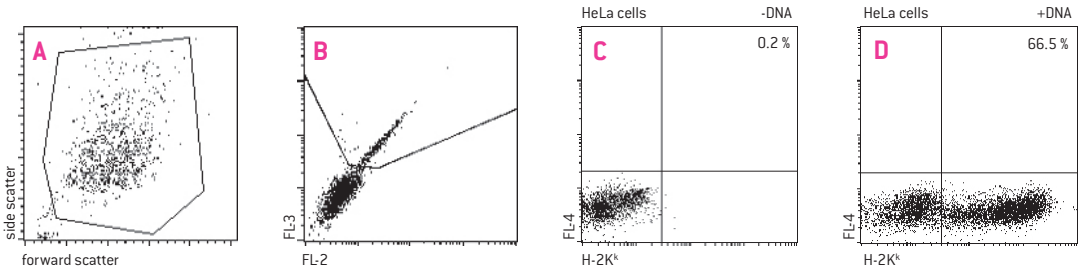
# Amaxa® Cell Line Nucleofector® Kit R

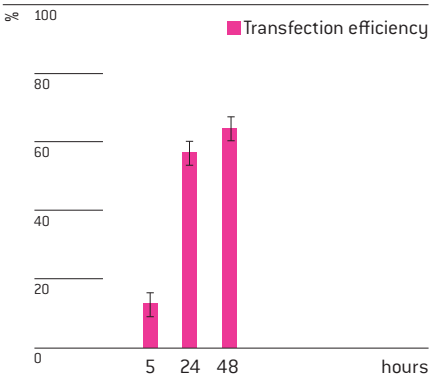
## For HeLa cells [DSMZ, Cat. No. ACC57]

Human cervix carcinoma (HeLa); epitheloid cells in monolayers

### Example for Nucleofection® of HeLa cells with H-2K$^k$ cDNA




**HeLa cells (DSMZ ACC57) were transfected with the Nucleofector® Kit R, Program A-028 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K$^k$.** Cells were analyzed 24 hours post Nucleofection®. Cells were stained with a Cy5-coupled antibody directed against H-2K$^k$ and were analyzed by flow cytometry. HeLa cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2K$^k$ expression is shown after Nucleofection® without (C) and with plasmid DNA (D).




**Average transfection efficiency of HeLa cells.** HeLa cells (DSMZ ACC57) were transfected with program A-028 and 5 µg of plasmid encoding the mouse MHC class I heavy chain molecule H-2K$^k$. Cells were analyzed 5, 24 and 48 hours post Nucleofection® by flow cytometry. Cell viability is around 95%.

## 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
- 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**: 90% MEM (Minimum Essential Medium with Earle's salts) with 2 mM Ultraglutamine I [Lonza, Cat. No. BE17-605E/U1], 100 U/ml Penicillin, 100 µg/ml Streptomycin and 10% FCS
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells ($5 \times 10^5$ – $1 \times 10^6$ cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection®

### Cell culture recommendations

1.1 Replace medium 2 – 3 times a week (20 – 30 ml medium per 162 $cm^2$ flask)
1.2 Passage cells after reaching 70 % confluency
1.3 Seed out 6 – 7 x $10^4$ cells per 25 $cm^2$ flask
1.4 Subculture 3 days before Nucleofection®
1.5 Cells should be transfected after reaching 70% confluency

### 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 ~5 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.8 Neutralize trypsinization reaction with supplemented culture medium or PBS/0.5% BSA once the majority of the cells (>90%) have been detached

## 2. Nucleofection®

**One Nucleofection® Sample contains**

| |
|---|
| 5 x $10^5$ – 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 1.6 – 1.8)
2.4 Count an aliquot of the cells and determine cell density
2.5 Centrifuge the required number of cells (5 x $10^5$ – 1 x $10^6$ cells per sample) at 200xg for 10 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 **A-028** (**A-28** 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

## 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-1001_2009-05