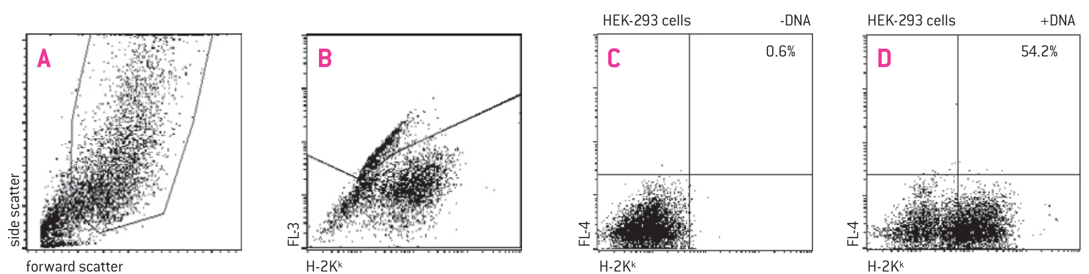
Amaxa® Cell Line Nucleofector® Kit V

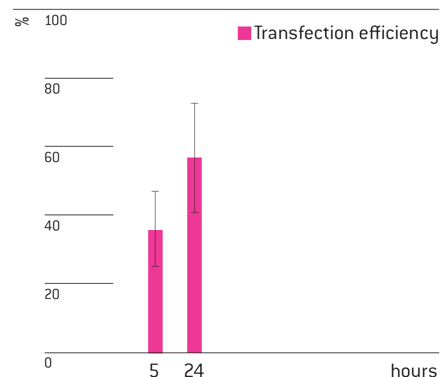
For HEK-293 [DSMZ ACC305, cryopreserved]

Human embryonic kidney; adherent fibroblastoid cells in monolayers

Example for Nucleofection® of HEK-293 cells



HEK-293 cells (DSMZ ACC305) were transfected with the Cell Line Nucleofector® Kit V, Program A-023 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kᵏ. Cells were analyzed 6 hours post Nucleofection® with a PE-coupled antibody directed against H-2Kᵏ and analyzed by flow cytometry. Cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2Kᵏ expression is shown post Nucleofection® without (C) and with plasmid DNA (D).



Average transfection efficiency of HEK-293 cells. HEK-293 cells (DSMZ ACC305) were transfected with program A-023 and 5 µg of a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kᵏ. Cells were analyzed 5 and 24 hours post Nucleofection® by flow cytometry. Cell Viability varies between 75 – 85%.

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**: PBS
- **Culture medium I**: 90% Dulbecco's modified Eagle medium (DMEM) [Lonza, Cat. No. BE12-604F] with UltraGlutamine I [Lonza, Cat. No. BE17-605E/U1] and 10% FCS
- **Culture medium II**: RPMI 1640 with 10% serum
- Prewarm appropriate volume of **culture medium I** (1.0 ml per sample) and **culture medium II** (0.5 ml per sample) to 37°C
- Appropriate number of cells (1 x 10^6 – 2 x 10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Use **culture medium I**
1.2 Passage interval: split confluent culture 1 : 5 to 1 : 6 every 2 – 3 days
1.3 Seed out 2.5 x 10^5 cells/25 cm^2 flask
1.4 Subculture 2 – 3 days before Nucleofection®
1.5 Optimal confluency for Nucleofection®: 50%. Higher cell densities may cause lower Nucleofection® Efficiencies

For detaching cells

1.6 Harvest the cells by incubating them in a small volume of PBS or by tapping the flask

2. Nucleofection®

One Nucleofection® Sample contains
1 – 2 x 10^6 cells
1 – 5 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 medium I** and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Harvest the cells (please see 1.7)
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 200xg 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 **1 – 5 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** (**A-23** 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 II** 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

For more technical assistance, contact our Scientific Support Team:

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References

1. Michaux G et al. [2003] Blood 102(7): 2452-8.

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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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