Technical Description

Users Manual

Specification MA1

Rev. 1.0 June, 4. 2004

1. Scope

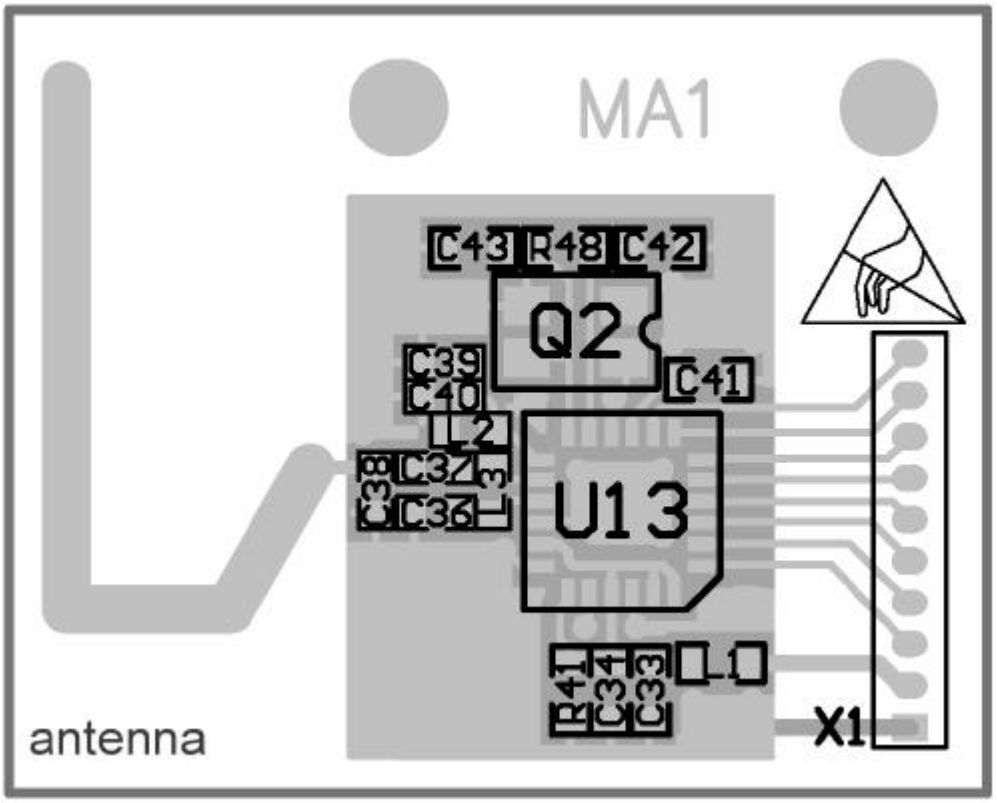
This specification covers the wireless communication module MA1 with integrated RF radio transceiver, power filtering and antenna.

2. product description

The MA1 module is a RF radio transceiver designed for low power short range communication applications.
It enables robust wireless communication in the 2.4 - 2.4835 GHz unlicensed ISM band.
It can be interfaced directly to a microcontroller powered with 3.3V.
The main operating parameters have to be programmed via 8 interface lines.
This specification defines pcb layout, mounting position, schematics and bill of material, electronic and software interface.
Applying these definitions, the module complies with the world-wide regulations covered by EN 300 440 (Europe) and CFR47 Part 15 (US)

