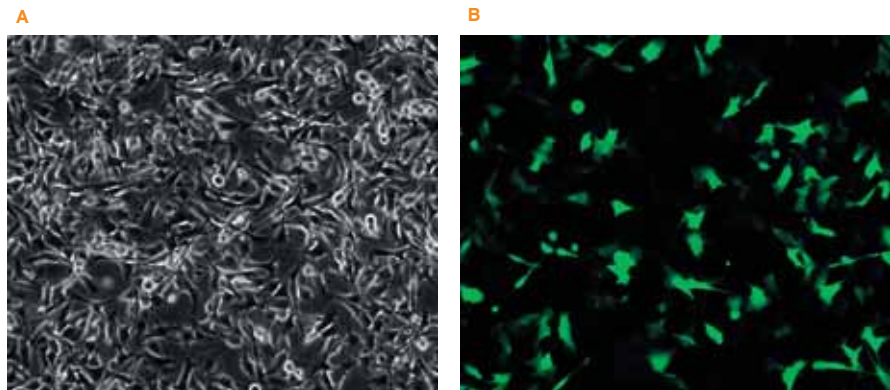
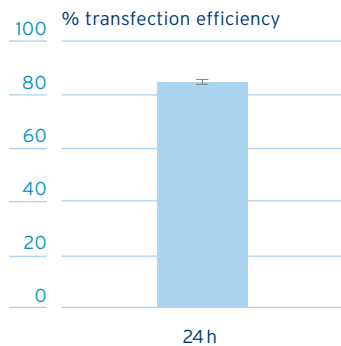


## Cell Line Nucleofector® Kit R

for B16-F0 Cell Line [ATCC]

<b>Cell type</b>	<b>Origin</b>	Mouse skin cell, melanoma [ATCC®; CRL-6322; frozen vial].
	<b>Morphology</b>	Spindle shaped cell.

### Example for nucleofection® of B16-F0 cells.



**Average transfection efficiencies of B16-F0 cells.** Cells were nucleofected with program **P-31/P-031** and 2 µg of pmaxGFP™. **24 hours** post nucleofection, the cells were analyzed by flow cytometry. Cell viability (% PI negative cells) is around 90% 24h post nucleofection.

B16-F0 cells were nucleofected using the Cell Line Nucleofector Kit R, program **P-31/P-031** and 2 µg of pmaxGFP. **24 hours** post nucleofection the cells were analyzed by fluorescence microscopy.

Chapter	Contents
<b>1</b>	Procedure outline & important advice
<b>2</b>	Product description
<b>3</b>	Protocol
	3.1 › Required reagents
	3.2 › DNA preparation and quality
	3.3 › Cell culture
	3.4 › Important controls and vector information
	3.5 › Nucleofection protocol
<b>4</b>	Recommended literature

1

**Procedure outline & important advice**

1.



**Procedure outline**

Culturing of cells before nucleofection.  
(For details see 3.3.)

**Important advice**

- › Replace medium every 2-3 days.
- › Cells should be passaged every 2-3 days.
- › Subculture ratio 1:6-1:15.
- › Passage 1-2 days before nucleofection.

2.



Combine the cells of interest, DNA or siRNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette.  
(For details see 3.5.)

**Contents of one nucleofection sample:**

- › 1 x 10<sup>6</sup> cells (optimal cell number)
- › 2 µg highly purified plasmid DNA (in max. 10 µl) or 0.5 - 3 µg siRNA
- › 100 µl Nucleofector Solution R

Perform each sample separately to avoid storing the cells longer than 15 min in Nucleofector Solution R.

3.

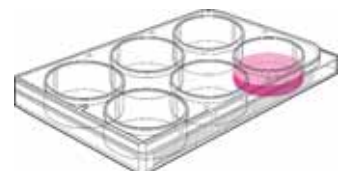


Choose the cell-type specific program. Insert the cuvette into the Nucleofector and press the "X" button to start the program.  
(For details see 3.5.)

