

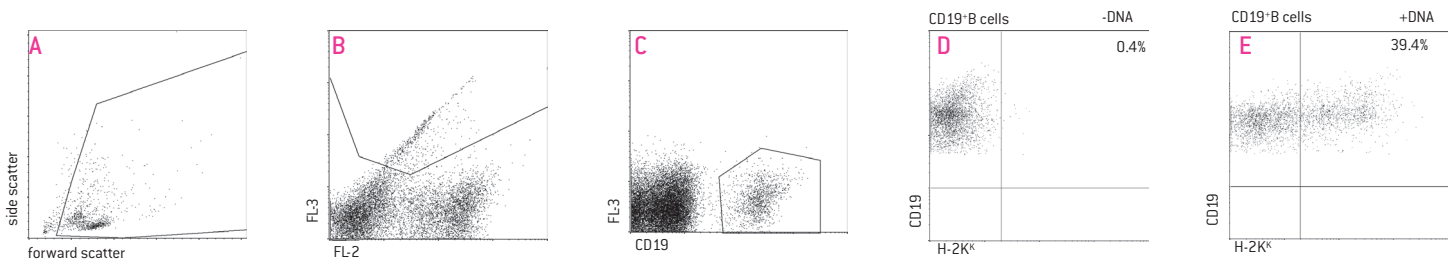
## Amaxa<sup>®</sup> Human B Cell Nucleofector<sup>®</sup> Kit

### For human B Cells

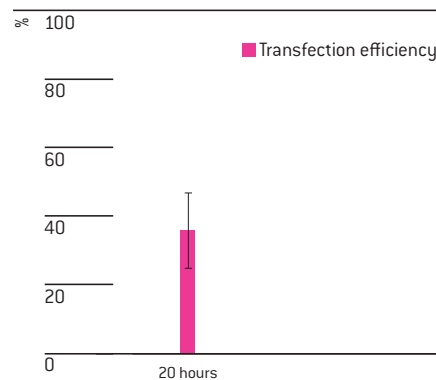
Unstimulated CD19<sup>+</sup> human B cells (small round lymphoblastoid cells) are a subpopulation of human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood treated with an anticoagulant or from leukocyte-rich buffy coat.

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

### Example for Nucleofection<sup>®</sup> of CD19<sup>+</sup> B cells with H-2K<sup>k</sup> cDNA



Fresh peripheral blood mononuclear cells (PBMC) were transfected by Nucleofection<sup>®</sup> using the Human B Cell Nucleofector<sup>®</sup> Kit and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K<sup>k</sup>. 16 hours post Nucleofection<sup>®</sup>, the cells were stained with an APC-coupled antibody directed against CD19, a PE-coupled antibody directed against H-2K<sup>k</sup> 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-2K<sup>k</sup> expression is shown post Nucleofection<sup>®</sup> without (D) and with plasmid DNA (E).



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

### Product Description

Cat. No.	VPA-1001
Size (reactions)	25
Human B Cell Nucleofector <sup>®</sup> Solution	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP <sup>®</sup> 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 <sup>®</sup> Solution, Supplement and pmaxGFP <sup>®</sup> Vector at 4°C. For long-term storage, pmaxGFP <sup>®</sup> Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector <sup>®</sup> Supplement is added to the Nucleofector <sup>®</sup> 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<sup>6</sup> 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

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1 – 5 x 10<sup>6</sup> cells

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1 – 5 µg plasmid DNA (in 1 – 5 µl H<sub>2</sub>O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)

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100 µl Human B Cell Nucleofector® Solution

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- 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<sub>2</sub> incubator
- 2.3 Count the cells and determine cell density
- 2.4 Centrifuge the required numbers of cells (1 – 5 x 10<sup>6</sup> 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<sub>2</sub> 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](http://www.amaxa.com/citations)

**For more technical assistance, contact our Scientific Support Team:**

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