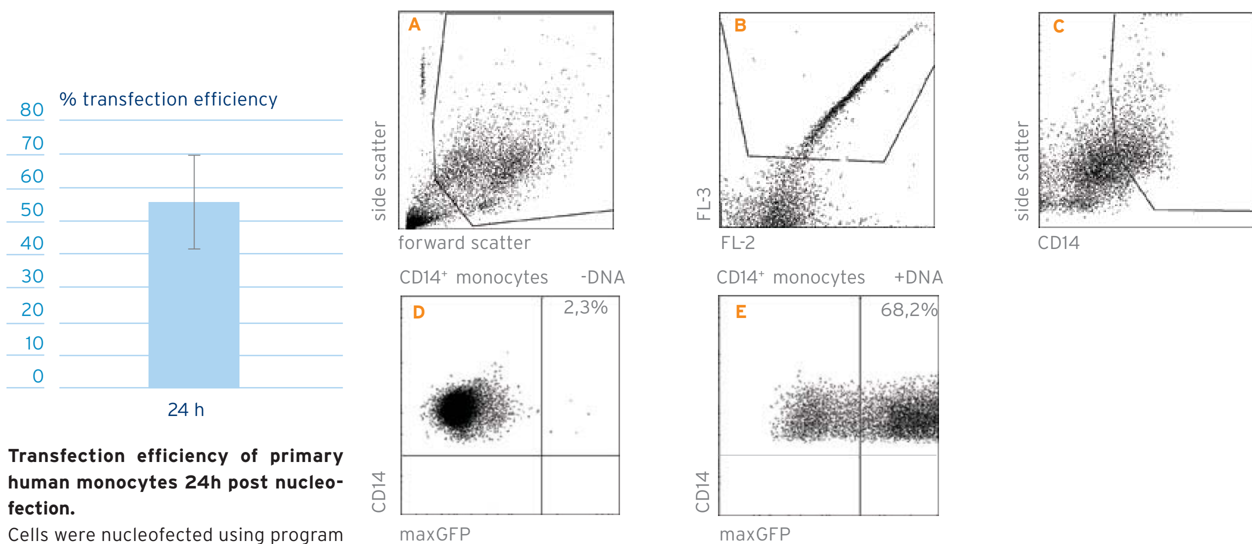
Human Monocyte Nucleofector® Kit

for Primary Human Monocytes

Cell type	**Origin**	This protocol is designed for human monocytes freshly isolated from blood samples or buffy coats.

Remarks	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 (see chapter 4).

Example for nucleofection® of CD14+ monocytes with pmaxGFP™

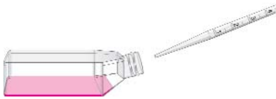
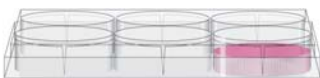


Transfection efficiency of primary human monocytes 24h post nucleofection.
Cells were nucleofected using program Y-01/Y-001 and 1 µg of pmaxGFP™. Cell viability is usually around 70% (%PI negative monocytes) after 24h.

Human monocytes freshly enriched from peripheral blood mononuclear cells (PBMC) were nucleofected with 1 µg pmaxGFP™ 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). pmaxGFP™ expression is shown after nucleofection without (D) and with plasmid DNA (E).

Chapter	Contents
1	Procedure outline & important advice
2	Product description
3	Protocol
3.1	› Required reagents
3.2	› DNA preparation and quality
3.3	› Preparation of cells and cell culture
3.4	› Important controls
3.5	› Nucleofection protocol
4	Recommended literature

1 Procedure outline & important advice

Step	Procedure outline	Important advice
1. 	Culturing of cells before nucleofection. (For details see 3.3.)	› use RosetteSep™ Isolation Kit for human monocytes › Supplement Human Monocyte Nucleofector Medium with 2 mM glutamine and 10% FCS.
2.	Combine the cells of interest, DNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette. (For details see 3.5.)	**Contents of one nucleofection sample:** › 3 x 10^6 to 1 x 10^7 monocyte-enriched PBMC › 1 µg pmaxGFP plasmid (in max. 5 µl H_2O or TE) › 100 µl Human Monocyte Nucleofector Solution Perform each sample separately to avoid storing the cells longer than 15 min in Human Monocyte Nucleofector Solution.
3.	Choose the cell-type specific program. Insert the cuvette into the Nucleofector and press the start button "X" (For details see 3.5.)	› Optimal Nucleofector program: **Y-01*** **or Y-001**** Software requirements: › *version **V2.3** for Nucleofector I Device › **version **S3.4** for Nucleofector II Device
4. 	Rinse the cuvette with culture medium and transfer the cells into the culture dish. (For details see 3.5.)	› remove sample from the cuvette immediately › use amaxa certified pipette › transfer directly to 37°C › **use amaxa Human Monocyte Nucleofector Medium**