- › Optimal Nucleofector program:  
**P-31\*/P-031\*\***

- › \*for Nucleofector I Device
- › \*\*for Nucleofector II Device

4.



Rinse the cuvette with culture medium using an amaxa certified pipette. Transfer the cells into the culture dish.  
(For details see 3.5.)

- › **After program has finished incubate the sample in the cuvette at room temperature for 10 min.**

- › Take a 500 µl aliquot of the prewarmed medium and add it to the cuvette. Immediately transfer the medium with cells back to the culture dish and incubate at 37°C/5% CO<sub>2</sub>.

**2**

**Product description**

Cat. No.	<b>VCA-1001</b>
Kit components	2.25 ml <b>Cell Line Nucleofector® Solution R</b> 0.5 ml Supplement 1 30 µg pmaxGFP™ (0.5 µg/µl in 10 mM Tris pH 8.0) 25 certified cuvettes 25 plastic pipettes
Size	25 reactions
Storage and stability	Store Nucleofector Solution, Supplement and pmaxGFP at 4°C. For long term storage pmaxGFP is ideally stored at -20°C. The expiry date is printed on the Solution Box.

**3**

**Protocol**

**3.1**

› **Required reagents**

**Medium**

Dulbecco's modified eagle's medium with 4 mM L-glutamine adjust to contain 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 90% [ATCC; Cat. No. 30-2002]; fetal bovine serum, 10% [ATCC; Cat. No. 30-2020].

**Trypsin**

0.5 mg/ml Trypsin; 0.2 mg/ml EDTA in PBS.

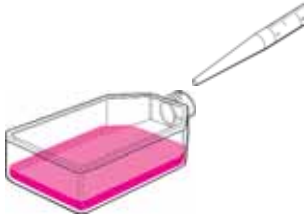
**Treatment**

**3.2**

› **DNA preparation and quality**

The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit]. The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN protocol.





### 3.3 › Cell culture

**Culture conditions** Replace medium every 2-3 days.

**Passage interval** Cells should be passaged every 2-3 days. A subcultivation ratio of 1:6 to 1:15 is recommended.

**Seeding conditions**  $1.3-3 \times 10^6$  cells per T162 cm<sup>2</sup> flask.

#### **Culture conditions before nucleofection**

- › The cells should be preferably passaged 1-2 days before nucleofection. A subculture ratio of 1:6 to 1:15 is recommended.
- › **Maximum confluency 70-80%. Do not allow the cells to become over 80% confluent as it causes the cells to detach.**

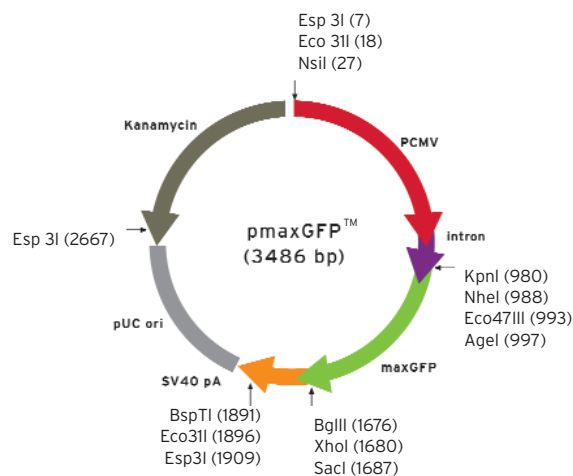
#### **Note**

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Normocin™ [Cat. No. VZA-1001], a new antibiotic formulation specifically developed to protect sensitive cell lines from mycoplasma infection and microbial contaminations. For more information and ordering info see [www.amaxa.com/antibiotics](http://www.amaxa.com/antibiotics).

### 3.4 › Important controls and vector information

#### **Positive control**

We strongly recommend establishing the Nucleofector technology with the positive control vector **pmaxGFP** as provided in this kit. pmaxGFP encodes the green fluorescent protein (GFP) from copepod *Pontellina p.* Just like eGFP expressing cells, maxGFP expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



### Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection or amount/purity of DNA on cell viability.

