

## Basic Nucleofector® Kit

for Primary Smooth Muscle Cells (SMC)

<b>Cell type</b>	<b>Origin</b>	This protocol is designed for primary mammalian smooth muscle cells from various organs.
	<b>Morphology</b>	Long tapering cells.

### Foreword

amaxes Nucleofector technology allows non-viral gene transfer directly into the nucleus using specific Nucleofector Solutions and applying specific electrical parameters (programs), delivered by the Nucleofector device. Optimized Protocols for numerous primary cells are already available from amaxa - to view an up-to-date list refer to our website at [www.amaxa.com/products](http://www.amaxa.com/products).

Please find some guidelines on SMC culture for nucleofection® and on the transfection procedure using our Basic Nucleofector® Kit below. However, **we recommend referring to more detailed culture protocols before you start the experiments.** Having tested various smooth muscle cell types, high transfection efficiencies could be achieved using one of the programs indicated below. In the event that you do not attain satisfying results with your SMC of interest please contact our Scientific Support Team for further help with the optimization.

<b>Chapter</b>	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	› Preparation of cells and cell culture
3.4	› Important controls
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

- › Do not use cells after passage 15 for nucleofection.
- › Cells should be nucleofected at 80% confluency. Higher confluency may reduce the number of viable cells, later.

2.



Combine the cells of interest, DNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette.  
(For details see 3.3.)

### Contents of one nucleofection sample:

- › 0.5-1 x 10<sup>6</sup> cells (optimal cell number).
- › 1-5 µg highly purified plasmid DNA or 2 µg pmaxGFP.
- › 100 µl Basic Nucleofector Solution.

Perform each sample separately to avoid storing the cells longer than 20 min in Basic Nucleofector Solution.

3.



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

- › For optimal results the following Nucleofector programs should **ALL** be tested:

**A-33, D-33, P-13, P-24, and U-25.**

4.



Rinse the cuvette with culture medium and transfer the cells into the culture dish.  
(For details see 3.3.)

- › Remove sample from the cuvette immediately.
- › Use amaxa certified pipette.
- › Transfer directly to 37°C.

**2**

**Product description**

Cat. No.	<b>VPI-1004</b>
Kit components	2.25 ml <b>Nucleofector® Solution</b> 0.5 ml Supplement 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 and Supplement at 4°C, pmaxGFP™ at -20°C. The expiry date is printed on the Solution Box.

**3**

**Protocol**



**3.1** › **Required reagents**

**Medium**

We recommend using a medium especially suited for the culture of primary smooth muscle cells, e.g. SmGM Bullet Kit [Cambrex, Cat. No. CC-3182]. Alternatively, DMEM supplemented with 10% FCS may be used.

**Trypsin treatment**

We recommend using Reagent Pack™ Subculture Reagent Kit containing trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS).[Clonetics/BioWhittaker; Cat. No. CC-5034], final trypsin concentration 0.25 %. If cells are very difficult to detach, trypsin 0.5 % - EDTA 0.2 % [e.g. Invitrogen/ Gibco Cat.No. 35400-019] may be used.



**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.





## Cell culture

### 3.3 › Preparation of cells and cell culture

- › Replace medium every second day.
- › Cells should be passaged after reaching 80% confluency.
- › **Do not use cells after passage 15** for nucleofection.
- › Cells should be nucleofected at 80% confluency. Higher confluency may reduce the number of viable cells after Nucleofection.

