BioResearch

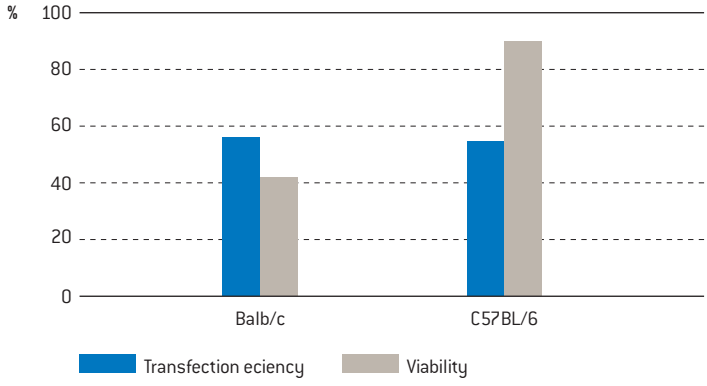
Lonza

Amaxa™ HT Nucleofector™ 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 DI-100-AA 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 HT Nucleofector™ kits

Cat. No.	V5SP-4002
Size (reactions)	2×384
P4 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-4010
Size (reactions)	10×384
P4 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well 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

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, 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

- HT Nucleofector™ system
- Supplemented Nucleofector™ solution at room temperature
- Supplied 384-well 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*
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 ß-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 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
- Pre-warm appropriate volume of culture medium I to 37°C (186 µl per sample)
- Appropriate number of cells (6×10^5 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 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 (1×10^8 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

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 H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–1000 nM siRNA (0.6–20 pmol/sample)
- 20 µl P4 primary cell HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution

2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to the HT Nucleofector™ manuals)

2.3 Select the appropriate HT Nucleofector™ program **DI-100-AA**

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium II, e.g. 146 µl for one well of a 96-well plate 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 (40 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 HT 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 384-well Nucleocuvette™ plates

B: Multiple substrates (e.g. library transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without airbubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.11 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position

2.12 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)

2.13 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

2.14 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 54 µl of resuspended cells to 146 µl pre-warmed media

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 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 % 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

Up-to-date List of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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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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