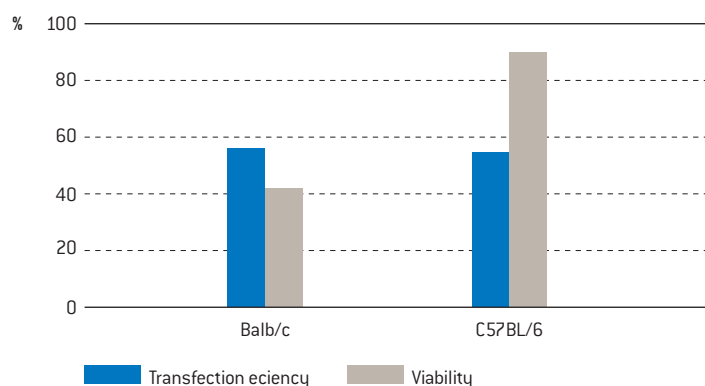


Amaxa™ 96-well Shuttle™ Protocol for Stimulated Mouse B Cells

Cell Description

This protocol is designed for stimulated mouse B cells, derived from mouse spleen (mice strain BALB/c and C57BL/6). Small round cells, suspension. This protocol is not suited for unstimulated B cells.

Example for Nucleofection™ of Stimulated Mouse B Cells



Average transfection efficiency and viability of mouse B cells 6×10^5 of stimulated cells were transfected with program 96-DI-100 using 0.4 μg of pmaxGFP™ Vector. 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 Kits

P4 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-4096
Size (reactions)	1×96
P4 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0)	50 μg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SP-4960
Size (reactions)	10×96
P4 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0)	50 μg
Nucleocuvette™ Plate(s)	10

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 expiry 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

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. 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

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plates
- Supplied pmaxGFP Vector, stock solution 1 µg/µl

Note

Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8*
- Nucleocuvette™ compatible tips: 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 Instrument, 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
- 96-well culture plate or culture system 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 β-mercaptoethanol, 1 % ITS (Sigma) and 50 µg/ml LPS (Sigma, Cat. No. L-4005) if desired. When using other LPS, please titrate optimal amount for stimulation.
- 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 β-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 I to 37°C (190 µl per sample)
- Appropriate number of cells (6×10⁵ cells per sample); lower or higher cell numbers may influence transfection results

1. Pre Nucleofection™

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 eukocytes
- 1.4 Do not overload the separation separation columns. As a rule of thumb use only 1 columns to separate 1.4 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 (1×10⁸ cells per T75 flask /20 ml) and cultivate the cells in a humidified 37°C / 5 % CO₂ 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

Note

Transfection results may be strain dependent.

2. Nucleofection™

One Nucleofection™ Sample Contains

- 6×10^5 cells
- 0.4–1 µg plasmid DNA (in 1–2 µl H₂O or TE) or 0.4 µg pmaxGFP™ Vector or 30–1000 nM siRNA (0.6–20 pmol/sample)
- 20 µl P4 Primary Cell 96-well Nucleofector™ Solution

- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
- 2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual “Nucleofector™ 96-well Shuttle™ System”)
- 2.3 Select the appropriate Nucleofector™ Program **96-DI-100**
- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium II, e.g. 175 µl for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
- 2.5 Pre-warm an aliquot of culture media to 37°C (110 µl per sample* see comments at the end of this chapter)
- 2.6 Prepare 0.4–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30–1000 nM siRNA (0.6–20 pmol/sample)
- 2.7 Centrifuge the required number of cells (6×10^5 cells per sample) at 90xg for 10 minutes at room temperature
- 2.8 Discard the supernatant completely and make sure that no residual medium covers the cell pellet
- 2.9 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well 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.

- 2.10 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
- 2.11 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well “A1” must be in upper left position
- 2.12 Start 96-well Nucleofection™ Process by either pressing “Upload and start” in the 96-well Shuttle™ Software or pressing “Upload” in the 96-well Shuttle™ Software and then the “Start” button at the 96-well Shuttle™ (for both options please refer to the respective Manual)
- 2.13 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer.
- 2.14 Resuspend cells with desired volume of pre-warmed culture medium I (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells with 80 µl of pre-warmed medium*
- 2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 90 µl of resuspended cells to 110 µl pre-warmed culture medium I prepared in 96-well culture plates*

* Note

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ results in most cases, however, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

- 3.1 Incubate the cells in a humidified 37°C/5% CO₂ 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

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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Reference

1. Rowland-Jones S.L. and McMichael A.J., Lymphocytes, A practical approach, Oxford University Press; ISBN-10:0-19-963816-0, ISBN-1. 13: 978-0-19-962816-1; Publication date: December 16, 1999

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