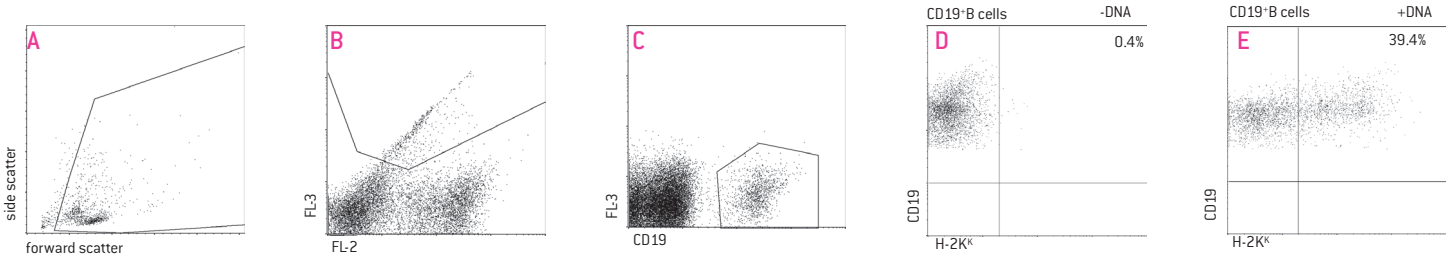
Amaxa® Human B Cell Nucleofector® Kit

For human B Cells

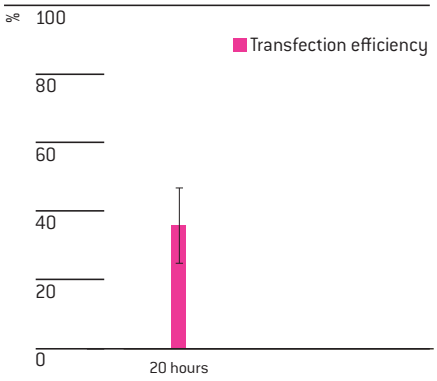
Unstimulated CD19+ human B cells (small round lymphoblastoid cells) are a subpopulation of human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood treated with an anticoagulant or from leukocyte-rich buffy coat.

Note **This Kit is not suitable for transfection of immortalized B cells (e.g. EBV immortalized LCLs). Please use the Cell Line Optimization Nucleofector® Kit instead.**

Example for Nucleofection® of CD19+ B cells with H-2Kk cDNA



Fresh peripheral blood mononuclear cells (PBMC) were transfected by Nucleofection® using the Human B Cell Nucleofector® Kit and a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kk. 16 hours post Nucleofection®, the cells were stained with an APC-coupled antibody directed against CD19, a PE-coupled antibody directed against H-2Kk and were analyzed by flow cytometry. Lymphocytes were gated according to forward/side scatter (A). Dead cells and CD19 negative cells were excluded by staining with propidium iodide and gating (B, C). H-2Kk expression is shown post Nucleofection® without (D) and with plasmid DNA (E).



Transfection efficiencies of fresh, un-stimulated human B cells 20 hours post Nucleofection®. Cells were transfected by Nucleofection® with program U-015 and 5 µg of a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kk.

Product Description

Cat. No.	**VPA-1001**
Size (reactions)	25
Human 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
- 12-well culture dish or culture system of your choice
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- **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. BE17-605E/U1]
- **For isolation:** Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]; PBS containing 0.5% [w/v] BSA (PBS/BSA)
- Prewarm appropriate volume of culture media to 37°C (2 ml per sample)
- Appropriate number of cells (1 – 5 x 10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Notes

- **This protocol is designed for fresh unstimulated primary human B cells from whole PBMCs.**
- **Transfection results may be donor-dependent.**
- **For preparation, do not perform protocols using hypo-osmolar buffers. This may lead to high cell mortality after Nucleofection®.**
- **For freshly isolated cells no cultivation is required prior to Nucleofection®.**
- **For cryopreserved cells we recommend incubating the thawed cells for 1 – 2 hours at 37°C in culture medium before 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 cell

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®

One Nucleofection® Sample contains

1 – 5 x 10^6 cells
1 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Human 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 1.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Count the cells and determine cell density
2.4 Centrifuge the required numbers of cells (**1 – 5 x 10^6 cells per sample**) at **200xg for 10 minutes** at room temperature. Discard supernatant completely so that no residual PBS/BSA covers the cell pellet
2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample. Avoid storing the cell suspension longer than 20 minutes in Human B Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
2.6 Combine 100 µl of cell suspension with **1 – 5 µg DNA** or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)
2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.8 Select the appropriate Nucleofector® Program **U-015** (**U-15** for Nucleofector® I Device)
2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.10 Take the cuvette out of the holder once the program is finished
2.11 Add ~500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample into the 12-well plate (final volume of 2 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 is often detectable after only 4 – 8 hours

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.amaxa.com/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

Lonza Cologne AG
50829 Cologne, Germany

DPA-1001_2009-08