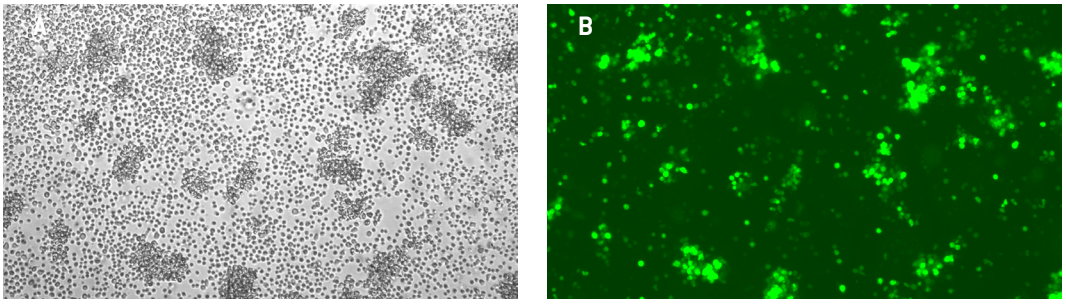
Amaxa® Mouse B Cell Nucleofector® Kit

For Stimulated Mouse B Cells

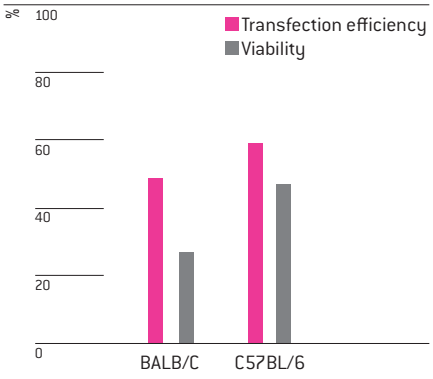
Cells derived from mouse spleen (mice strain BALB/c and C57BL/6); small round cells; suspension

Note **This Kit is NOT suited for unstimulated B cells.**

Example for Nucleofection® of stimulated mouse B cells



Mouse B cells were transfected using the Mouse B Cell Nucleofector® Kit . Cells were stimulated with LPS for 24 hours to induce blast formation. Stimulated cells were transfected with program Z-001 and 2 µg of pmaxGFP® Vector. 24 hours post transfection maxGFP® Reporter Protein expression was analyzed by fluorescence microscopy.



Average transfection efficiency and viability of mouse B cells 3 x 10^6 cells were transfected with program Z-001 using 2 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed on a Becton Dickinson FACSCalibur™. Cell viability was analyzed by using the CellTiter GLO® -Assay [Promega] 24 hours post Nucleofection®.

Product Description

Cat. No.	**VPA-1010**
Size (Reactions)	25
Mouse B Cell Nucleofector® Solution	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
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.

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. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- 12-well culture dish or culture system of your choice
- **Culture medium I**: RPMI1640 [Lonza; Cat. No. 12-167F] supplemented with 10% FCS, 2mM 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
- Prewarm appropriate volume of culture medium I to 37°C [1 ml per sample]
- Appropriate number of cells [3 x 10^6 cells per sample]; lower or higher cell numbers may influence transfection results

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 and cell culture 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. Avoid the erythrocyte lysis step

1.2 Purify and enrich the B cells by using the Miltenyi B Cell Isolation Kit. Do not overload the Miltenyi separation separation columns. As a rule of thumb use only 1 column to separate the B cells isolated from 2 spleens

1.3 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. After incubation with LPS the B cells should have formed visible clusters, showing the blast formation has been induced successfully

2. Nucleofection®

One Nucleofection® Sample contains

3 x 10^6 cells
2 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Mouse B Cell Nucleofector® Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution

2.2 Prepare 12-well plates by filling appropriate number of wells with 1ml of supplemented culture medium I and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator

2.3 Centrifuge the required number of cells (3 x 10^6 cells per sample) **at 90xg for 10 minutes** at room temperature. Make sure that no residual medium covers the cell pellet

2.4 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample. Avoid storing the cell suspension longer than 15 minutes in Nucleofector® Solution, as this may reduce cell viability

2.5 Combine 100 µl of cell suspension with **2 µg DNA** or pmaxGFP® Vector or **30 – 300 nM siRNA** (3 – 30 pmol/sample) or other substrates

2.6 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap

2.7 Select the appropriate Nucleofector® Program **Z-001** (Z-01 for Nucleofector® I Device)

2.8 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program

2.9 Take the cuvette out of the holder once the program is finished

2.10 Add ~500 µl of the pre-equilibrated culture medium I to the cuvette and gently transfer the sample immediately into the 12-well plate (final volume 1.5 ml media per well/sample). Use the supplied pipettes and avoid repeated aspiration of the sample

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. 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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