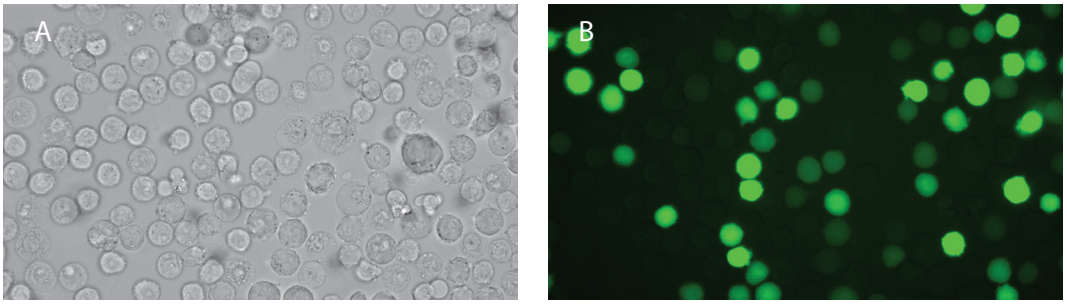
Amaxa® Cell Line Nucleofector® Kit V

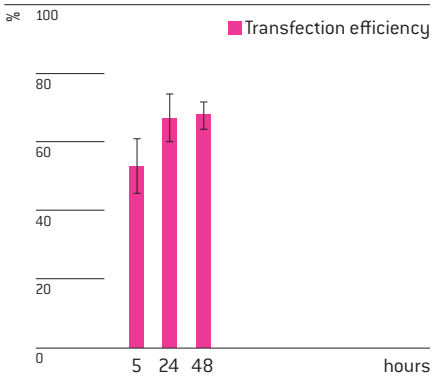
For THP-1

Human acute monocyte leukemia cell line; monocytoid cells

Example for Nucleofection® of THP-1 cells



THP-1 cells were transfected with the Cell Line Nucleofector® Kit V, Program V-001 and 0.5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 5 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of THP-1 cells. THP-1 cells were transfected with program V-001 and 0.5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 5, 24 and 48 hours post Nucleofection® by flow cytometry. Cell viability is around 40% for V-001 and 60% for U-001 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1003**
Size (reactions)	25
Cell Line Nucleofector® Solution V	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; Software requirements: version V2.3 for Nucleofector® I Device; version S 3-4 for Nucleofector® II 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
- 12-well culture dish or culture system of your choice
- **Culture medium**: RPMI 1640 [Lonza Cat. No.: BE12-167F], 2 mM UltraGlutamine I [Lonza Cat. No. BE17-605E/U1], 10 mM HEPES, 1 mM sodium pyruvate [Lonza Cat. No. BE13-115E], 4.5 g/l glucose, 100 µg/ml streptomycin, 100 U/ml penicillin, 0.05 mM 2-mercaptoethanol and 10% fetal calf serum (FCS)
- **Differentiation medium (optional)**: Culture medium supplemented with 20 nM PMA (Phorbol 12-myristate 13-acetate; Promega; Cat.No. V1171)
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10^6 cells per sample; minimal recommended cell number is 8 x 10^5, a lower cell number leads to a major increase in cell mortality; maximal cell number is 2 x 10^6)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media 2 – 3 times a week (30 ml per 162 cm^2 flask)
1.2 Passage cells at a density of 6 – 7 x 10^5 cells/ml
1.3 Seed out 2 x 10^5 cells/ml
1.4 Subculture 2 – 3 days before Nucleofection®
1.5 Cells should be grown to a density of 3 – 4 x 10^5 cells/ml before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains
1 x 10^6 cells
0.5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 0.5 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution V

Note **0.5 µg is the maximum recommended DNA amount per Nucleofection® Sample for THP-1 cell line. A higher DNA amount will cause a tremendous increase in cell death.**

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 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Count an aliquot of the cells and determine cell density
2.4 Centrifuge the required number of cells (1 x 10^6 cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.5 Resuspend the cell pellet carefully in 100 µl room-temperature Nucleofector® Solution per sample

Note **Avoid leaving the cells in Nucleofector® Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability and gene transfer efficiency.**

2.6 Combine 100 µl of cell suspension with **0.5 µg DNA**, 0.5 µg pmaxGFP® Vector or **30 nM – 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 **U-001** (for high viability) or **V-001** (for high expression level) (**U-01** or **V-01** for Nucleofector® I Device)
2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.10 Take the cuvette out of the holder once the program is finished
2.11 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 12-well plate (final volume 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 or down regulation, respectively, is often detectable after only 4 – 8 hours
3.2 THP-1 cells can be differentiated into macrophage-like cells by culturing in differentiation medium immediately post Nucleofection®. Cells should be differentiated and become adherent 1 – 3 days after adding PMA

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:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: 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

Lonza Cologne AG
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 AG.

Amaxa, Nucleofector, Nucleofection and maxGFP are either registered trademarks or trademarks of the Lonza Cologne AG 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.

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 2009, Lonza Cologne AG. All rights reserved DCV-1012 05/09

DCV-1012_2009-05