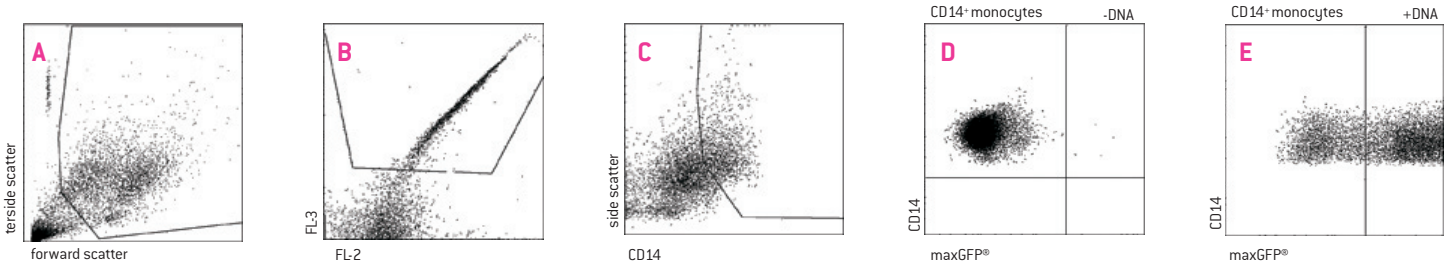
Amaxa® Human Monocyte Nucleofector® Kit

For Human Monocytes

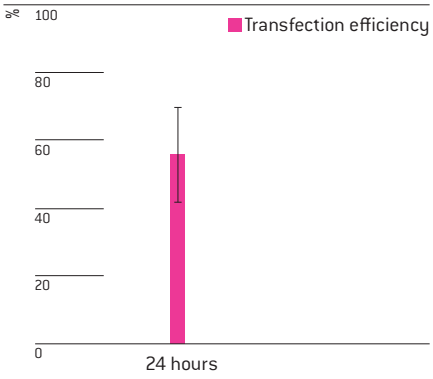
Human monocytes freshly isolated from blood samples or buffy coats.

Note **This protocol only gives an outline for the handling and the Nucleofection® of human monocytes. Please refer to more detailed preparation and cultivation protocols before starting the experiments**

Example for Nucleofection® of CD14+ monocytes with pmaxGFP® Vector



Human monocytes freshly enriched from peripheral blood mononuclear cells (PBMC) were transfected with 1 µg pmaxGFP® Vector using the Human Monocyte Nucleofector® Kit. 22 hours post Nucleofection®, the cells were stained with an antibody directed against CD14 and analyzed by flow cytometry. PBMC were gated according to forward/side scatter (A). Dead cells were excluded by propidium iodide staining and gating (B). CD14 positive cells were identified by staining with CD14-antibody (C). Expression of maxGFP® Reporter Protein is shown after Nucleofection® without (D) and with plasmid DNA (E).



Transfection efficiency of primary human monocytes 24 hours post Nucleofection®. Cells were transfected using Nucleofector® Program Y-001 and 1 µg of pmaxGFP® Vector. Cell viability is usually around 70% (%PI negative monocytes) after 24 hours.

Product Description

Cat. No.	**VPA-1007**
Size (reactions)	25
Human Monocyte 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
- 24-well culture dish or culture system of your choice
- **Culture medium:** Clonetics® Lymphocyte Growth Media-3 LGM-3® for serum-free culture [Lonza, Cat. No. CC-3211] or BioWhittaker® IMDM media for addition of 10% serum [Lonza, Cat.No. BE12-722F]
- **For isolation:** Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]; PBS containing 0.5% [w/v] BSA [PBS/BSA]
- **For enrichment:** We recommend using the Rosette Sep™ Isolation Kit for human monocytes [Stem Cell Technologies, 1.1 Cat.No. 15028]. Please note: It is also possible to use the Monocyte Isolation Kit II [Miltenyi Biotec, Cat.No. 130-091-153] to purify the monocytes Appropriate number of cells [3 x 10^6 – 1 x 10^7 cells per sample]
- Prewarm appropriate volume of culture medium to 37°C [1.5 ml per sample]
- Appropriate number of cells [3 x 10^6 – 1 x 10^7 cells per sample; lower or higher cell numbers may influence transfection results]

1. Pre Nucleofection®

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

Enrichment of monocytes from buffy coats

1.1 Centrifuge one buffy coat [~60 ml] in two 50 ml tubes at 1200xg for 20 minutes at RT [brake off]
1.2 Remove most of the serum in the upper layer
1.3 Transfer the interphases [PBMC] together with traces of serum and erythrocytes [~15 ml] into two fresh 50 ml tubes
1.4 Add 1000 µl cold Rosette-Cocktail [4°C] to each PBMC-Mix and vortex
1.5 Incubate 20 minutes at RT
1.6 Dilute 15 ml of the PBMC-Mix with 15 ml PBS/BSA and mix gently
1.7 Prepare two 50 ml tubes with 15 ml Ficoll-Paque™ and place 30 ml of the diluted PBMC-Mix as a layer on top of the Ficoll-Paque™
1.8 Centrifuge at 1200xg for 20 minutes at RT with brake off
1.9 Collect the interphase and transfer it to a fresh 50 ml tube on ice
1.10 Wash the enriched cells 2x with ice-cold PBS/BSA
1.11 Resuspend cells in 5 ml PBS/BSA

Note **If you want to enrich monocytes from whole blood please refer to the Rosette Sep® Procedure for Human Monocyte Enrichment Cocktail (www.stemcell.com).**

1.12 Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully

1.13 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully

2. Nucleofection®

One Nucleofection® Sample contains

3 x 10^6 – 1 x 10^7 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 Monocyte Nucleofector® Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution

2.2 Prepare 24-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 (3 x 10^6 – 1 x 10^7 cells per sample) at 200xg for 10 – 12 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 Human Monocyte 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-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 supplemented culture medium to the cuvette and **gently** transfer the sample into the 24-well plate (final volume of 1.5 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 or 48 hours

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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