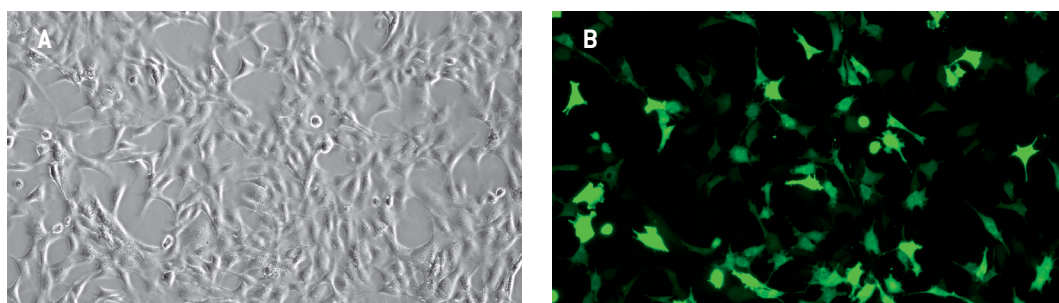


## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit V

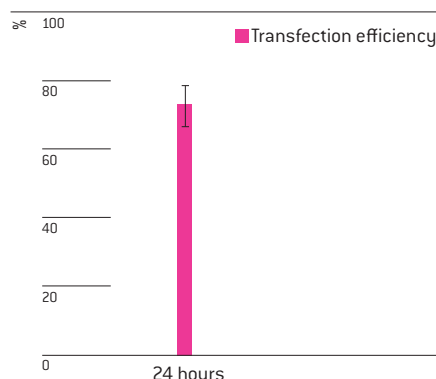
For undifferentiated 3T3-L1 [ATCC<sup>®</sup> CL-173<sup>™</sup>, cryopreserved]

Mouse embryonal fibroblast; fibroblastoid cells

Example for Nucleofection<sup>®</sup> of 3T3-L1 cells



3T3-L1(pre-ad) cells [ATCC<sup>®</sup> CL-173<sup>™</sup>] were transfected with the Cell Line Nucleofector<sup>®</sup> Kit V, Program T-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 3T3-L1 (pre-ad) cells. 3T3-L1 (pre-ad) cells [ATCC<sup>®</sup> CL-173<sup>™</sup>] were transfected with program T-030 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell viability is around 59% 24 hours post Nucleofection<sup>®</sup>.

### 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; Software requirements: version V2.3 or higher for Nucleofector® I Device; version S3-4 or higher for Nucleofector® II 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:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose [ATCC®, Cat. No. 30-2002], 90%; bovine calf serum, 10% [ATCC®, Cat. No. 30-2030]
- 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 Replace media every 2 – 3 days
- 1.2 Passage cells 3 times a week at 85 % confluency. Avoid confluency
- 1.3 Seed out 4 x 10<sup>5</sup> cells/T75 flask (see ATCC® protocol)
- 1.4 Subculture 2 days before Nucleofection®
- 1.5 Optimal confluency for Nucleofection®: 80%. Higher cell densities may cause lower Nucleofection® Efficiencies

### 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 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

1 x 10 <sup>6</sup> cells
2 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 1.6 – 1.8)
- 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 90xg 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 2 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 T-030 (T-30 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)

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