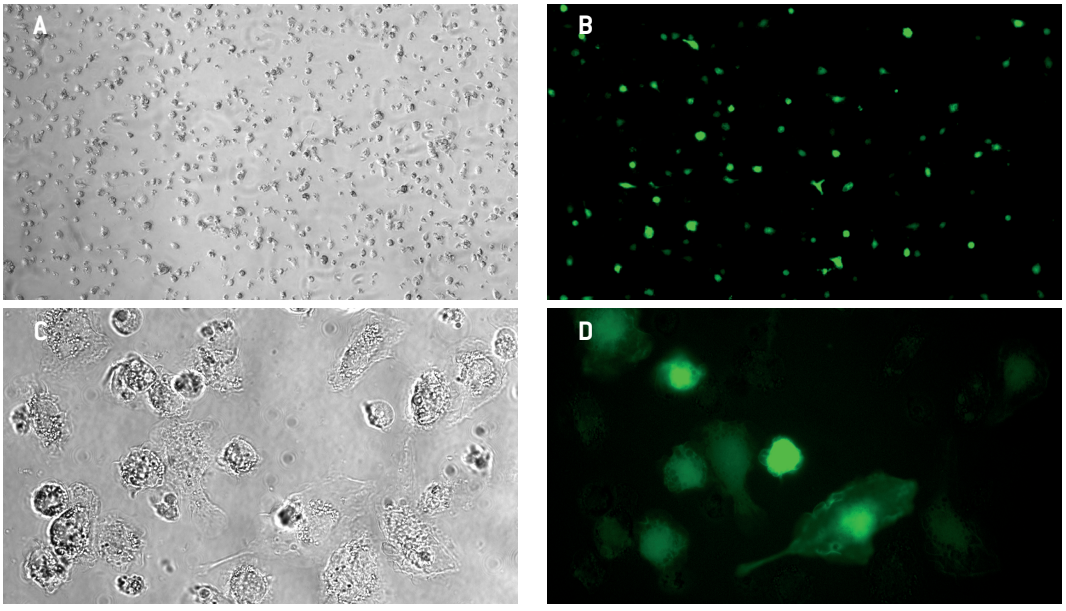
Amaxa® Mouse Macrophage Nucleofector® Kit

For mouse bone marrow derived macrophages

Mouse macrophages differentiated from freshly isolated bone marrow of C57BL/6 & BALB/c mice. Mouse macrophages are large granular cells with filament extrusions which adhere to plastic surfaces

Example for Nucleofection® of mouse macrophages



Example for the transfection of mouse macrophages with pmaxGFP® Vector. Primary mouse macrophages (isolated from C57BL/6 mice) transfected using the Mouse Macrophage Nucleofector® Kit with a plasmid encoding maxGFP® Protein. 24 hours post Nucleofection® cells were analyzed by light (A, C) and fluorescence microscopy (B, D). A and B show cells at 10x magnification. At 40x magnification (C, D) transfected macrophages reveal cytoplasmic extrusions important for phagocytic function of macrophages.

Product Description

Cat. No.	**VPA-1009**
Size (Reactions)	25
Mouse 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 moints 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 dish for differentiation:** Poly-D-Lysine coated flasks [Becton Dickinson; Cat. No. 354537]
- **Culture medium:** RPMI 1640 [Lonza; Cat.No. 12-167F] or alternatively DMEM [Lonza; cat. No. 12-604F] supplemented with 20% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, 2 mM UltraGlutamine I [Lonza; Cat. No. BE17-605E/U1]
- **Differentiation medium:** Culture medium supplemented with 40 ng/ml rHu M-CSF (working range: 10 – 50 ng/ml) [Promokine; Cat. No. C-60442]
- **For detaching cells:** 0.5 mg/ml trypsin, 0.2 mg/ml EDTA in PBS for 25 minutes
- Prewarm appropriate volume of culture medium to 37°C (2.0 ml per sample)
- Appropriate number of cells (1 x 10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Note **Transfection results may be donor – dependent.**

Cell sample

1.1 Aseptically remove femura from 7 – 11-week old mice
1.2 Wash femura thoroughly in petri dish with PBS to avoid possible contamination with cells outside the bone
1.3 Place bone in a fresh petri dish (cut off muscles and tibia)
1.4 Cut off one end of the bone
1.5 Use a 27G needle attached to a syringe containing 10 ml culture medium
1.6 Flush the bone marrow cells carefully from the bone directly into a 15 ml conical tube (ca. 2 ml medium per femur)
1.7 Resuspend the cells by gentle pipetting
1.8 Pass through a 70 µm filter into a 50 ml conical tube, wash with medium
1.9 Spin down at 300xg for 10 minutes at 4°C. Remove the supernatant carefully
1.10 Resuspend cell pellet in 10 ml medium (for 10 femura)
1.11 Adjust to a concentration of 6 x 10^6 bone marrow cells/ml (typically 1 – 2 x 10^7 cells are obtained per femur)

Differentiation

1.12 Plate 6 x 10^6 bone marrow cells per 75 cm^2 Poly-D-Lysin coated flask (minimun 2 x 10^6 bone marrow cells)
1.13 Add 10 ml differentiation medium and incubate at 37°C in 5% CO_2 atmosphere
1.14 Feed every 2 – 3 days by adding fresh medium to the culture

Trypsinization (for flow cytometry analysis)

1.15 Wash adherent macrophages once with PBS
1.16 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
1.17 Stop trypsinization by addition of supplemented culture medium

2. Nucleofection®

One Nucleofection® sample contains

1 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 Mouse 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 culture medium 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 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 15 minutes in Mouse 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** or **appropriate amount of siRNA** 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-001** (**Y-01** 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 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 Incubate the cells in humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable after only 4 – 8 hours. If this is not the case, the incubation period may be prolonged to 24 hours

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

3.2 For activation experiments replace medium 6 hours post Nucleofection® and add 1 µg/ml LPS to the fresh medium

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

Note **It is known that macrophages respond to intracellular foreign DNA by activation [Stacey KJ et al., 1996, J Immunol.; 157(5):2116-22]. Nucleofection® of plasmid DNA causes activation which is indicated by transient TNFα secretion for up to 6 hours after Nucleofection®. It is possible to reactivate macrophages after medium change 6 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. Stacey, KJ et al. (1996). J Immunol 157(5):2116-22

Lonza Cologne AG
50829 Cologne, Germany

DPA-1010_2009-09