**control 1** Recommended amount of cells in Nucleofector Solution with DNA but without application of the program (alternatively: untreated cells)  
**(Cells + Solution + DNA - program)**

**control 2** Recommended amount of cells in Nucleofector Solution without DNA with application of the program **(Cells + Solution - DNA + program)**

### Vector information

If using IRES sequences in your vectors, please remember that the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some cell types may not be expressed at all. As alternatives we suggest either: co-transfecting two (or more) plasmids, using one plasmid with each gene under the control of its own promoter, or making a GFP fusion.

## 3.5 › Nucleofection protocol

<b>Preparation of Nucleofector Solution</b>	Add 0.5 ml Supplement to 2.25 ml Nucleofector Solution and mix gently. The Nucleofector Solution is now ready to use and is stable for 3 months at 4°C. <b>Note the date of addition on the vial.</b>
<b>One nucleofection sample contains</b>	› <b>1 x 10<sup>6</sup> cells</b> › <b>2 µg plasmid DNA (in 1-5 µl H<sub>2</sub>O or TE) or 2 µg pmaxGFP or 0.5-3µg siRNA</b> › <b>100 µl Nucleofector Solution R</b>
	For more details about the nucleofection of siRNA: <a href="http://www.amaxa.com/RNAi">www.amaxa.com/RNAi</a>

### Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **2 µg** DNA or **0.5 - 3 µg** siRNA for each sample.
3. Pre-warm the supplemented Nucleofector Solution **R** to room temperature. Pre-warm an aliquot of culture medium at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6-well plates by filling appropriate number of wells with 1 ml of culture medium containing supplements and serum. Pre-incubate plates in a humidified 37°C/5% CO<sub>2</sub> incubator.
5. Remove the medium from the cell culture. Wash cells once with PBS.

6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with supplemented culture medium or PBS/0.5% BSA (see Nucleofector Manual for details).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**1 x 10<sup>6</sup>** cells per nucleofection sample) at **90xg at room temperature for 10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature Nucleofector Solution **R** to a final concentration of **1 x 10<sup>6</sup> cells/100 µl**. Avoid storing the cell suspension longer than **15 min** in Nucleofector Solution as this reduces cell viability and gene transfer efficiency.  
**Important: Steps 10-15 should be performed for each sample separately.**



#### **Nucleofection**

10. Mix 100 µl of cell suspension with **2 µg** DNA or **0.5-3 µg** siRNA.
11. Transfer the nucleofection sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Select the appropriate Nucleofector program, **P-31/P-031** (see Nucleofector Manual for details). Insert the cuvette into the cuvette holder (Nucleofector I : rotate carousel to final position) and press the "X" button to start the program.
13. After the program has finished (display showing "OK") take the cuvette out of the holder **and incubate the sample in the cuvette for 10 min at room temperature**.
14. After the post nucleofection incubation step, add 500 µl of the prewarmed culture medium to the cuvette and gently transfer the sample into the prepared 6-well plates. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells.
15. Press the "X" button to reset the Nucleofector.
16. Repeat steps 10-15 for the remaining samples.
17. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6-well plates.
18. Incubate cells in a humidified 37°C/5% CO<sub>2</sub> incubator. Following nucleofection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 4-8 hours. If this is not the case, the incubation period may be prolonged up to 24 hours.



#### **Cultivation after nucleofection**

## Recommended literature

For an up-to-date list of all Nucleofector references, please refer to:  
[www.amaxa.com/citations](http://www.amaxa.com/citations)

- \* amaxa's Nucleofector® process, Nucleofector® device and Nucleofector® Solutions are covered by PCT applications PCT/EP01/07348, PCT/DE02/01489, PCT/DE02/01483 and other pending patents and domestic or foreign applications corresponding thereto.
- \* amaxa, Nucleofector, nucleofection and maxGFP are trademarks of amaxa GmbH.
- \* This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this amaxa product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this amaxa product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at [license@evrogen.com](mailto:license@evrogen.com).
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