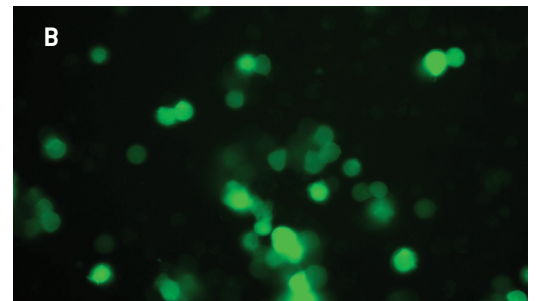
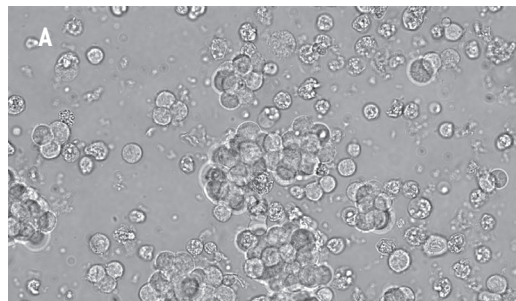


Amaxa[®] Cell Line Nucleofector[®] Kit L

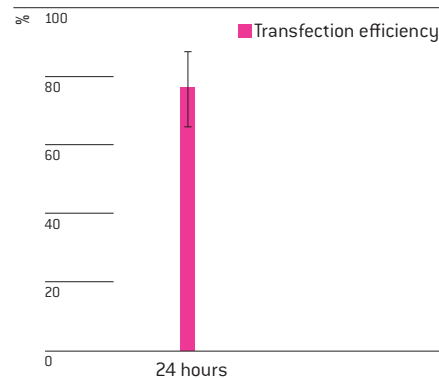
For WEHI-231

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 μ g 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 μ g 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 μ g/ μ l in 10 mM Tris pH 8.0)	30 μ g
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 10⁶ 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 ₂ O 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% CO₂ 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 10⁶ 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% CO₂ 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:

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