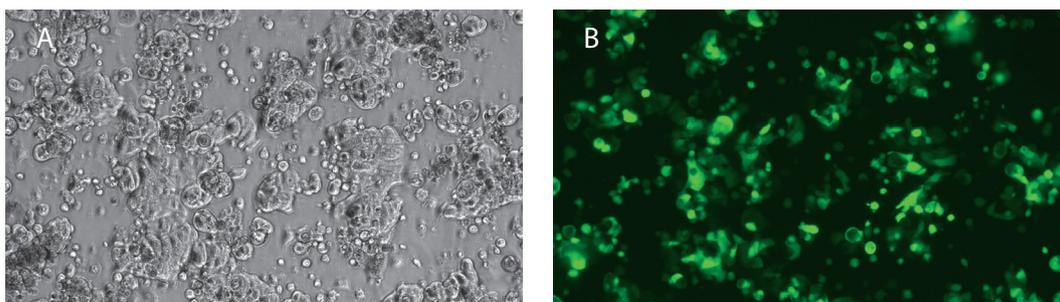


Amaxa[®] Cell Line Nucleofector[®] Kit V

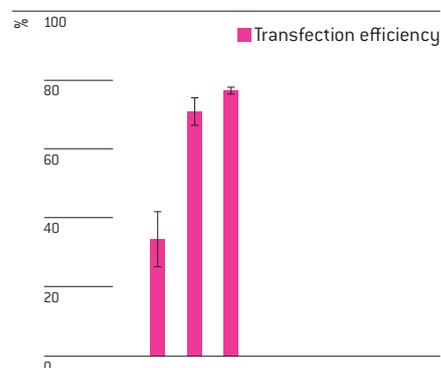
For MCF7

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 10⁶ cells per sample)
Minimal cell number: 8 x 10⁵ cells (a lower cell number may lead to a major increase in cell mortality)
Maximum cell number: 4 x 10⁶

1. Pre Nucleofection®

Cell culture recommendations

- 1.1 Replace media 2 times a week (30 ml per 162 cm² flask)
- 1.2 Cells should be passaged at 75 – 80% confluency
- 1.3 Seed out 2 x 10⁵ cells/cm²
- 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 10⁶ cells

1 – 2 µg plasmid DNA (in 1 – 5 µl H₂O 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% CO₂ 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 10⁶ 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% CO₂ 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

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

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