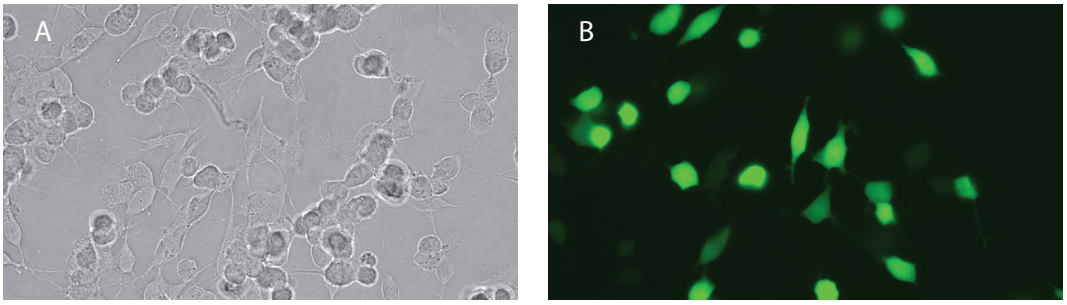
Amaxa® Cell Line Nucleofector® Kit V

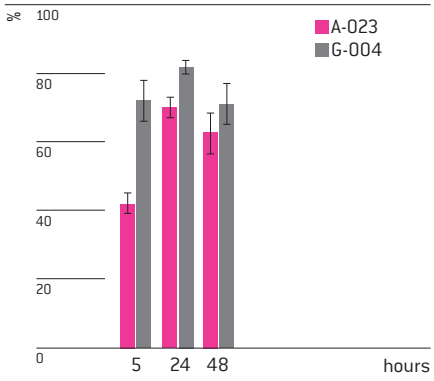
For SH-SY5Y

Human neuroblastoma; epithelial cells

Example for Nucleofection® of SH-SY5Y cells



SH-SY5Y cells were transfected with the Cell Line Nucleofector® Kit V, Program A-023 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 SH-SY5Y cells. SH-SY5Y cells were transfected with program A-023 or G-004 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 5, 24 and 48 hours post Nucleofection® by flow cytometry. Cell Viability (compared to non-transfected control) is around 60% using program A-023 and 40% using program G-004 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
- 6-well culture dish or culture system of your choice
- **For detaching cells**: 0.5 mg/ml Trypsin and 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- **Culture medium**: 1 : 1 mixture of EMEM, Ham's F12 Nutrient-Mixture [Lonza BE12-615F] and 10% fetal calf serum [FCS]
- Prewarm appropriate volume of culture medium to 37°C [1.5 ml per sample]
- Appropriate number of cells [1 x 10^6 – 2 x 10^6 cells per sample; minimal recommended cell number is 8 x 10^5 cells per sample; a lower cell number leads to increased cell mortality; maximal cell number: 4 x 10^6 cells per sample]

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media twice a week
1.2 Passage cells at 75 – 80 % confluency
1.3 Seed out 2 x 10^5 cells/cm^2
1.4 Subculture 3 – 4 days before Nucleofection®
1.5 Optimal confluency for Nucleofection®: 75 – 80%. Higher cell densities may cause lower Nucleofection® Efficiencies

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.7 For harvesting, incubate the cells ~5 minutes at 37°C with indicated trypsinization reagent [please see required material]
1.8 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5% BSA once the majority of the cells [>90%] have been detached

2. Nucleofection®

One Nucleofection® Sample contains

1 – 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 V

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 6-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 by trypsinization (please see 1.6 – 1.8)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (1 – 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 **A-023** (for high viability and analysis up to 4 – 6 days) or **G-004** (for high expression level and analysis up to 24 hours) (**A-23** or **G-04** 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 culture medium to the cuvette and gently transfer the sample into the prepared 6-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

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