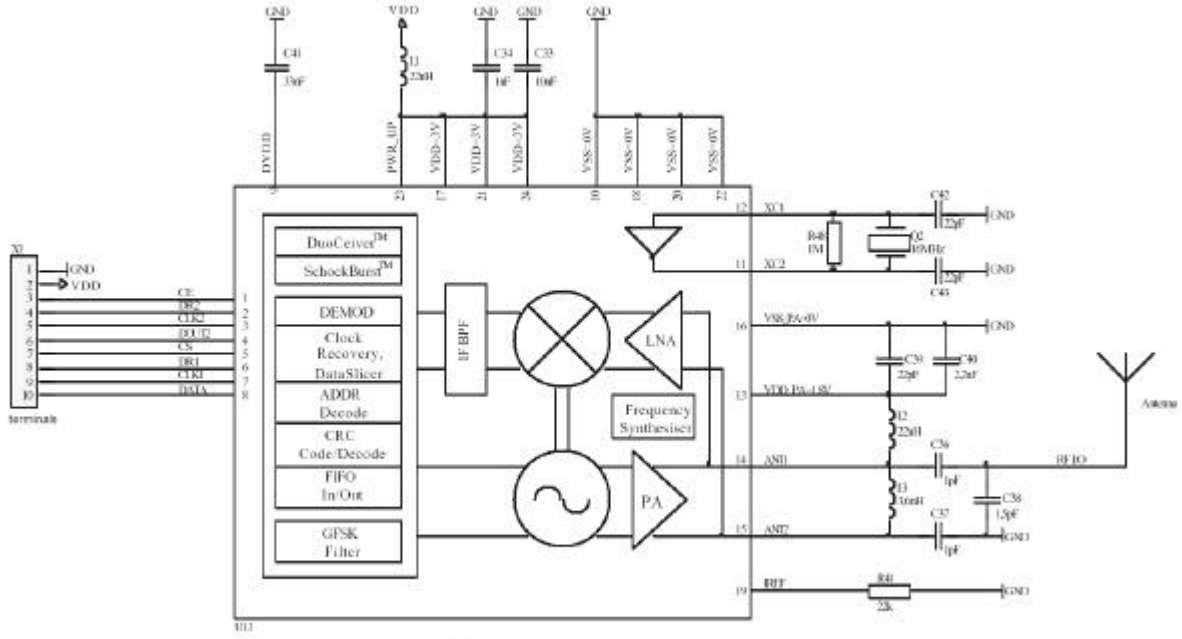
3. mechanical specification

pcb	module size 24mm x 30mm (with terminals) used pcb area 24mm x24 mm (terminal-free design) pcb thickness 1.5mm pcb material: FR4 layout: Cu 35u, covered with solder resist, partly HAL
mounting	Any angle possible
environment	required distance between the antenna and other metallic parts: min. 50mm



4. electrical specification

absolut maximum rating:		
Voltage supply	VDD	3.6V
Storage temperature	T	-20°C to 95°C
interface voltage	VI	-0.3V to VDD+0.3V, max. 3.6V
Interface output current	IO	<0.5mA
Operating conditions:		
Temperature	TOP	0°C to 70°C
Voltage supply	VDD	3.3V ±0.3V, max. noise 50mV
Current consumption	IDD	Max. 13mA
Input low level	VIL	0V to 0.3V
Input ligh level	VIH	VDD-0.3V to VDD
Output low level	VOL	0V to 0.3V (IOL=0.5mA)
Output high level	VOH	VDD-0.3V to VDD (IOH= -0.5mA)
Operating frequency	fOP	2402 and 2410 MHz
Frequency deviation	Df	±156 kHz
Data rate (ShockBurst™)	RGFSK	250kbps
Duty cycle	Dc	≤ 10%



nRF2401

Pos.	Pcs.	Name	Designation	Value	mechanics	manufacturer	Order Code
1	1	U13	Single Chip 2,4GHz Radio Trans.	NRF2401IC/24PIN	QFN5X5-24	Nordic	
2	1	Q2	crystal	16MHz, CI=12pF, ESR<100Ohm, +/-30ppm	L=4.0, W=2.5, H=0.8mm	SMI	42SMX Series
3	2	L1, L2	Inductor	22nH / 5% / SFR >2.5GHz	0603	Würth Elektr.	744761122
4	1	L3	Inductor	3,6nH / 5% / SFR >2.5GHz	0603	Würth Elektr.	744761033
5	1	R41	Resistor	22K/1%	0603		
6	1	R48	Resistor	1M/1%	0603		
7	1	C33	Capacitor	10nF/50V/10%/X7R	0603		

8	1	C34	Capacitor	1nF/50V/10%/X7R	0603		
9	2	C36, C37	Capacitor	1pF / 50V / +/-0,25pF / NPO	0603		
10	3	C39, C42, C43	Capacitor	22pF/50V/5%/NPO	0603		
11	1	C40	Capacitor	2,2nF/50V/10%/X7R	0603		
12	1	C41	Capacitor	33nF/50V/10%/X7R	0603		
13	1	X1	Connector	10-pole	Grid=50mil		

5. software specification

device configuration by software:

RF output power	0dB
data rate	250 kBit/sec
communication mode	ShockBurst
number of used channels	2
frequency channel #1	2402MHz
frequency channel #2	2410MHz (=channel 1 + 8MHz)
Crystal frequency	16MHz

For detailed information refer to nRF2401 data sheet, Nordic VLSI ASA (www.nvlsi.no)

6. Liability disclaimer

Kellendonk Elektronik GmbH reserves the right to make changes without further notice to the product to improve reliability, function or design. Kellendonk Elektronik GmbH does not assume any liability arising out of the application or use of any product or circuits described herein.

