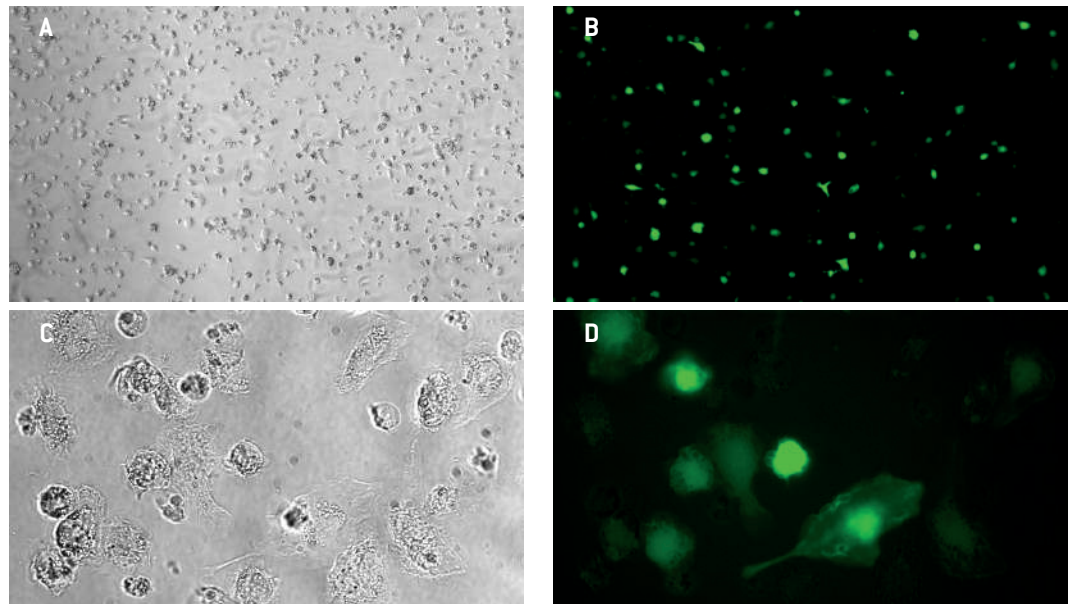


Amaxa[®] Mouse Macrophage Nucleofector[®] Kit

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 months at 4°C.

Required Material

Note Please make sure that the entire supplement is added to the Nucleofactor® Solution. The ratio of Nucleofactor® Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofactor® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.

- Nucleofactor® Device
- Supplemented Nucleofactor® 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 10⁶ 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 10⁶ bone marrow cells/ml (typically 1 – 2 x 10⁷ cells are obtained per femur)

Differentiation

- 1.12 Plate 6×10^6 bone marrow cells per 75 cm^2 Poly-D-Lysin coated flask (minimum 2×10^6 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 $37^\circ\text{C}/5\% \text{CO}_2$ 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% CO₂ 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:

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References:

1. Stacey, KJ et al. (1996). J Immunol 157(5):2116-22

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