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2 Product description

Cat. No.	**VPA-1007**
Kit components	2.25 ml **Human Monocyte Nucleofector Solution**
	0.5 ml Supplement
	10 µg pmaxGFP™ (0.5 µg/µl in 10 mM Tris pH 8.0)
	100 ml **Human Monocyte Nucleofector Medium**
	25 certified cuvettes
	25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector Solution and Supplement at 4°C, pmaxGFP™ at -20°C. Store Human Monocyte Nucleofector Medium at 4-8°C. The expiry date is printed on the Solution Box.

3 Protocol

3.1 › Required reagents

Medium The Human Monocyte Nucleofector Kit is delivered with 100 ml Human Monocyte Nucleofector Medium. This medium is specially developed to provide consistent high-yield transfection results. The medium is used for the culture post nucleofection and is essential for survival of nucleofected monocytes. Before use, supplement medium with 2 mM glutamine and 10% FCS.

3.2 › DNA preparation and quality

The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN® EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit). The purified DNA should be resuspended in deionised water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN® protocol.

3.3 › Preparation of cells and cell culture

This protocol only gives an outline for the handling and the nucleofection of human monocytes.

Experimental results and viability may vary within different blood samples or buffy coats.

Enrichment

We recommend using the RosetteSep™ Isolation Kit for human monocytes [Stem Cell Technologies, 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.

Enrichtment of monocytes from buffy coats

1. Centrifuge one buffy coat (~60 ml) in two 50 ml tubes at 1200xg for 20 min at RT (brake off).
2. Remove most of the serum in the upper layer.
3. Transfer the interphases (PBMC) together with traces of serum and erythrocytes (~15 ml) into two fresh 50 ml tubes.
4. Add 1000 µl cold Rosette-Cocktail (4°C) to each PBMC-Mix and vortex.
5. Incubate 20 min at RT.
6. Dilute 15 ml of the PBMC-Mix with 15 ml PBS/BSA and mix gently.
7. Prepare two 50 ml tubes with 15 ml Ficoll Hypaque™ and place 30 ml of the diluted PBMC-Mix as a layer on top of the Ficoll Hypaque™.
8. Centrifuge at 1200xg for 20 min at RT with brake off.
9. Collect the interphase and transfer it to a fresh 50 ml tube on ice.
10. Wash the enriched cells 2x with ice-cold PBS/BSA.
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).

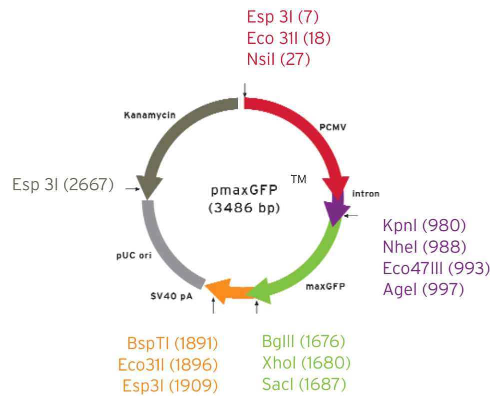
Note

Contamination of cell culture with mycoplasma is a widely spread phenomenon that might negatively influence experimental results. We recommend the use of Plasmocin™ [Cat. No. VZA-1021], a new antibiotic formula specifically developed to protect sensitive primary cells from mycoplasma infection and microbial contaminations. For more information and ordering info see **www.amaxa.com/antibiotics**.

