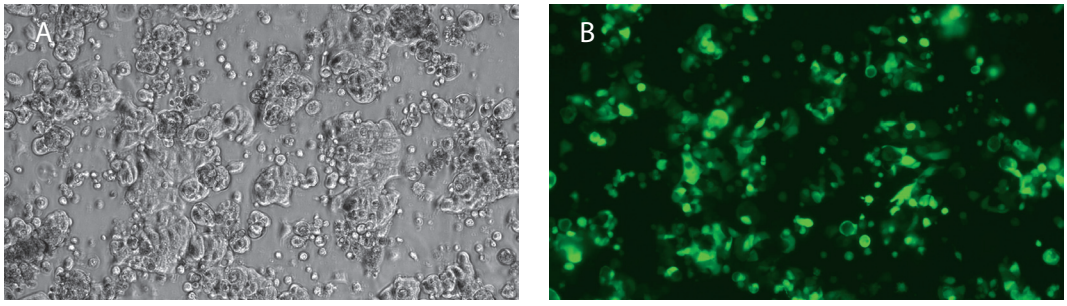
Amaxa® Cell Line Nucleofector® Kit V

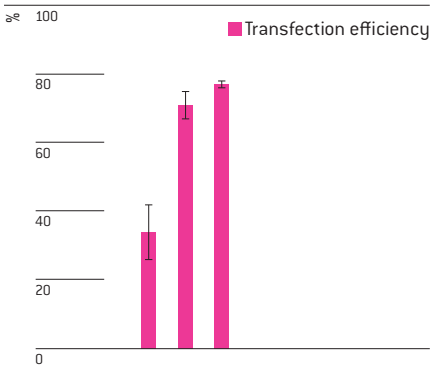
For MCF7

Human mammary gland adenocarcinoma cell line; adherent epithelial

Example for Nucleofection® of MCF7 with eGFP cDNA



MCF7 cells were transfected using the Cell Line Nucleofector® Kit V, program P-020 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. 24 hours post Nucleofection® the cells were analyzed by light (A) and fluorescence microscopy (B).



Average transfection efficiency of MCF7 cell line. Cells were transfected with Nucleofector® Program P-020 and 2 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. 5, 24 and 48 hours post Nucleofection® cells were analyzed by flow cytometry. Cell viability is around 60% 24 hours post Nucleofection®.

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:** 0.05% trypsin/0.02% EDTA and supplemented culture media or PBS/0.5% BSA
- **Culture medium:** Eagle's Minimum Essential Media (EMEM) , 0.01 mg/ml bovine insulin and 10% fetal calf serum (FCS))
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml media per sample)
- Appropriate number of cells (2 x 10^6 cells per sample)
  Minimal cell number: 8 x 10^5 cells (a lower cell number may lead to a major increase in cell mortality)
  Maximum cell number: 4 x 10^6

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media 2 times a week (30 ml per 162 cm^2 flask)
1.2 Cells should be passaged at 75 – 80% confluency
1.3 Seed out 2 x 10^5 cells/cm^2
1.4 Subculture 3 – 4 days before Nucleofection®
1.5 For Nucleofection® Cells should be 75 – 80% confluent

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.7 For harvesting, incubate the cells at 37°C with e.g. 0.05% trypsin/0.02% EDTA
1.8 Inactivate trypsinization reaction with supplemented culture media or PBS/0.5% BSA

2. Nucleofection®

One Nucleofection® Sample contains

2 x 10^6 cells
1 – 2 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM 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 1.5 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 1.6 – 1.8)

2.4 Count an aliquot of the trypsinized cells and determine cell density

2.5 Centrifuge the required number of cells (2 x 10^6 cells per sample) at 90xg for 10 minutes at room temperature

2.6 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample. As leaving cells in Nucleofector® Solution for extended periods of time (longer than 15 minutes) may lead to reduced transfection efficiency and viability it is important to work as quickly as possible

2.7 Combine 100 µl of cell suspension with **1 – 2 µg** DNA 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 **P-020** for high transfection efficiency or **E-014** for high viability and short term expression (P-20 or E-14 for Nucleofector® I Device)

2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program

2.11 Take the cuvette out of the holder once the program is finished

2.12 Add ~500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the 6-well plate (final volume 1.5 ml media per well/sample). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

3.1 Incubate the cells in a humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable already 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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