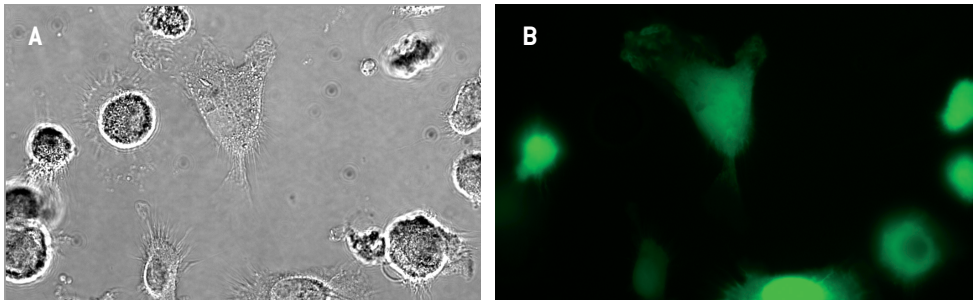
Amaxa® Human Macrophage Nucleofector® Kit

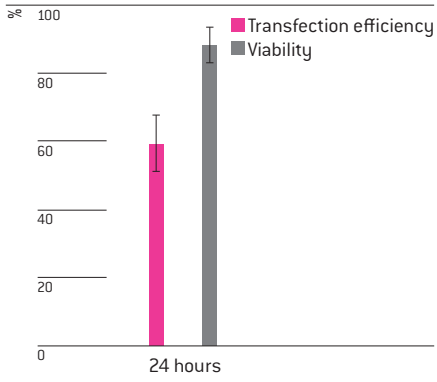
For Human Macrophages

Suitable for Macrophages differentiated from human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood samples treated with an anticoagulant or from leukocyte-enriched buffy coat. Macrophages are large granular cells which adhere to plastic surfaces.

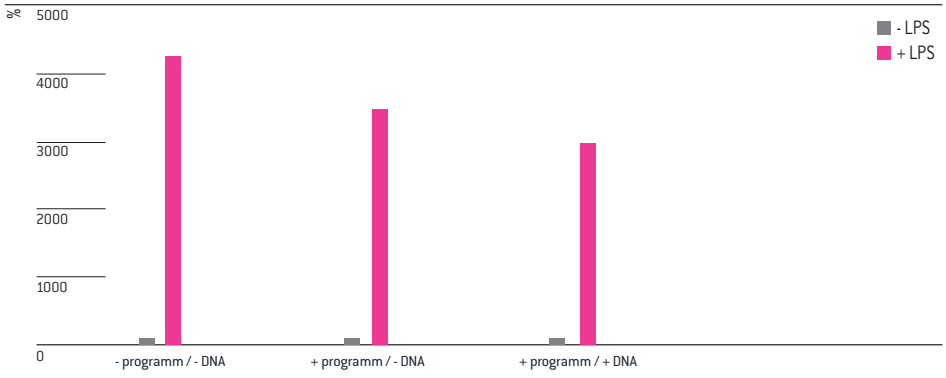
Example for Nucleofection® of Human Macrophages



Example for the transfection of human macrophages with pmaxGFP® Vector. Primary human macrophages were transfected by Nucleofection® using the Human Macrophage Nucleofector® Kit with pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed by light (A) and fluorescence microscopy (B) at 40x magnification. Transfected macrophages reveal cytoplasmic extrusions important for phagocytic function of macrophages.



Transfection efficiency of human macrophages 24 hours post Nucleofection®. Cells were transfected by Nucleofection® with program Y-010 and 2 µg of pmaxGFP® Vector. Cell viability (%7-AAD negative) is 88%.



Human Macrophages can be stimulated after Nucleofection®. Primary human macrophages were transfected with 2 µg pmaxGFP® Vector. 24 hours post Nucleofection® the culture medium was changed and cells were stimulated with LPS. 48 hours post Nucleofection® TNF α secretion was determined by ELISA. In all three samples stimulated with LPS a comparable TNF α production could be detected. In non-stimulated samples no TNF α was detected.

