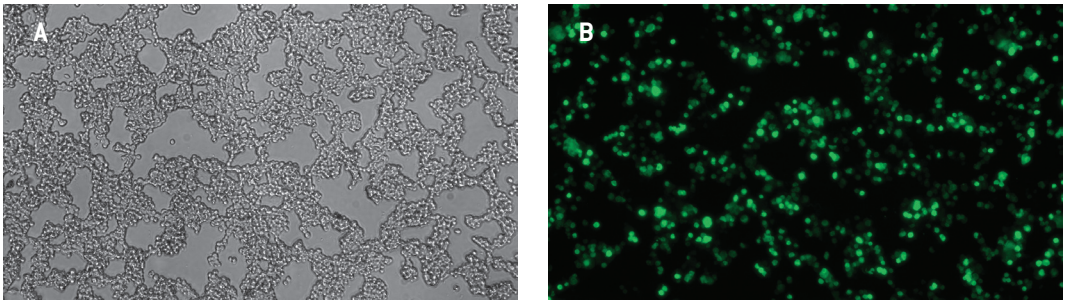
Amaxa® Cell Line Nucleofector® Kit V

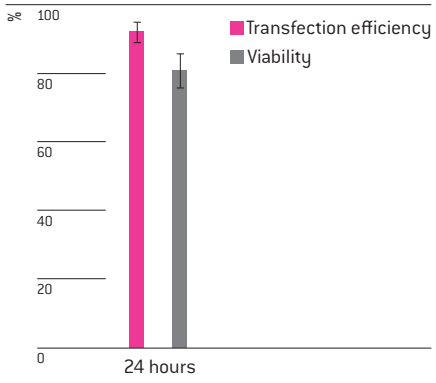
For PC-12

Pheochromocytome cells from rat adrenal gland; polygonal cells

Example for Nucleofection® of PC-12 cells



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

Product Description

Cat. No.	**VCA-1003**
Size (reactions)	25
Cell Line Nucleofector® Solution V	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
- 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 10^6 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 10^4 cells/cm^2
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 10^6 cells
2 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 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_2 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 10^6 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 **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_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

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