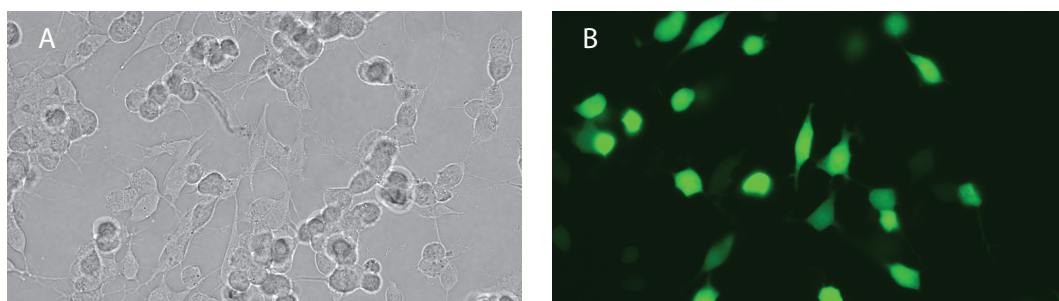


Amaxa[®] Cell Line Nucleofector[®] Kit V

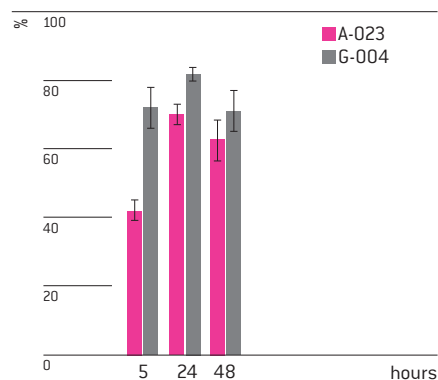
For SH-SY5Y

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 10⁶ – 2 x 10⁶ cells per sample; minimal recommended cell number is 8 x 10⁵ cells per sample; a lower cell number leads to increased cell mortality; maximal cell number: 4 x 10⁶ 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 10⁵ cells/cm²
- 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 ₂ O 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% CO₂ 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 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 **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% 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

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