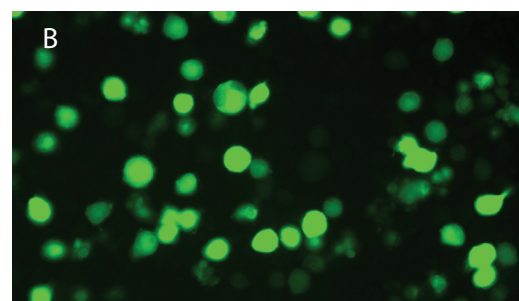
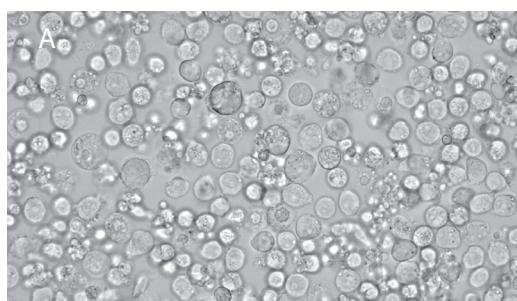


Amaxa[®] Cell Line Nucleofector[®] Kit V

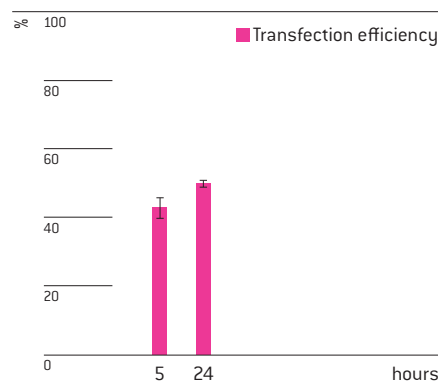
For HL-60

Acute promyelocytic leukemia; myeloblastic cells

Example for Nucleofection[®] of HL-60 cells



HL-60 cells were transfected with the Cell Line Nucleofector[®] Kit V, Program T-019 and 2 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 HL-60 cells. HL-60 cell were transfected with program T-019 and 1 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 5 and 24 hours post Nucleofection[®] by flow cytometry. Cell Viability is around 60-65% 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
- 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 DMEM [Lonza; Cat. No. BE12-722F], 100 µg/ml streptomycin, 100 U/ml penicillin and 20% fetal calf serum (FCS)
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (2 x 10⁶ cells per Nucleofection® sample; minimal recommended cell number: 8 x 10⁵ cells per sample; a lower cell number leads to a major increase in cell mortality; maximal cell number: 4 x 10⁶)

1. Pre Nucleofection®

Cell culture recommendations

- 1.1 Replace media 2 – 3 times per week (30 ml medium per T162 flask)
- 1.2 Passage cells at a density of 7 – 8 x 10⁵ cells/ml. Do not use cells after passage 25 for Nucleofection®
- 1.3 Seed out 1 x 10⁵ cells/ml
- 1.4 Subculture 3 days before Nucleofection®
- 1.5 Cells should be grown to a density of 5 – 7 x 10⁵ cells/ml before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

2 x 10⁶ cells

1 – 2 µg plasmid DNA (in 1 – 5 µl H₂O or TE) or 1 – 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% 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 1 – 2 µg DNA, 1 – 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 T-019 (T-19 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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