

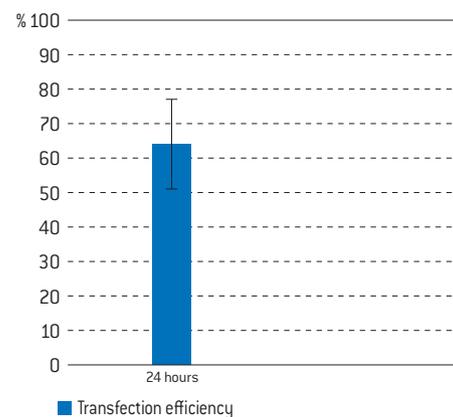
# Amaxa™ 4D-Nucleofector™ Protocol for Primary Human Monocytes For 4D-Nucleofector™ X Unit—Transfection in suspension

Human monocytes freshly isolated from blood samples or buffy coats

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 primary human monocytes

Average transfection efficiency of primary human monocytes 24 hours post Nucleofection™. Enriched human monocytes were transfected with program EA-100 and 0.4 µg of pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ [Becton Dickinson]. Cell viability is usually around 75% [% PI-negative monocytes] after 24 hours.



## Product Description

### Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032
Transfection volume	100 µl	100 µl	20 µl
Size [reaction]	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µl)	12	24	-
16-well Nucleocuvette™ Strips (20 µl)	-	-	2

### 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.

### Note

4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. They are not compatible with the Nucleofector™ II/2b Device.

## 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 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips:** epT.I.P.S. [US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266], Matrix TallTips® [Matrix Technologies Corp., Cat. No. 7281] or LTS Tips [Rainin Instruments, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S]. Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- **Supplied pmaxGFP™ Vector, stock solution 1 µg/µl**

### Note

For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260:A280 ratio should be at least 1.8
- Cell culture plates of your choice
- **Culture medium:** Please use your established human monocyte culture medium and required supplements.
- **For enrichment:** We recommend using the RosetteSep™ Isolation Kit for human monocytes [Stem Cell Technologies, Cat. No 15028]. Alternatively, it is also possible to use the Monocyte Isolation Kit II [Miltenyi Biotec, Cat. No. 130-091-153] to purify the monocytes
- **PBS/BSA for isolation:** PBS containing 0.5% BSA FicolI-Paque™ (GE Healthcare; cat. No. 17-1440-03)
- Prewarm appropriate volume of culture medium to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

## 1. Pre Nucleofection™

### Note

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 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 FicolI-Paque™ and place 30 ml of the diluted PBMC-Mix as a layer on top of the FicolI-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 2 x 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 RosetteSep® Procedure for Human Monocyte Enrichment Cocktail ([www.stemcell.com](http://www.stemcell.com)).

## 2. Nucleofection™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
- 2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
- 2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.5 Pre-warm an aliquot of culture medium to 37°C (see table 4)
- 2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
- 2.7 Count an aliquot of the cells and determine cell density
- 2.8 Centrifuge the required number of cells (see table 3) at 90xg for 10 minutes at room temperature. Remove supernatant completely
- 2.9 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
- 2.10 Prepare mastermixes by dividing cell suspension according to number of substrates
- 2.11 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
- 2.12 Transfer mastermixes into the Nucleocuvette™ Vessels

## Note

As leaving cells in Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

- 2.13 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
- 2.14 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
- 2.15 Start Nucleofection™ Process by pressing the “Start” on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
- 2.16 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
- 2.17 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
- 2.18 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

## 3. Post Nucleofection™

- 3.1 Incubate the cells in humidified 37°C/5% CO<sub>2</sub> incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours

## Additional Information

For an up-to-date list of all Nucleofector™ References, please refer to:  
[www.lonza.com/nucleofection-citations](http://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](mailto: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](mailto:scientific.support.eu@lonza.com)

Lonza Cologne GmbH  
50829 Cologne, Germany

Please note that the Amaxa™ Nucleofector™ Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

The Nucleofector™ Technology, comprising Nucleofection™ Process, Nucleofector™ Device, Nucleofector™ Solutions, Nucleofector™ 96-well Shuttle™ System and 96-well Nucleocuvette™ plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne GmbH.

Amaxa, Nucleofector, Nucleofection, 4D-Nucleofector, Nucleocuvette and maxGFP are registered trademarks of the Lonza Cologne GmbH in Germany and/or U.S. and/or other countries.

Other product and company names mentioned herein are the trademarks of their respective owners.

This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this Lonza product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this Lonza 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](mailto:license@evrogen.com). The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

The use of this product in conjunction with materials or methods of third parties may require a license by a third party. User shall be fully responsible for determining whether and from which third party it requires such license and for the obtainment of such license.

No statement is intended or should be construed as a recommendation to infringe any existing patent.

© Copyright 2010, Lonza Cologne GmbH. All rights reserved.

**Table 1: Volumes required for a single reaction**

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

**Table 2: Required amounts of cells and media for Nucleofection™**

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	2 ml	230 µl
Cell number per Nucleofection™ Sample	3 x 10 <sup>6</sup> –1 x 10 <sup>7</sup> [Lower or higher cell numbers may influence transfection results]	1 x 10 <sup>6</sup> [Lower or higher cell numbers may influence transfection results]

**Table 3: Contents of one Nucleofection™ Sample and recommended program**

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells	3 x 10 <sup>6</sup> –1 x 10 <sup>7</sup>	1 x 10 <sup>6</sup>
Substrate*	pmaxGFP™ Vector	2 µg
	or plasmid DNA (in H <sub>2</sub> O or TE)	1–5 µg
	or siRNA	30–300nM siRNA [3–30 pmol/sample]
P3 Primary Cell 4D-Nucleofector™ X Solution	100 µl	20 µl
Program	EA-100	EA-100

\* Volume of substrate should comprise maximum 10% of total reaction volume

**Table 4: Culture volumes required for post Nucleofection™ Steps**

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
24-well culture plate	1.5 ml	-
96-well culture plate	-	150 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl

\* Maximum cuvette volume 200 µl

**Table 5: Recommended volumes for sample transfer into culture plate**

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	50 µl

\* Maximum cuvette volume 200 µl