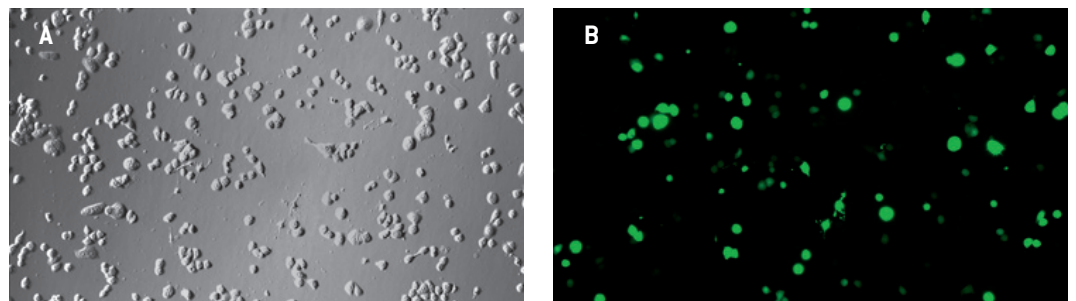


Amaxa[®] Cell Line Nucleofector[®] Kit R

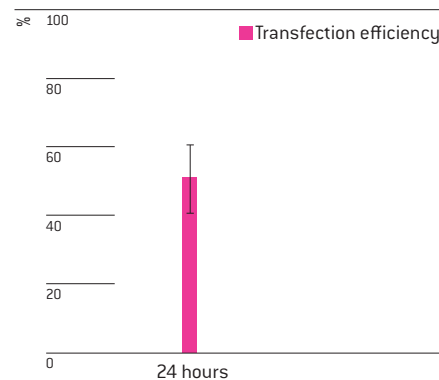
For HT-29

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 10⁶ cells per sample
Minimum recommended cell number: 5 x 10⁵ cells per sample
Maximum cell number: 2 x 10⁶ 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 10⁴ cells/cm²
- 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 ₂ O 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% CO₂ 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 10⁶ 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% 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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