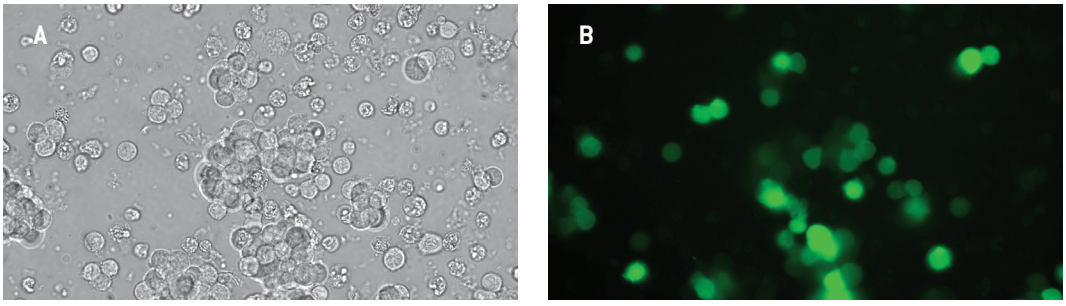
Amaxa® Cell Line Nucleofector® Kit L

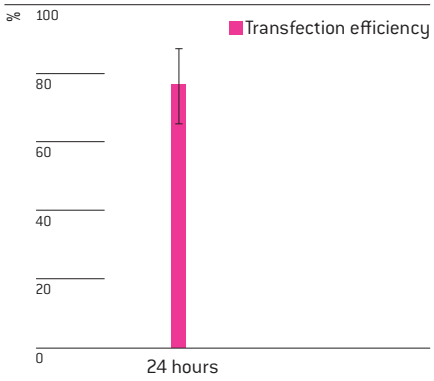
For WEHI-231

Mouse B lymphocyte; lymphoblastoid cells

Example for Nucleofection® of WEHI-231 cells



WEHI-231 cells were transfected with the Cell Line Nucleofector® Kit L, Program C-005 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of WEHI-231 cells. WEHI-231 cells were transfected with program C-005 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell Viability (% PI negative) is around 62% after 24 hours post Nucleofection®.

Product Description

Cat. No.	**VCA-1005**
Size (reactions)	25
Cell Line Nucleofector® Solution L	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
- 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
- **For trypsin treatment**: 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- **Culture medium I** (before Nucleofection®): Dulbecco's modified Eagle's medium with 4mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate and 4,5 g/L glucose and supplemented with 0.05mM 2-Mercaptoethanol,90%; fetal bovine serum, 10%
- **Culture medium II** (post Nucleofection®): RPMI 1640 medium with 2mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4,5 g/L glucose, 10mM Hepes, 1mM sodium pyruvate and supplemented with 0.05mM 2-Mercaptoethanol, 90%; fetal bovine serum, 10%
- Prewarm appropriate volume of culture medium II to 37°C (1.5 ml per sample)
- Appropriate number of cells (2 x 10^6 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. A subcultivation ratio of 1 : 8 to 1 : 10 is recommended
1.3 Subculture 2 – 3 days before Nucleofection®. Do not passage more than 30 times
1.4 Trypsinize cells prior to Nucleofection®

Trypsinization

1.5 Harvest the cells by centrifuging cell suspension at 90xg. Discard supernatant and add Trypsin (0.5 mg/ml Trypsin; 0.2 mg/ml EDTA in PBS). Wait for 3 to 4 minutes and then add medium

2. Nucleofection®

One Nucleofection® Sample contains

2 x 10^6 cells
2 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample)
100 µl Cell Line Nucleofector® Solution L

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 Harvest the cells and treat them with trypsin (please see 1.5)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (2 x 10^6 cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.6 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.**

2.7 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
2.8 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
2.9 Select the appropriate Nucleofector® Program **C-005** (**C-05** for Nucleofector® I Device)
2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
2.11 Take the cuvette out of the holder once the program is finished
2.12 Immediately add ~500 µl of the pre-equilibrated RPMI1640 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

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

DCL-1003_2009-05