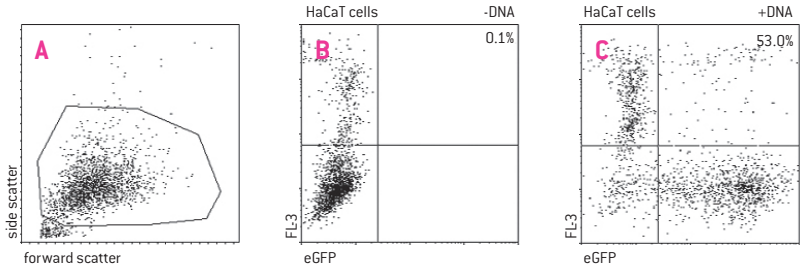
Amaxa® Cell Line Nucleofector® Kit V

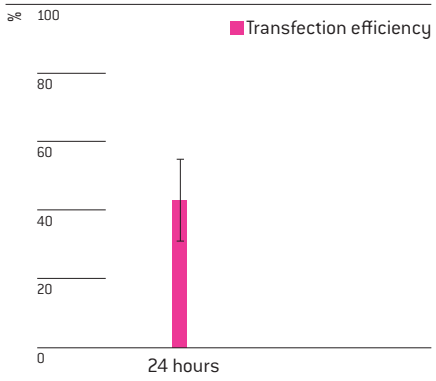
For HaCat [DKFZ, cryopreserved]

Spontaneously transformed human keratinocyte cell line; related to keratinocytes (cobble stone-like), but more fibroblastoid cells

Example for Nucleofection® of HaCat cells



HaCat cells (DKFZ) were transfected with the Cell Line Nucleofector® Kit V, Program U-020 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. HaCaT cells were gated according to forward/side scatter (A). Dead cells were visualized by staining with propidium iodide. eGFP expression of HaCaT is shown post Nucleofection® with (B) and without plasmid DNA (C). (Courtesy of Dr. T Wachter and PD Dr. M Leverkus, Dermatology Department, University of Wuerzburg, Germany).



Average transfection efficiency of HaCat cells. HaCat cells (DKFZ) were transfected with program U-020 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed 24 hours post Nucleofection® by flow cytometry.

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; Software requirements: version V2.3 for Nucleofector® I Device; version S 3.4 for Nucleofector® II 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**: RPMI 1640 [Lonza, Cat. No. BE12-167F] or DMEM [Lonza, Cat. No. BE12-604F], supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, and 2 mM UltraGlutamine I [Lonza, Cat. No. BE17-605E/U1]
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1×10^6 – 5×10^6 cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Cell culture recommendations

1.1 Passage cells at 70 – 80 % confluency.
1.2 Seed out 2.5×10^5 cells/25cm² flask
1.3 Subculture 2 – 3 days before Nucleofection®
1.4 Transfect cells after reaching 80 – 100% confluency. Transfection efficiency and mortality may vary dependent on passage number

Trypsinization

1.5 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.6 Optional: Treat cells with 0.2 mg/ml EDTA in PBS (20 minutes at 37°C). This helps to detach the desmosomes. Afterwards remove EDTA in PBS
1.7 For harvesting, incubate the cells ~5 minutes at 37°C with recommended volume of 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 – 5 x 10^6 cells
1 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 1 – 5 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 1.5 – 1.8)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (1 – 5 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 **1-5 µg DNA**, 1-5 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 **U-020** (**U-20** 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

For more technical assistance, contact our Scientific Support Team:

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References

1. Leverkus M, Sprick MR, Wachter T, Mengling T, Baumann B, Serfling E, Bröcker EB, Goebeler M, Neumann M, Walczak H, *et al.*, FASEB J. 2003; 17(3):440-2.

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