7. Life support applications

These products are not designed for use in life support appliances, devices, or systems where malfunction of these products can reasonably be expected to result in personal injury. Kellendonk Elektronik GmbH customers using or selling these products for use in such applications do so at their own risk and agree to fully indemnify Kellendonk Elektronik GmbH for any damages resulting from such improper use or sale.

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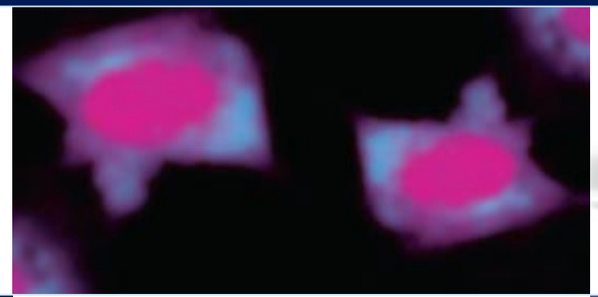

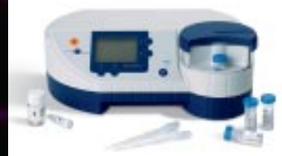

Nucleofector™ II Manual

amaxa biosystems

gene transfer begins here

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DMA-1001_05.2004

For more information and future program updates, check www.amaxa.com/program_update

Nucleofector™ Chip Card

For future protocol updates, also check www.amaxa.com/celldatabase

Nucleofector™ CD-ROM

Appendix	Technical data
Power supply:	110 VAC +10%/-20% or
	230 VAC +10%/-20%
	50 - 60 Hz
	self-regulating
Power consumption:	20VA/fuse 0.4 A
Temperature range:	+1°C to +40°C, non-condensing
Altitude:	to 2000 m above sea level
Protection:	IP 22
Protection class:	I
Weight:	2.6 kg
Dimensions (w x d x h):	30 x 23 x 11 cm
Radio data communication unit:	frequency 2.4-2.4835 GHz
	field intensity < 35 µV/m
	integrated antenna
	Activated only upon radio request by appppropriate amaxa modules.

1. amaxa – your link to transfection

Since the introduction of the Nucleofector™ technology in 2001 a constantly growing interest has helped to establish amaxa's technology as a leading and widely used transfection method in labs worldwide. An increasing number of publications from many different research areas reflect how the Nucleofector™ technology drives research in numerous different cell types and applications, such as gene silencing in primary cells and hard-to-transfect cell lines.

The Nucleofector™ technology allows easy and efficient gene transfer into a multitude of different cell types, many of which were formerly considered non-transfectable. The technology is equally efficient for cell lines and primary cells as well as for the delivery of various substrates such as plasmid DNA, RNA or siRNA. This flexibility offers numerous possibilities to study gene function and regulation in eukaryotic cells. The Nucleofector™ technology offers you more opportunities than any other transfection method to approach your research issues from many different angles.

Listening to your scientific needs has always kept amaxa moving ahead as your partner in gene transfer. Our aim to constantly improve the Nucleofector™ technology has led to the development of the second generation of Nucleofector™ hardware – the Nucleofector™ II Device. It provides, for example, a larger graphic display and a new motor-driven carousel, both offering easier handling and more convenience. The possibility of storing individual programs for optimized cell lines also ensures greater flexibility in a multiple user setting.

amaxa's mission has always been constant innovation in gene transfer. As such, we will continue with our goal to be your link to transfection.

The Nucleofector™ technology

The Nucleofector™ technology is based on two unique components, the Nucleofector™ Device that delivers the specifically developed electrical parameters and Nucleofector™ Kits that contain cell-specific and optimized Nucleofector™ Solutions.

