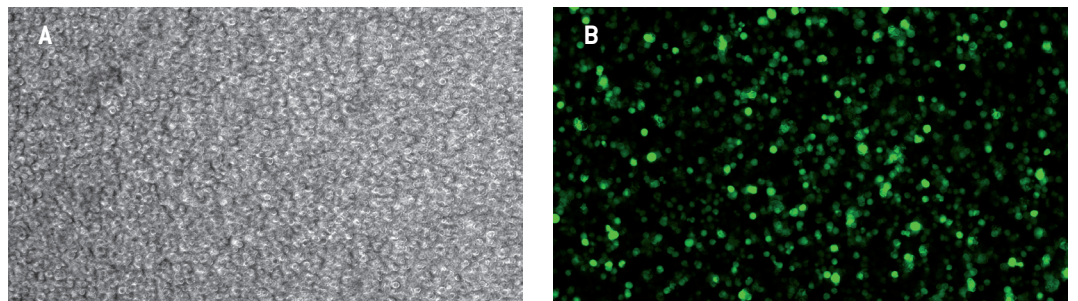


## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit C

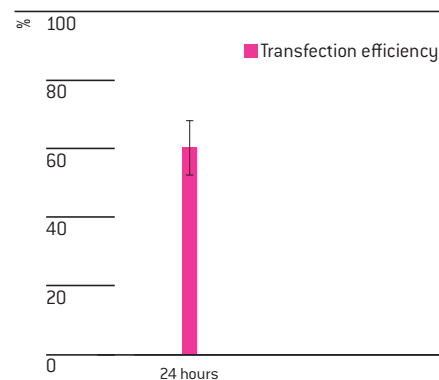
### For T2

Human cloned hybrid between B-lymphoblast and T-lymphoblast; lymphoblastoid cells

#### Example for Nucleofection<sup>®</sup> of T2 cells



T2 cells were transfected with the Nucleofector<sup>®</sup> Kit C, program A-030 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> using light (A) and fluorescence microscopy (B).



**Average transfection efficiency of T2 cells.** T2 cells were transfected with program A-30 or A-030 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell viability (compared to non-transfected control) is around 68% 24 hours post Nucleofection<sup>®</sup>.

### Product Description

Cat. No.	VCA-1004
Size (reactions)	25
Cell Line Nucleofector <sup>®</sup> Solution C	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
- 12-well culture dish or culture system of your choice
- **Culture medium:** Iscove's Modified Dulbecco's Medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 80%; fetal bovine serum, 20%
- Prewarm appropriate volume of culture medium to 37°C (2.0 ml per sample)
- Appropriate number of cells (2 x 10<sup>6</sup> cells per sample; lower or higher cell numbers may influence transfection results)

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### 1. Pre Nucleofection®

#### Cell culture recommendations

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- 1.1 Passage cells 3 times a week
- 1.2 Maintain cultures between 3 x 10<sup>5</sup> and 1 x 10<sup>6</sup> viable cells/ml
- 1.3 Seed out 3 x 10<sup>5</sup> cells/ml
- 1.4 Subculture 2 days before Nucleofection®

### 2. Nucleofection®

#### One Nucleofection® Sample contains

2 x 10 <sup>6</sup> cells
2 µg plasmid DNA (in 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 Nucleofector® Solution C

- 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 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.3 Count an aliquot of the cells and determine cell density
- 2.4 Centrifuge the required number of cells (2 x 10<sup>6</sup> cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely
- 2.5 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.

- 2.6 Combine 100 µl of cell suspension with 2 µg DNA, 2 µg pmaxGFP® Vector or 30 nM – 300 nM siRNA (3 – 30 pmol/sample) or other substrates
- 2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
- 2.8 Select the appropriate Nucleofector® Program A-030 (A-30 for Nucleofector® I Device)
- 2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program by pressing the X-button
- 2.10 Take the cuvette out of the holder once the program is finished
- 2.11 Immediately add ~500 µl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 12-well plate (final volume 2 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)

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