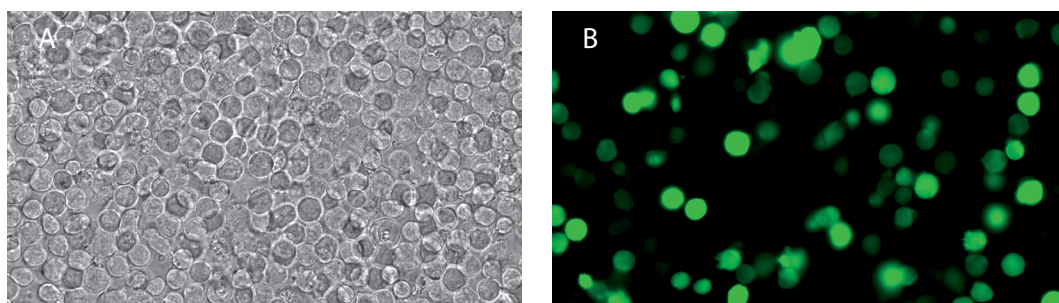


# Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit V

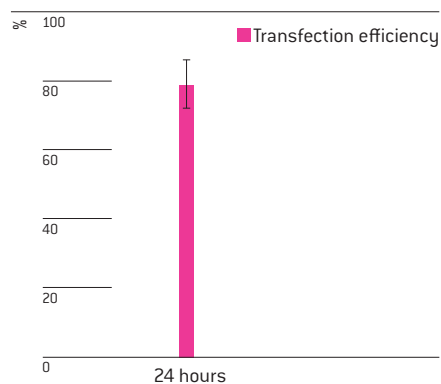
For 32 D

Mouse bone marrow; lymphoblastoid cells

Example for Nucleofection<sup>®</sup> of A-431 cells of 32 D cells



32 D cells were transfected with the Cell Line Nucleofector<sup>®</sup> Kit V, Program E-032 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> using light (A) and fluorescence microscopy (B).



Average transfection efficiency of 32 D cells. 32 D cells were transfected with program E-032 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell viability is around 61% 24 hours post Nucleofection<sup>®</sup>.

## Product Description

Cat. No.	VCA-1003
Size (reactions)	25
Cell Line Nucleofector <sup>®</sup> Solution V	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP <sup>®</sup> 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 <sup>®</sup> Solution, Supplement and pmaxGFP <sup>®</sup> Vector at 4°C. For long-term storage, pmaxGFP <sup>®</sup> Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector <sup>®</sup> Supplement is added to the Nucleofector <sup>®</sup> 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 or higher for Nucleofector® I Device; version S3-4 or higher 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:** complete growth medium: RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate supplemented with 10% heat-inactivated fetal bovine serum, 10% mouse Interleukin-3 culture supplement [Becton Dickinson Cat. No. 354040]
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10<sup>6</sup> cells per sample; lower or higher cell numbers may influence transfection results)

### 1. Pre Nucleofection®

#### Cell culture recommendations

- 1.1 Replace media every 2 – 3 days
- 1.2 Passage cells every 2 – 3 days.
- 1.3 Seed out 2 x 10<sup>5</sup> – 1 x 10<sup>6</sup> viable cells/ml
- 1.4 Subculture 1 – 2 days before Nucleofection®
- 1.5 Optimal confluency for Nucleofection®: 75%. Higher cell densities may cause lower Nucleofection® Efficiencies

#### For detaching cells:

- 1.6 Scrape off the attached cells and transfer along with floating cells into new flasks

### 2. Nucleofection®

#### One Nucleofection® Sample contains

1 x 10 <sup>6</sup> cells
2 µg plasmid DNA (in 1 – 5 µl H <sub>2</sub> O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution V

- 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<sub>2</sub> incubator
- 2.3 Count an aliquot of the cells and determine cell density
- 2.4 Centrifuge the required number of cells (1 x 10<sup>6</sup> 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 2 µg DNA, 2 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 E-032 (E-32 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<sub>2</sub> incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours

**Note** For detaching the adherent cells post Nucleofection® in 12-well plates, we recommend incubating the adherent cells in ice-cold PBS for 5 – 10 min and then rinsing the well.

### 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 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](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 2009, Lonza Cologne AG. All rights reserved DCV-1019 05/09