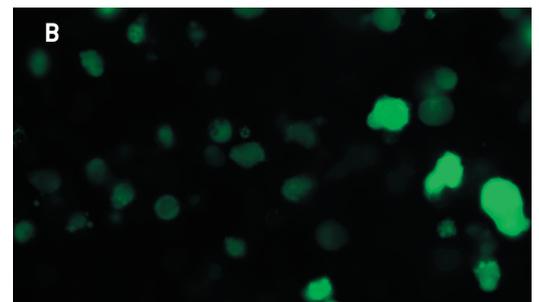
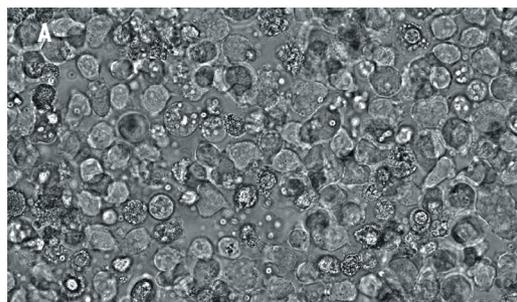


Amaxa[®] Cell Line Nucleofector[®] Kit R

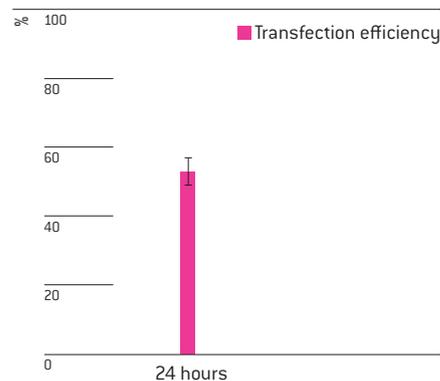
For HuT 78

Human cutaneous T cell lymphoma; lymphoblastoid cells

Example for Nucleofection[®] of HuT 78 cells



HuT 78 cells were transfected with the Cell Line Nucleofector[®] Kit R, Program V-001 and 2 μ g of pmxGFP[®] Vector. Cells were analyzed 24 hours post Nucleofection[®] using light (A) and fluorescence microscopy (B).



Average transfection efficiency of HuT 78 cells. HuT 78 cells were transfected with program V-001 and 2 μ g of pmxGFP[®] Vector. Cells were analyzed 24 hours post Nucleofection[®] by flow cytometry. Cell Viability (% PI negative) is around 86% 24 hours post Nucleofection[®].

Product Description

Cat. No.	VCA-1001
Size (reactions)	25
Cell Line Nucleofector [®] Solution R	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmxGFP [®] 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 pmxGFP [®] Vector at 4°C. For long-term storage, pmxGFP [®] 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 S3-4 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:** Iscove's modified Dulbecco's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 80%; fetal bovine serum, 20%
- Prewarm appropriate volume of culture medium 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 2 – 3 times a week. A subcultivation ratio of 1 : 2 to 1 : 4 is recommended
- 1.3 Maintain cell density at less than 1 x 10⁶ cells/ml
- 1.4 Seed out 3 – 5 x 10⁴ viable cells/ml
- 1.5 Subculture 2 – 3 days before Nucleofection® with a ratio of 1 : 3 – 1 : 4
- 1.6 In the first 15 to 20 passages cell debris is often present in culture. For Nucleofection® Experiments use cells passaged a minimum of 20 times

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 R

- 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 Count an aliquot of the cells and determine cell density
- 2.4 Centrifuge the required number of cells (2 x 10⁶ 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 **V-001** (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% 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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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

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