As part of the Nucleofector™ technology, amaxa provides cell-type specific Optimized Protocols for many different cell lines and primary cells. An up-to-date database of the continuously extended range of Optimized Protocols can be found on our homepage at www.amaxa.com/celldatabase. If no Optimized Protocol for the cell line of interest is available, we offer a Cell Line Optimization Nucleofector™ Kit enabling you to easily establish the Nucleofector™ technology for your specific cell line. For fine-tuning after the optimization process, please contact our Scientific Support Teams:

phone	Europe/World: +49 (0)221-99199-400 USA: 240-632-9110
e-mail	Europe/World: scientific-support@amaxa.com USA: scientific-support.US@amaxa.com

2. Operating instructions

2.1 Restrictions

Medical use restrictions

The Nucleofector™ technology is intended for research and investigational use by professionals only. The Nucleofector™ Device and Solutions are not approved for any clinical application and must not, under any circumstances, be used for testing or treatment in humans, or as a therapeutic or diagnostic tool, or as an accessory or complement to such a tool.

License statement

amaxa GmbH is holder of various patent applications and technical and scientific experience with respect to the Nucleofector™ technology. Use of amaxa's Nucleofector™ technology requires a license from amaxa GmbH.

Purchasers are granted a non-exclusive, non-transferable license for the use of amaxa's Nucleofector™ technology for research and development purposes, the terms of which are disclosed in detail in the license agreement accompanying the shipped Nucleofector™ Device. Commercial application is allowed under amaxa's license for for-profit-entities. Both licenses exclude in particular any right to manufacture, copy, reproduce, transmit, distribute, sell, lease, transfer or sublicense amaxa's Nucleofector™ technology to any third party. For license information contact amaxa GmbH by phone +49 (0)221-99199-0 or e-mail licenses@amaxa.com.

2.2 Maintenance

The Nucleofector™ II requires very little maintenance to assure reliable operation. To clean and disinfect the case, first unplug the power supply. Use a damp cloth to wipe down the outer case (water or 70 – 80% ethanol). Avoid wetting the cuvette holder within the cuvette carousel and the connectors located on the rear of the device.

The Nucleofector™ II has been designed for use under a sterile hood including UV radiation. Prolonged exposure of the outer casing to UV light will lead to discoloration with no functional impairment of the Nucleofector™.

When laminar flows are sterilized on a regular basis by UV radiation overnight we recommend protection of the device by appropriate shielding or removal during prolonged UV radiation.

The Nucleofector™ II is protected by two main fuses. Both are inside a receptacle incorporated in the inner power socket (see Fig. 2, page 7). In case of a blown fuse you can easily exchange it. Insert a small flat screwdriver into the slot on the right-hand side of the receptacle to pull it open. Both the upper and lower part of the receptacle, have to hold working fuses in the inner positions. Blown fuses can usually be identified by molten interrupted wires inside the glas tube. Only use T400L 250 V fuses to substitute blown fuses.

2.3 Safety instructions – please read carefully!

This symbol means that there is a risk of electric shock. An electric shock could cause death or personal injury.

The Nucleofector™ II has been certified by international safety standards and is safe to use when operated in accordance with this manual.

This device is designed to deliver variable high voltage electrical impulses for the purpose of introducing DNA into eukaryotic cells. **These electrical impulses can be DEADLY!**

Therefore, use this device with care and take the following PRECAUTIONS:

- Do NOT attempt to open either the device or the cuvette holder. The device does not contain user-serviceable parts. Under NO circumstances should circuit components be interfered with, as they can deliver an electric shock even when the start button is not pressed. Do NOT alter the device in any manner.
- Do NOT plug in any non-amaxa-certified connectors into the external electrode sockets at the rear side of the Nucleofector™ device. The sockets are high-voltage outputs that can deliver electric shocks via any non-amaxa device that is connected to the Nucleofector™.
- Only use the device once you have read and understood the Nucleofector™ manual. The manual should be accessible for all users. Make sure that each potential user reads and understands it.
- Only use the device when it is set on top of a safe and stable table or bench.
- Do not expose the device to a humid environment.
- Set up the device in a dry place. Avoid spilling liquid onto or into the Nucleofector™. Do not use the device if it is wet.
- Place the device such that easy removal of the power cord is possible at any time.
- The device is not approved for use in fire or explosion endangered areas, nor for use with inflammable or explosive media.
- Use the device with amaxa's certified Nucleofector™ Solutions and amaxa certified cuvettes only. Use of any other solution or cuvette from any other source than amaxa will preclude all warranty and liability claims.

