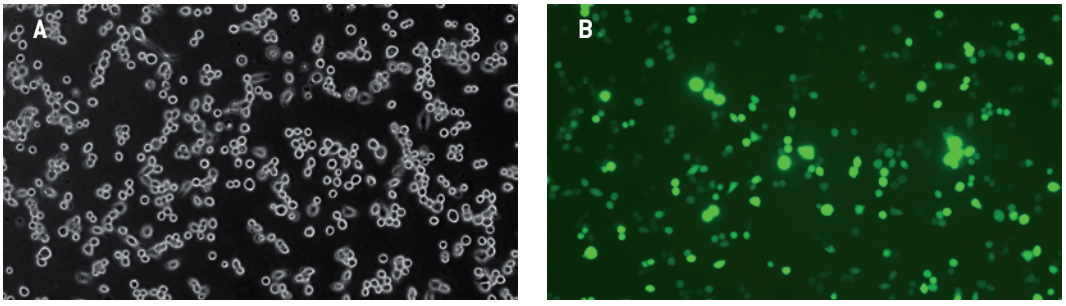
Amaxa® Cell Line Nucleofector® Kit L

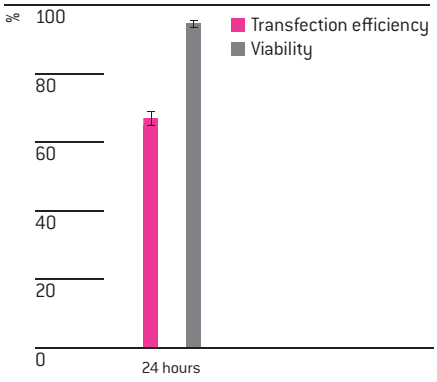
For HeLa S3 [ATCC® CCL-2.2™, cryopreserved]

Human cervix adenocarcinoma; adherent epithelial cell line

Example for Nucleofection® of HeLa S3 cells



HeLa S3 cells [ATCC® CCL-2.2™] were transfected using the Cell Line Nucleofector® Kit L, program V-001 and 2 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed by light (A) and fluorescence microscopy (B).



Average transfection efficiency and viability of HeLa S3 cells. HeLa S3 ATCC® CCL-2.2™] cells were transfected using Nucleofector® Program V-001 and 2 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed by flow cytometry. Cell viability was determined as % PI negative cells.

Product Description

Cat. No.	**VCA-1005**
Size (reactions)	25
Cell Line Nucleofector® Solution L	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
- 12-well culture dish or culture system of your choice
- **For detaching cells:** 0.5 mg/ml trypsin; 0.2 mg/ml EDTA in PBS and supplemented culture media or PBS/0.5% BSA
- **Culture medium**: Ham's F12K media with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 90% [ATCC® 30-2004]; fetal bovine serum, 10% [ATCC® 30-2020])
- 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); lower or higher cell numbers may influence transfection results

1. Pre Nucleofection®

Cell culture recommendations

1.1 Replace media every 2 – 3 days
1.2 Cells should be passaged three times a week
1.3 Seed out 2 – 3 x 10^6 cells per T162 flask
1.4 For Nucleofection® cells should be passaged 1 day before
1.5 Cells will not reach a 100% confluency growing in a flask

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. 0.5 mg/ml trypsin; 0.2mg/ml EDTA in PBS
1.8 Inactivate trypsinization reaction with supplemented culture media or PBS/0.5% BSA

2. Nucleofection®

One Nucleofection® Sample contains

1 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 L

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 12-well plates by filling appropriate number of wells with 1.5 – 2 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 (1 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
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 **V-001** (V-01 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 12-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

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

References

1. Johnson JL et al, Traffic 2005; 6(8): 667-681.
2. Voss OH et al, J Biol Chem 2005; 280(17): 17371-17379

Lonza Cologne AG
50829 Cologne, Germany

DCL-1017_2009-05