BioResearch

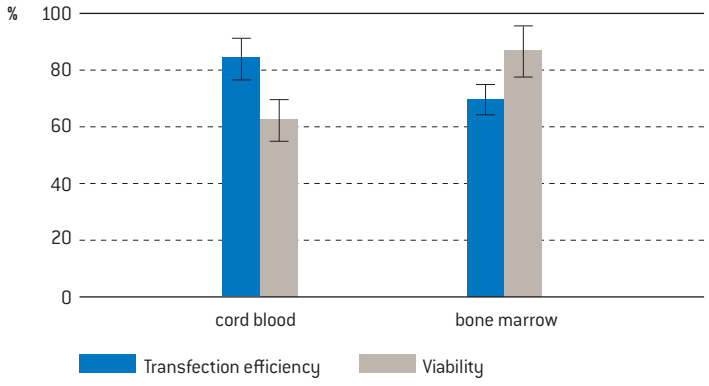
Lonza

# Amaxa™ 96-well Shuttle™ Protocol for Human CD34+ Cells

## Cell Description

This protocol is designed for unstimulated human CD34+ cells; self-isolated or Poietics™ Human Cord Blood CD34+ Progenitor Cells (Lonza, Cat. No. 2C-101) or Poietics™ Human Bone Marrow Blood CD34+ Progenitor Cells (Lonza, Cat. No. 2M-101 (frozen) or Lonza, Cat. No. 1M-101C (fresh)).

### Example for Nucleofection™ of Human CD34+ Cells




**Average transfection efficiency and viability of CD34+ cells 24 hours post Nucleofection™.** $5\times10^4$ CD34+ cells from cord blood (2C-101, Lonza) or bone marrow (2M-101, Lonza) were transfected with program 96-E0-100 and 0.4 µg of pmaxGFP™ Vector. 24 hours post Nucleofection™, cells were analyzed on a FACSCalibur™ with HTS option. Cell viability was determined as a relative portion of untreated control (measured with the ViaLight™ Plus Bioassay Kit; LT07-221, Lonza).

## Product Description

### Recommended Kits

P3 Primary Cell 96-well Nucleofector™ Kits

| Cat. No. | V4SP-3096 |
|---|---|
| Size (reactions) | 1×96 |
| P3 Primary Cell 96-well Nucleofector™ Solution | 2.25 ml |
| Supplement | 0.5 ml |
| pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0) | 50 µg |
| Nucleocuvette™ Plate(s) | 1 |

| Cat. No. | V4SP-3960 |
|---|---|
| Size (reactions) | 10×96 |
| P3 Primary CellI 96-well Nucleofector™ Solution | 22.5 ml |
| Supplement | 5 ml |
| pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0) | 50 µg |
| Nucleocuvette™ Plate(s) | 10 |

### 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

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. 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.

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ plate(s)
- Nucleocuvette™ compatible tips: 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 Instrument, 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**
Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 96-well culture plates or culture plates of your choice
- Culture medium: X-VIVO™ 15 (Lonza, Cat. No. 04-418Q) supplemented with SCF (25 ng/ml, TPO (50 ng/ml) and FLT-3 (50 ng/ml)
- Differentiation medium (for myeloid differentiation): X-VIVO™ 15 (Lonza, Cat. No. 04-418Q) supplemented with GM-CSF (10 ng/ml), G-CSF (10ng/ml), IL-6 (10 ng/ml), IL-3 (10 ng/ml) and SCF (100 ng/ml)
- Differentiation medium (for lymphoid differentiation): X-VIVO™ 15 (Lonza, Cat. No. 04-418Q) supplemented with IL-2 (1000 U/ml), IL-3 (5 ng/ml), IL-7 (20 ng/ml), SCF (20 ng/ml) and FLT3 (10 ng/ml)
- Prewarm appropriate volume of culture medium to 37°C (190 µl per sample)
- Appropriate number of cells ($5\times10^4$ cells) per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection™

**Note**
It is recommended to transfect cells immediately after thawing. Alternatively, cells may be expanded in culture medium (please see cell culture recommendations below). However, expansion of cells may lead to a decrease of CD34+ expression. Transfection results may be donor-dependent.

### Cell Culture Recommendations

1.1 Replace media every 3–4 days
1.2 For passaging please spin cells down at 300×g for 10 minutes and resuspend them in fresh media. Passage cells 2 times a week
1.3 Maintain cultures between $0.1–1.5\times10^6$ cells/ml
1.4 Seed out $1\times10^5$ cells/ml for expansion

**Note**
For preparation of self isolated CD34+ cells, please follow the respective literature.

# 2. Nucleofection™

## One Nucleofection™ Sample Contains

- $5\times10^4$ cells
- 0.2–1 µg plasmid DNA (in 1–2 µl $H_2O$ or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 Primary Cell 96-well Nucleofector™ Solution

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

2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see device and software manuals)

2.3 Select the appropriate 96-well Nucleofector™ Program **96-EO-100**

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture medium, e.g. 10 µl* (see note at the end of this chapter) for one well of a 96-well plate and preincubate/equilibrate plates in a humidified 37°C/5 % $CO_2$ incubator

2.5 Pre-warm and equilibrate an aliquot of culture medium to 37°C (180 µl per sample*) in a cell culture plate or flask

2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Count an aliquot of the cells and determine cell density

2.8 Centrifuge the required number of cells ($5\times10^4$ cells per sample) at 300×g for 10 minutes at room temperature. Remove supernatant completely

2.9 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

**A: One or several substrates (DNAs or RNAs) in multiples**

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

**B: Multiple substrates (e.g. Library Transfection)**

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

**Note**

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well 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.

2.10 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.11 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well "A1" must be in upper left position

2.12 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.13 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.14 Resuspend cells with 180 µl /per sample* (recommendation for 96-well plates) or desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 90 µl of resuspended cells to 10 µl pre-warmed medium prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases. However, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

## 3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5 % $CO_2$ incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours

3.2 For differentiation of CD34+ cells, you may plate cells directly in the respective differentiation medium (see required material section) post Nucleofection™.

## Additional Information

### Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

### Technical Assistance and Scientific Support

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## References

1. Von Levetzow et al, 2007 (Stem cells and development)
2. Wiehe et al, 2007 (J.Cell.Mol.Med. 2007)

**www.lonza.com**

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