3.4 › Important controls

1. Positive control

We strongly recommend establishing the Nucleofector technology with the positive control vector **pmaxGFP**™ as provided in this kit. pmaxGFP™ encodes the green fluorescent protein (GFP) from copepod *Pontellina p.* Just like eGFP expressing cells, maxGFP expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



2. Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection or amount/purity of DNA on cell viabilty.

control 1 Recommended amount of cells in Nucleofector Solution with DNA but without application of the program (alternatively: untreated cells) **(Cells + Solution + DNA - program).**

control 2 Recommended amount of cells in Nucleofector Solution without DNA with application of the program **(Cells + Solution - DNA + program).**

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3.5 › Nucleofection protocol

Preparation of Nucleofector Solution	Add 0.5 ml Supplement to 2.25 ml Nucleofector Solution and mix gently. The Nucleofector Solution is now ready to use and is stable for 3 months at 4°C. **Note the date of addition on the vial**.
One nucleofection sample contains	› **3×10^6 to 1×10^7 monocyte enriched PBMC** › **1 - 5 µg plasmid DNA (in 1-5 µl H_2O or TE) or 1 µg pmax GFP or 0.5 - 3 µg siRNA** › **100 µl Human Monocytes Nucleofector Solution** Minimal cell number: 1×10^6 cells (a lower cell number may lead to a major increase in cell mortality). Maximal cell number: 2×10^7 cells.
	For more details about the nucleofection of siRNA: **www.amaxa.com/RNAi**

Preparation of samples

1. Isolate monocytes (for details see 3.3).
2. Prepare 1-5 µg plasmid DNA, 1 µg pmaxGFP DNA or 0.5-3 µg siRNA for each sample.
3. Pre-warm the supplemented Human Monocyte Nucleofector Solution to room temperature.
4. Prepare 24-well plates by filling the appropriate number of wells with 1 ml of Human Monocyte Nucleofector Medium supplemented with 2 mM glutamine and 10% FCS. Pre-incubate plates in a humidified 37°C/5% CO_2 incubator for at least 30 min.
   Note: Using any other medium after nucleofection will most likely result in less cell viability and lower transfection efficiency.
5. Count the monocyte-enriched resuspended cells.
6. Centrifuge the required number of cells (3×10^6-1×10^7 cells per nucleofection sample) at 200xg for 10-12 min. Discard supernatant completely so that no residual PBS/BSA covers the cell pellet.
7. Resuspend monocyte-enriched PBMC in room temperature Human Monocyte Nucleofector Solution to a final concentration of 3×10^6-1×10^7 monocytes/100 µl. Avoid storing the cell suspension longer than 15 min in Human Monocyte Nucleofector Solution as this reduces cell viability and gene transfer efficiency.
   Important: Steps 8-12 should be performed for each sample separately.

Nucleofection

8. Mix 100 µl of cell suspension (see step 9) with 1-5 µg plasmid DNA, **1 µg** pmaxGFP DNA or 0.5 - 3 µg siRNA (in **1-5 µl** H_2O or TE).
9. Transfer the sample into an amaxa certified cuvette. Make sure that the sample

covers the bottom of the cuvette, avoid air bubbles while pipetting. Close cuvette with the blue cap.

10. Select the appropriate Nucleofector program, **Y-01 or Y-001** (see Nucleofector I or Nucleofector II manual for details). Insert the cuvette into the cuvette holder (Nucleofector I: rotate carousel to final position) and press the "X" key to start the program.
11. To avoid damage to the cells remove the samples from the cuvette immediately after program has finished (display showing "OK"). Take the cuvette out of the holder. To transfer the cells from the cuvettes, we strongly recommend using the provided plastic pipettes to prevent damage and loss of cells. Add 500 µl of the pre-incubated Human Monocyte Nucleofector Medium containing 2 mM glutamine and 10% FCS to the cuvette and transfer the sample into the prepared 24-well plate.
12. Press the "X" button to reset the Nucleofector.

Cultivation post nucleofection

13. Incubate cells in a humidified 37°C/5% CO_2 incubator in Human Monocyte Nucleofector Medium containing 2 mM glutamine and 10% FCS.
14. Following nucleofection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 4-8 hours. If this is not the case, the incubation period may be prolonged to 24 or 48 hours.

4 Recommended literature

For an up-to-date list of all Nucleofector references, please refer to:
www.amaxa.com/citations

* amaxa's Nucleofector® process, Nucleofector® device and Nucleofector® Solutions are covered by PCT applications PCT/EP01/07348, PCT/DE02/01489, PCT/DE02/01483 and other patents in addition to domestic or foreign applications corresponding thereto.

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* This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this amaxa product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this amaxa product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at license@evrogen.com

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