› Unpack the cuvettes just prior to the experiment. Make sure that the outer contact areas are dry.
› amaxa disclaims all warranties and shall in no event be liable for any kind of damages caused by or arising out of any operation or use in violation with the above safety and handling instructions.

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

2.4 Nucleofector™ II components

The Nucleofector™ II is delivered with the following components:
› 1 Nucleofector™ II Device
› 1 Power cord
› 1 Nucleofector™ II manual

Front panel of the Nucleofector™ II

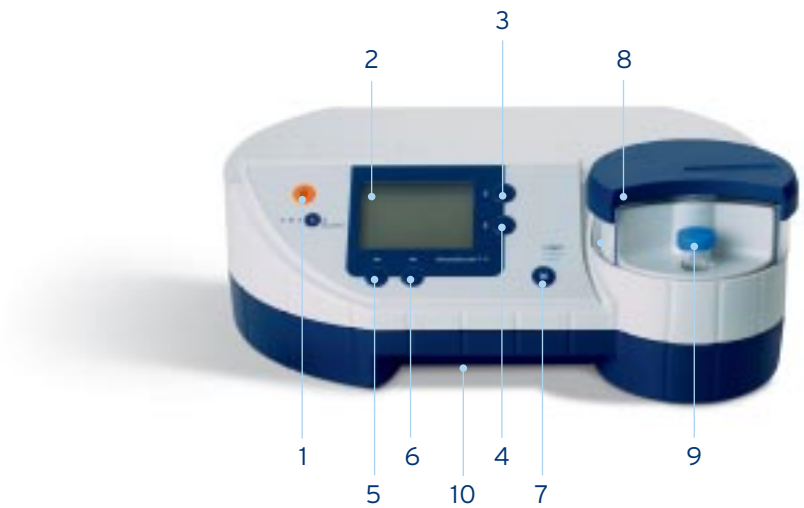

Fig. 1.

1 Power button
2 Graphic display
3 "Up" button
4 "Down" button
5 Functional button "menu"
6 Functional button "options"
7 "X" button with start, exit or enter function
8 Automatic cuvette carousel
9 Cuvette holder
10 Chipcard slot

Rear panel of the Nucleofector™ II

Fig. 2.

11 Power cord receptacle
12 Spare fuse
13 External electrode sockets

The Nucleofector™ II comes with default programs and contains a chipcard reader (10) below the handle for program updates.

2.5 Set-up instructions

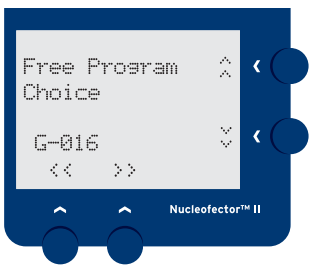

Fig. 3.

1. Remove all packing material.
2. Attach the power cord to the receptacle on the rear side of the Nucleofector™ II (12) and plug it into an appropriate electrical outlet. The device will automatically be in the stand-by mode. Turn on the device by pressing the orange power button in the upper left corner of the front panel (1). The device performs a self test accompanied by a short intro in the front graphic display (2). After power up the display shows the main menu "Free Program Choice" (Fig. 3.). The start mode can be changed to the last program used as described in chapter 2.7.5.

2.6 Instructions for using the Nucleofector™ II Device

2.6.1 Main menu and program execution

The Nucleofector™ II Device offers two separate ways for program selection. Programs can either be chosen by direct selection of the program code, e.g. A-023, in the "Free Program Choice" mode (Fig. 3), which is comparable to program selection in Nucleofector™ I. As a convenient alternative, programs can also be selected by cell type code using the "Cell Type List" (Fig. 4). In this mode all programs from currently available Optimized Protocols are filed, in alphabetical order under the specific cell type name (Fig. 4). In addition, custom programs can be filed in this cell type list in chronological order. Furthermore custom programs are listed separately in the "Custom Programs" mode. You can switch program modes by pressing the functional button "menu" (5) several times until the required program mode appears and confirm by pressing the functional button "go" (6).

› **Note:** Only custom programs can be stored or deleted by the user. The program codes of the Nucleofector™ II Device consist of two parts: a one-digit alphabetical code followed by a three-digit numerical code, e.g. A-023. This corresponds to the program code A-23 of the Nucleofector™ II Device.

