Cell Line Nucleofector™ Kit V

for HaCaT Cells

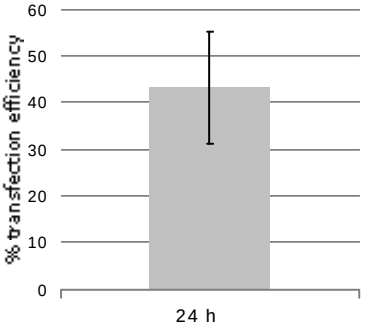
Product description

Cat. No.	**VCA-1003**
Kit components	2.25 ml **Cell Line Nucleofector™ SolutionV** 0.5 ml Supplement 25 certified cuvettes 25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector™ Solution and Supplement at 4°C. The expiry date is printed on the Solution Box.

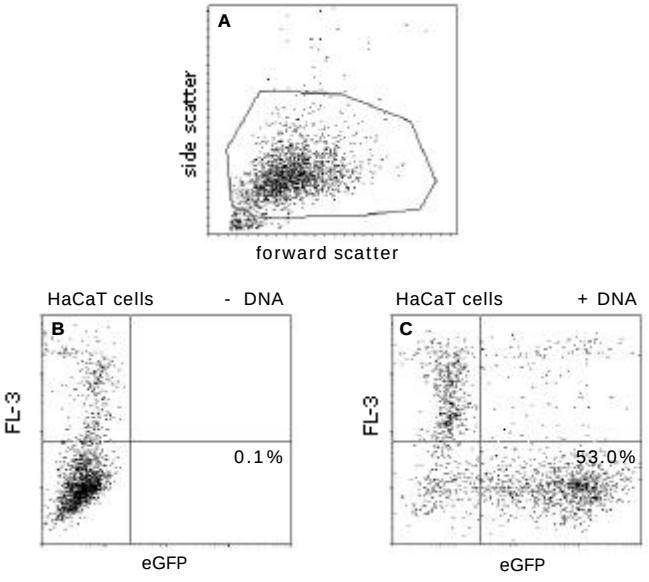
Cell type

Origin	Spontaneously transformed human keratinocyte cell line.
Morphology	Related to keratinocytes (cobble stone-like), but more fibroblastoid.

Optimal Nucleofector™ program **U-20**



Transfection efficiency of HaCaT cells.
HaCaT cells were nucleofected using program U-20 and a plasmid encoding the enhanced green fluorescent protein eGFP. **24 hours** post nucleofection, the cells were analyzed by flow cytometry.



Example for nucleofection of HaCaT cells with eGFP cDNA.
HaCaT cells were nucleofected using the Cell Line Nucleofector™ Kit V, program U-20 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. **24 hours** post nucleofection, the cells were analyzed 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 without (B) and with plasmid DNA (C).

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Cell culture

Medium	RPMI 1640 [Invitrogen/Gibco, Cat. No. 31870-025] supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, and 2 mM GlutaMAX [Invitrogen/Gibco, Cat. No. 35050-038].
Trypsin treatment	0.5 mg/ml Trypsin, 0.2 mg/ml EDTA in PBS.
Passage interval	Cells should be passaged at 70-80% confluency.
Seeding conditions	$2.5x10^5$ per 25 cm^2 flask.

Protocol

Culture conditions before nucleofection

- **Transfection efficiency and mortality may vary dependent on passage number.**
- The cells should be passaged 2-3 days before nucleofection.
- Cells should be nucleofected after reaching 80-100% confluency. Lower confluency may lead to lower nucleofection efficiencies.

DNA quality

The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like Marligen Bioscience CONCERT™ High Purity Plasmid Prep Purification System or QIAGEN® EndoFree® Plasmid Kits. The purified DNA should be resuspended in deionised water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio. The ratio should be at least 1.8 for nucleofection.

Positive control

The easiest way to establish the Nucleofection™ technology is to use the enhanced green fluorescent protein (eGFP) for your first experiments. We strongly recommend using of a eGFP-plasmid like pEGFP-C1 [BD Clontech; Cat.No. 6084-1] or pEYFP-C1 [BD Clontech; Cat.No. 6005-1]. Please do not use eGFP plasmids with IRES sequences for your first experiments.
We also propose using the eGFP plasmid as a positive control for all following experiments.

Preparation of the Nucleofector™ Solution

- Add 0.5 ml Supplement to 2.25 ml Nucleofector™ Solution and mix gently. The Nucleofector™ Solution is now ready to use and is stable for 3 months at 4°C. **Note date of addition on the vial.**

Nucleofection protocol:

One nucleofection sample contains:

- **1-5x10^6 cells**
- **1-5 µg plasmid DNA (in 1-5 µl H_2O or TE)**
- **100 µl Nucleofector™ Solution V**

1. Prepare the required number of cells.
2. Prepare 1-5 µg DNA (in **1-5 µl** H_2O or TE) for each sample.
3. Pre-warm the supplemented Nucleofector™ Solution V to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6 well plates by filling appropriate number of wells with 1.5 ml of culture medium containing supplements and serum and pre-incubate plates in a humidified 37°C/5% CO_2 incubator.
5. Remove the medium from the cell culture. Wash cells once with PBS. **Optional:** treat cells with 0.2 mg/ml EDTA in PBS (20 min at 37°C). This helps to detach the desmosomes. Afterwards remove EDTA in PBS.
6. Add trypsin-EDTA solution and incubate at 37°C. As soon as the cells are floating stop trypsinization with supplemented culture medium or PBS/0.5% BSA (see Nucleofector™ Manual, section 3.5.1). Take an aliquot and count the cells.
7. Centrifuge the required number of cells (1-5x10^6 cells per nucleofection sample) at 200xg for 10 min. Discard supernatant completely so that no residual medium covers the cell pellet.
8. Resuspend the pellet in room temperature Nucleofector™ Solution to a final concentration of 1-5x10^6 cells/100 µl. Avoid storing the cell suspension longer than 15-20 min in Nucleofector™ Solution, as this reduces cell viability and gene transfer efficiency.
   Important: Steps 9-13 should be performed for each sample separately.
9. Mix 100 µl of cell suspension (see step 8) with 1-5 µg DNA (in 1-5 µl H_2O or TE).
10. Transfer the sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
11. Insert the cuvette into the cuvette holder and rotate the turning wheel clockwise to the final position. Select program **U-20** (see Nucleofector Manual, section 2.6). Press the "X" key to start the program.
12. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells. Add 500 µl of the pre-warmed culture medium containing serum and supplements to the cuvette and transfer the sample into the prepared 6 well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.

13. Press any key to reset the Nucleofector™.
14. Repeat steps 9-13 for the remaining samples.
15. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6 well plates.
16. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following nucleofection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 3-8 hours. If this is not the case, the incubation period may be prolonged to 24 hours.

    Just like freshly thawed cells, nucleofected HaCaT cells may need longer to attach to culture dish.