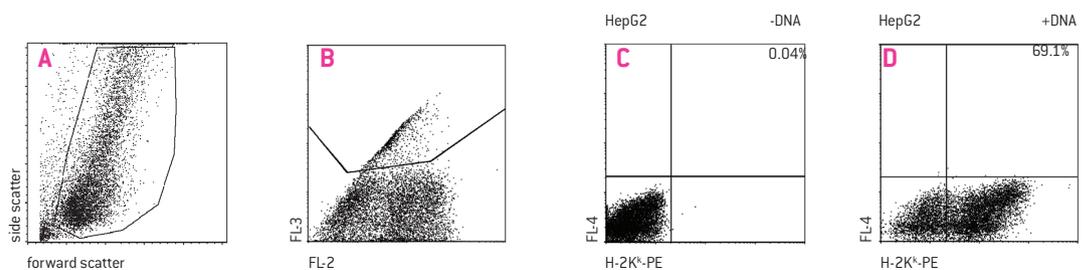


## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit V

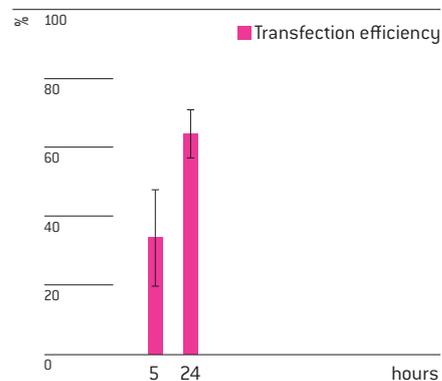
### For HepG2

Human hepatocellular liver carcinoma; epithelial cells

#### Example for Nucleofection<sup>®</sup> of HepG2 cells



HepG2 cells were transfected with the Cell Line Nucleofector<sup>®</sup> Kit V, Program T-028 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K<sup>k</sup>. Cells were stained 24 hours post Nucleofection<sup>®</sup> with a PE-coupled antibody directed against H-2K<sup>k</sup> and analyzed by flow cytometry. HepG2 cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2K<sup>k</sup> expression is shown after Nucleofection<sup>®</sup> without (C) and with plasmid DNA (D).



Average transfection efficiency of HepG2 cells. HepG2 cells were transfected with program T-028 and 5 µg of a plasmid encoding the mouse MHC class I heavy chain molecule H-2K<sup>k</sup>. Cell Viability is around 80 – 90%.

### Product Description

|  |  |
|--|--|
| Cat. No.   | VCA-1003   |
| Size (reactions)   | 25   |
| Cell Line Nucleofector <sup>®</sup> Solution V               | 2.25 ml (2.05 ml + 10% overfill)   |
| Supplement   | 0.5 ml (0.45 ml + 10% overfill)  |
| pmaxGFP <sup>®</sup> 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 <sup>®</sup> Solution, Supplement and pmaxGFP <sup>®</sup> Vector at 4°C. For long-term storage, pmaxGFP <sup>®</sup> Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector <sup>®</sup> Supplement is added to the Nucleofector <sup>®</sup> 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:** Minimum Essential Medium with Earle's BSS (Lonza Cat. No. BE12-125F) and 2 mM UltraGlutamine I [Lonza, Cat. No. BE17-605E/U1], 0.1 mM neAA; 1 mM Sodium pyruvate; 10% FCS
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (1 x 10<sup>6</sup> cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection®

### Cell culture recommendations

- 1.1 Passage cells at 80% confluency. HepG2 cells should not be used for Nucleofection® after passage number 20
- 1.2 Seed out 1 – 2 x 10<sup>4</sup> cells/cm<sup>2</sup>
- 1.3 Subculture 4 – 5 days before Nucleofection®
- 1.4 Optimal confluency for Nucleofection®: 70 – 80%. Higher cell densities may cause lower Nucleofection® Efficiencies

### Trypsinization

- 1.5 Remove media from the cultured cells and wash cells once 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 or PBS/0.5% BSA once the majority of the cells (>90%) have been detached

### 2. Nucleofection®

#### One Nucleofection® Sample contains

|  |
|--|
| 1 x 10 <sup>6</sup> cells  |
| 5 µg plasmid DNA (in 1 – 5 µl H <sub>2</sub> O or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM 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 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> 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 10<sup>6</sup> 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 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 H-022 (for high viability) or T-028 (for high expression level) (H-22 or T-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<sub>2</sub> 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](http://www.lonza.com/nucleofection-citations)

For more technical assistance, contact our Scientific Support Team:

|  |  |
|--|--|
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## References

1. Forges M et al. [2003]. Mol Cell Biol.;23(15):5282-92.

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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

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