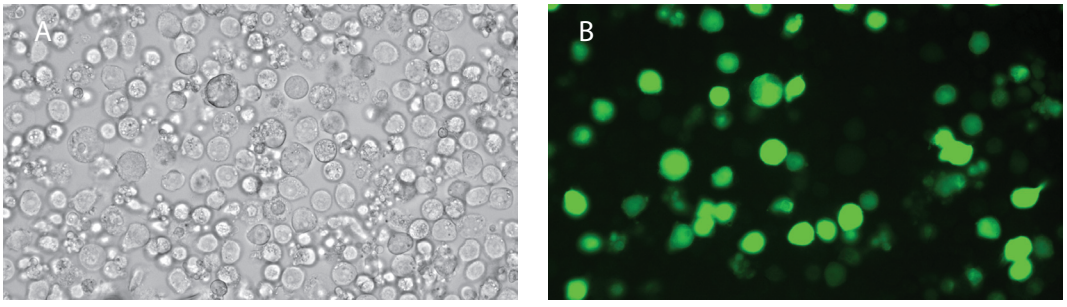
Amaxa® Cell Line Nucleofector® Kit V

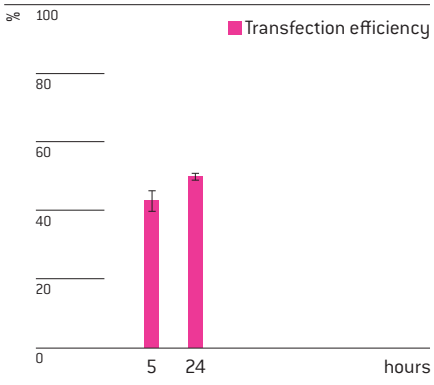
For HL-60

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 10^6 cells per Nucleofection® sample; minimal recommended cell number: 8 x 10^5 cells per sample; a lower cell number leads to a major increase in cell mortality; maximal cell number: 4 x 10^6)

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 10^5 cells/ml. Do not use cells after passage 25 for Nucleofection®
1.3 Seed out 1 x 10^5 cells/ml
1.4 Subculture 3 days before Nucleofection®
1.5 Cells should be grown to a density of 5 – 7 x 10^5 cells/ml before Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

2 x 10^6 cells
1 – 2 µg plasmid DNA (in 1 – 5 µl H_2O 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% CO_2 incubator
2.3 Count an aliquot of the cells and determine cell density
2.4 Centrifuge the required number of cells (2 x 10^6 cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.5 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% 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:

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