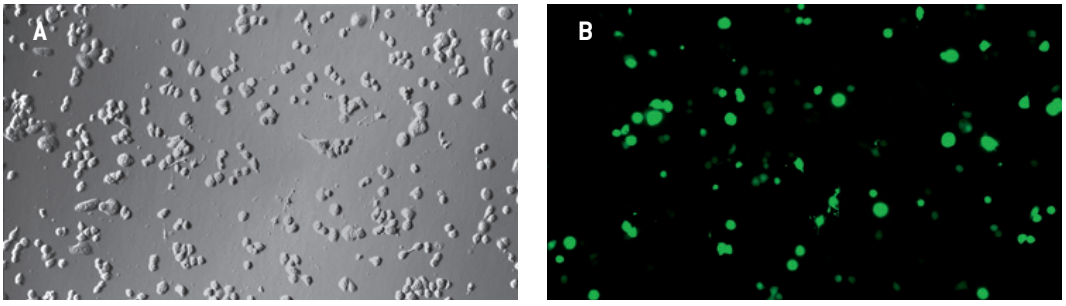
Amaxa® Cell Line Nucleofector® Kit R

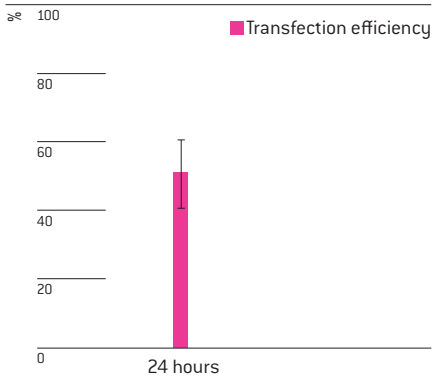
For HT-29

Human colorectal adenocarcinoma; epithelial cells

Example for Nucleofection® of HT-29 cells



HT-29 cells were transfected with the Cell Line Nucleofector® Kit R, Program W-017 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® using light (A) and fluorescence microscopy (B).



Average transfection efficiency of HT-29 cells. HT-29 cells were transfected with program W-017 and 2 µg of pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell viability is around 60% 24 hours post Nucleofection®. A cell viability of around 90% can be reached using program Q-09 (with a transfection efficiency of around 20%).

Product Description

Cat. No.	**VCA-1001**
Size (reactions)	25
Cell Line Nucleofector® Solution R	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 or higher** for Nucleofector® I Device; version **S3-4 or higher** 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:** McCoy's 5A Medium, supplemented with 10% FBS
- **Recovery medium**: RPMI 1640
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells: 1 x 10^6 cells per sample
  Minimum recommended cell number: 5 x 10^5 cells per sample
  Maximum cell number: 2 x 10^6 cells per sample

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media every 2 – 3 days
1.2 Passage cells 2 – 3 times a week. Cells should not be passaged more than 30 times
1.3 Seed out 3 x 10^4 cells/cm^2
1.4 Subculture 2 – 3 days before Nucleofection®. Optimal confluency for Nucleofection®: 70 – 90%

Trypsinization

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

2. Nucleofection®

One Nucleofection® Sample contains

1 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 R

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 – 1.7)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (1 x 10^6 cells per sample) at 300xg for 6 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 **Q-009** (for high viability) or **W-017** (for high transfection efficiency) (**Q-09** or **W-17** 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 . If very low efficiency is observed, a "recovery step" can be a useful option: Immediately after Nucleofection®, add 80 µl pre-equilibrated low-calcium media such as RPMI (recovery medium) and gently transfer it to the reaction tube. Place the cell suspension in an incubator for 15 – 30 minutes. Transfer the sample to the prepared culture dish containing the usual culture medium

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:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

Lonza Cologne AG
50829 Cologne, Germany

DCR-1007_2009-05