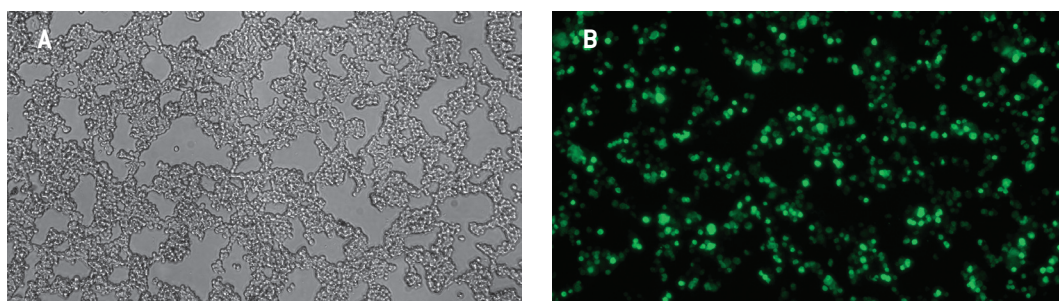


## Amaxa<sup>®</sup> Cell Line Nucleofector<sup>®</sup> Kit V

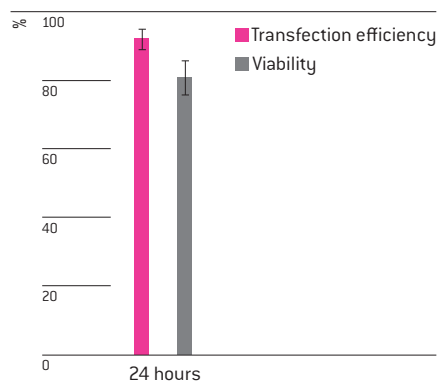
For PC-12

Pheochromocytome cells from rat adrenal gland; polygonal cells

Example for Nucleofection<sup>®</sup> of PC-12 cells



PC-12 cells were transfected with the Cell Line Nucleofector<sup>®</sup> Kit V, Program U-029 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 PC-12 cells. PC-12 cells were transfected with program U-029 and 2 µg of pmaxGFP<sup>®</sup> Vector. Cells were analyzed 24 hours post Nucleofection<sup>®</sup> by flow cytometry. Cell Viability was measured by using the CellTiter-Blue<sup>™</sup> Assay (Promega).

### 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
- 12-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:** F-12K Medium, Kaighn's Modification of Ham's F-12 Medium supplemented with 2mM L glutamine, 1.5 g/l sodium bicarbonate, 15% Horse serum and 2.5% FCS
- Prewarm appropriate volume of culture medium to 37°C (1.9 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)

### 1. Pre Nucleofection®

#### Cell culture recommendations

- 1.1 Culture cells in T75 or T162 flasks
- 1.2 Passage cells every 3 – 4 days. High passage numbers (above 20) will reduce transfection efficiency and viability
- 1.3 Seed out 1 – 3 x 10<sup>4</sup> cells/cm<sup>2</sup>
- 1.4 Subculture 3 – 5 days before Nucleofection®

- Note**
1. PC-12 cells adhere poorly to plastic and tend to grow in small clusters of loosely attached cells. The attachment of PC-12 cells can be enhanced by coating the culture vessels with bovine collagen or using Corning® CellBIND® Surface Flasks. If cells are loosely attached resuspend them by pipetting for passaging
  2. To obtain single cell suspension pass the PC-12 clusters through a 22-gauge needle (10 – 15 times)
  3. If PC-12 cells grow adherent you may passage them by trypsin treatment or by scraping (see below)

#### For trypsin treatment:

- 1.5 Remove and discard culture medium and replace it by Trypsin/EDTA
- 1.6 Incubate for 5 minutes at 37°C and stop the reaction by adding fresh medium
- 1.7 Resuspend cells and centrifuge the suspension at 200xg for 10 minutes
- 1.8 Remove supernatant and resuspend cells in 5 ml of fresh medium. Singularize cells by passing them 10 – 15 times through a 22 -gauge needle

### For scraping:

- 1.9 Remove and discard culture medium and replace it by fresh medium
- 1.10 Add 10 ml of fresh medium and scrape cells from the vessel surface
- 1.11 Resuspend cells and centrifuge the suspension at 200xg for 10 minutes
- 1.12 Remove supernatant and resuspend cells in 5 ml of fresh medium. Singularize cells by passing them 10 – 15 times through a 22-gauge needle

## 2. Nucleofection®

### One Nucleofection® Sample contains

2 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 12-well plates by filling appropriate number of wells with 1.4 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.3 If PC-12 cells grow adherent, harvest the cells by trypsinization or scraping (please see 1.5 – 1.12)
- 2.4 Count an aliquot of the cells and determine cell density
- 2.5 Centrifuge the required number of cells (2 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 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 U-029 (U-29 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 12-well plate (final volume 1.9 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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