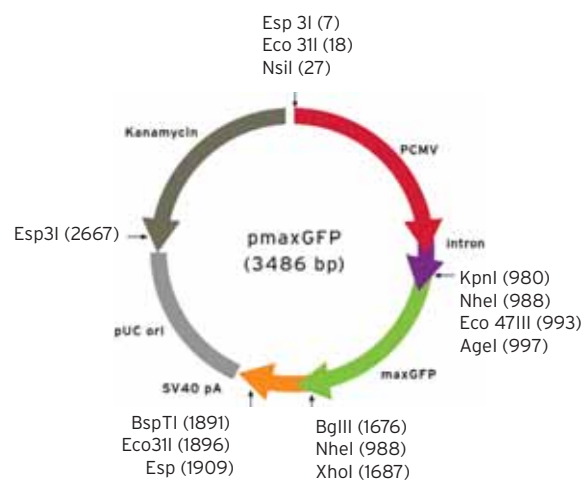
#### Note

Contamination of cell culture with mycoplasma is a widely spread phenomenon that might negatively influence experimental results. We recommend the use of Primocin™ [Cat. No. VZA-1021], a new antibiotic formula specifically developed to protect sensitive primary cells from mycoplasma infection and microbial contaminations. Add it directly to the cell culture medium without further need of Pen/Strep or other antibiotics. For more information and ordering info see [www.amaxa.com/antibiotics](http://www.amaxa.com/antibiotics).

### 3.4 › Important controls

#### 1. 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.



## 2. 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)**

### 3.5 › Nucleofection protocol

#### Preparation of Nucleofector Solution

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.  
**Note the date of addition on the vial.**

#### One nucleofection sample contains

- › **0.5-1 x 10<sup>6</sup> cells**
- › **1-5 µg plasmid DNA (in 1-5 µl H<sub>2</sub>O or TE) or 2 µg pmax GFP or 0.5-3 µg siRNA**
- › **100 µl Nucleofector Solution**

Minimal cell number: 2 x 10<sup>5</sup> cells (a lower cell number may lead to a major increase in cell mortality). Maximum cell number: 2 x 10<sup>6</sup>.

For more details about the nucleofection of siRNA: [www.amaxa.com/RNAi](http://www.amaxa.com/RNAi)

#### Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **1-5 µg DNA** for each sample.
3. Pre-warm the supplemented Nucleofector Solution to room temperature. For rinsing the cuvette pre-warm 500 µl of culture medium containing serum and supplements per sample at 37°C in an Eppendorf tube.
4. Prepare 6 well plates (3 wells/sample) by filling each well with 1 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO<sub>2</sub> incubator.
5. Remove the medium from the tissue culture. Wash cells once with HBSS [Reagent Pack], using at least the same volume of HBSS as culture medium. Aspirate and discard the HBSS.

6. Harvest the cells by trypsinization: Add trypsin-EDTA solution [Reagent Pack] to cover the cell monolayer, and gently swirl the dish/flask to ensure an even distribution of the solution. Place the dish/flask in an incubator at 37°C until the cells start to detach (usually after 10-15 minutes). Remove the dish/flask from the incubator. Tap against the sides of the dish/flask to improve detachment. Carefully check the process under a microscope to avoid overexposure of cells to trypsin. If necessary, prolong the incubation time for two more minutes at 37°C or add trypsin 0.5% - EDTA 0.2%. Once the majority of cells (>90%) have been dislodged, add TNS [Reagent Pack] to inactivate trypsin. Gently resuspend and remove the cells from the flasks by pipetting.
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Take the number of cells required for one Nucleofector Solution (**0.5-1 x 10<sup>6</sup> cells**). Centrifuge the cells at **100 xg** for **1 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in the room temperature Basic Nucleofector Solution to a final concentration of **0.5-1 x 10<sup>6</sup> cells/100 µl**. Avoid storing the cell suspension longer than **20 min** in Nucleofector Solution, as this reduces cell viability and gene transfer efficiency.



#### Nucleofection

**Important: Steps 10-14 should be performed for each sample separately.**

10. Mix 100 µl of cell suspension with **1-5 µg** DNA.
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 (**A-33, D-33, P-13, P-24** or **U-25**) (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. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. 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. Add 500 µl of the pre-warmed culture medium containing serum and supplements and incubate in the Eppendorf tubes at 37°C.
14. Press the "X" button to reset the Nucleofector.
15. Repeat steps 10-14 for the remaining samples.
16. Transfer the samples from the Eppendorf tubes into the prepared 6-well plates.  
**Seed one sample in 3 wells of one 6-well plate.**
17. Incubate cells in a humidified 37°C/5% CO<sub>2</sub> incubator. Change medium 16-18h post nucleofection.



#### Cultivation post 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).
- \* The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.
- \* QIAGEN and EndoFree are trademarks of QIAGEN.