Product Description

Cat. No.	**VPA-1008**
Size (Reactions)	25
Human Macrophage 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 dish of your choice
- Culture dish for differentiation: Poly-D-Lysine coated flasks [Becton Dickinson; Cat.No. 354537]
- **For detaching cells:** 0.5 mg/ml trypsin, 0.2 mg/ml EDTA in PBS for 25 minutes
- **Differentiation medium:** RPMI 1640 [Lonza; Cat.No. 12-167F] supplemented with 10% fetal calf serum (FCS), 100µg/ml streptomycin, 100 U/ml penicillin, and 2 mM glutamine, 1% Na-pyruvate, 1% NEAA (Non-Essential Amino Acids) and 50 ng/ml rHu M-CSF
- **Culture medium:** Macrophage-SFM [Invitrogen; Cat.No. 12065-074] supplemented with 10% FCS and 2 mM glutamine
- **For isolation:** PBS with 0.5% BSA (PBS/BSA); Ficoll-Paque™ Plus [GE Healthcare; Cat.No. 17-1440-03]
- Prewarm appropriate volume of culture media to 37°C (2 ml per sample)
- Appropriate number of cells (5 – 7 x 10^5 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre 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/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 cells

Differentiation of macrophages

1.9 Plate 5 x 10^7 PBMC per 75 cm^2 Poly-D-Lysine coated flask
1.10 Enrich monocyte population by plastic adherence for 1 – 2 hours in an incubator at 37°C in a 5% CO_2 atmosphere
1.11 Discard supernatant with non-adherent cells and wash adherent monocytes 1 x with 15 ml prewarmed PBS per flask. Aspirate washing solution.
1.12 Add 10 ml differentiation media to each flask and differentiate monocytes for 7 days into macrophages
1.13 Replace media 2 – 3x during the differentiation period

Trypsinization

1.14 Wash adherent macrophages once with PBS
1.15 Add Trypsin/EDTA solution (0.5 mg/ml trypsin and 0.2 mg/ml EDTA in PBS) to cover the cell monolayer (~3 ml per 75 cm^2 flask), and gently swirl the dish/flask to ensure an even distribution of the solution. Incubate the flask for 25 – 30 minutes at RT
1.16 Stop trypsinization by addition of supplemented RPMI without rHu M-CSF

2. Nucleofection®

One Nucleofection® Sample contains

5 – 7 x 10^5 cells
2 – 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 Macrophage 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 (**5 – 7 x 10^5 cells per sample**) at **200xg for 10 minutes** at room temperature. Discard supernatant completely so that no residual media 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 Macrophage Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
2.6 Combine 100 µl of cell suspension with **1 – 5 µg** DNA , 2 µg pmaxGFP® Vector or **30 – 300 nM siRNA** (3 – 30 pmol sample) or other substrates
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 **Y-010** (**Y-10** 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 supplemented culture medium to the cuvette and gently transfer the sample into the 12-well plate (final volume of 2 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

3.1 Gene expression should be analyzed at different time points

Note **For flow cytometry analysis we recommend harvesting cells by trypsin treatment. Do not use cell scraper.**

3.2 For activation experiments replace medium 24 hours post Nucleofection® and add 1 µg/ml LPS to the fresh culture medium

3.3 Activation markers (e.g. TNF α) can be analyzed 24 hours after activation

Note **It is known that macrophages respond to intracellular foreign DNA by activation (see additional information). Nucleofection® of plasmid DNA causes transient activation of macrophages which is indicated by transient TNF α secretion for up to 24 hours after Nucleofection®. It is possible to reactivate macrophages after medium change 24 hours post Nucleofection®.**

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:

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

References:

1. Hacker G. et al. (2002). Immunology 105(3):245-251

Lonza Cologne AG
50829 Cologne, Germany

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