; Cat. No. 12-167F) supplemented with 10 % FCS, 2 mM UltraGlutamine I (Lonza, Cat. No. 17-605E/U1), 50 µM ß-mercaptoethanol, 1 % ITS (Sigma) and LPS (50 µg/ml) if desired
- **Culture medium II:** RPMI 1640 (Lonza; Cat. No. 12-167F) supplemented with 10 % FCS, 2 mM UltraGlutamine I (Lonza, Cat. no. 17-605E/U1), 50µM ß-Mercaptoethanol and 50 µg/ml LPS (Sigma)
- **For isolation:** B Cell Isolation Kit, mouse (Milteny; Cat. No. 130-090-862; negative selection); PBS/BSA for B cell isolation
- 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 strain dependent.

Preparation and stimulation of mouse B cells

This section provides an outline for the isolation, cell culture and stimulation of primary mouse B cells. For further details we recommend the established preparation and cultivation protocols described in literature (e.g. Lymphocytes, A practical approach, Rowland-Jones S. L. and McMichael A.J., Oxford University Press)

1.1 Isolate mouse lymphocytes from spleens of 8–11 weeks old mice in cold PBS/BSA
1.2 Avoid the erythrocyte lysis step
1.3 Purify and enrich the B cells by using the B Cell Isolation Kit for mouse leukocytes
1.4 Do not overload the separation separation columns. As a rule of thumb use only 1 columns to separate the B cells isolated from 2 spleens
1.5 After isolation of pure B cells (around 95 %) incubate the cells for 24 hours in culture medium II. Use a culture flask for suspension cells (1 x 10^8 cells per T75 flask /20 ml) and cultivate the cells in a humidified 37 °C/5 % CO_2 incubator
1.6 After incubation with LPS the B cells should have formed visible clusters, showing the blast formation has been induced successfully
1.7 Take an aliquot of the cell suspension, count the cells and determine cell density

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 media 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. If this is not the case, the incubation time can be prolonged up to 48 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. Rowland-Jones S.L. and McMichae A.J., Lymphocytes, A practical approach, Oxford University Press; ISBN-10:0-19-963816-0, ISBN 13: 978-0-19-962816-1; Publication date: December 16, 1999

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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)	1.5 ml	190 µl
Cell number per Nucleofection™ Sample	3 x 10^6 (Lower or higher cell numbers may influence transfection results)	6 x 10^5 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		3 x 10^6	6 x 10^5
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	2 µg	0.4–1 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		DI-100	DI-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*
12-well culture plate	1 ml culture medium I	-
96-well culture plate	-	110 µl culture medium I
Culture medium to be added to the sample post Nucleofection™	500 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™	500 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