Mode	Program can be	
	edited	deleted
"Free Program Choice"	Yes	No
"Cell Type List" (pre-programmed)	No	No
"Custom programs"	Yes	Yes

2.6.2 Selection of Nucleofector™ programs by program code – "Free Program Choice"

1. Turn on the Nucleofector™ by using the power button (1). Nucleofector™ II Device starts in the "Free Program Choice" mode (Fig. 3). If it does not start in the "Free Program Choice" press the functional button "menu" (5) repeatedly until "Go to Free Program Choice" appears and confirm this pressing the functional button "go" (6).
2. The alphabetical first digit blinks. Choose the appropriate letter of the program code by using the "Up" and "Down" buttons (3,4). Then move the cursor with the functional buttons "<<" and ">>" (5,6) to the numerical three-digit code. Select the appropriate number of the program code with the "Up" and "Down" buttons (3,4). Holding button down gives fast-scrolling. Confirm the program by pressing "X" button (7). Your Nucleofector™ is now ready for program execution (see 2.6.5).

2.6.3 Selection of Nucleofector™ programs by name of cell type – "Cell Type List"

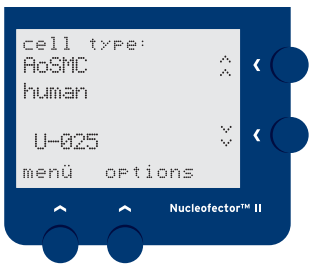

Fig. 4.

1. Turn on the Nucleofector™ by using the power button (1). If the Nucleofector™ II Device starts in the "Free Program Choice" mode (Fig. 3), exit this mode by pressing the "X" button (7). Press the functional button "menu" (5) several times until "Go to Cell Type List" appears and confirm this pressing the functional button "go" (6).
2. Choose the appropriate program for your cell type (Fig. 4) by navigating through the pre-programed list using the "Up" and "Down" buttons (3, 4). The programs are displayed in alphabetical order. The list is organized by first the cell name (e.g. T cells), descriptions of the species and further details of the cell type in line two. Line four shows the corresponding program itself. In cases where two or more programs are available, the third line indicates the specificity of the program such as for higher transfection efficiency or for higher cell viability. Your Nucleofector™ is now ready for program execution (see 2.6.5).

2.6.4. Selection and storage of Nucleofector™ custom programs

The Nucleofector™ II software provides the option to add programs to the cell type list by storing the appropriate program under a new individual name. By this way information about an optimized program for a particular cell type can be stored directly in the Nucleofector™ II Device.

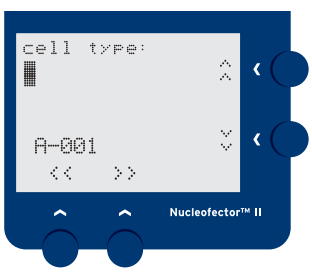

Fig. 5.

1. Turn on the Nucleofector™ by using the power button (1). If the Nucleofector™ II Device starts in the "Free Program Choice" mode (Fig. 3), exit this mode by pressing the "X" button (7).
2. Press the functional button "options" (6). Select "new" from the popup menu by using the "Up" and "Down" buttons (3, 4) and confirm with "X" button (7). The menu jumps to a free custom program memory location (Fig. 5).
3. The cursor blinks at the starting position. Scroll through the character set by using the "Up" and "Down" button (3,4) to enter the desired text and move to the consecutive character positions with the functional button ">>" (6). By using the functional button "<<" (5) the cursor moves

one step back and deletes the entered character.

4. After entering the desired name for the program, confirm the name by pressing the "X" button (7). The cursor automatically jumps to the program code position. Then choose the appropriate letter of the program code, e.g. A-023 by using the "Up" and "Down" buttons (3,4). Then move the cursor with the functional button ">>" (6) to the numerical three-digit code. Select the appropriate program number with "Up" and "Down" buttons (3,4). In order to save the custom program press the "X" button (7) and choose "yes" in the arising window by pressing the left functional button (5). Exit and save by pressing the "X" button (7).
5. Saved custom programs can be selected by navigating through the "Cell Type List" using the "Up" and "Down" buttons (3, 4). Custom programs are listed after the pre-programmed cell-type list, in chronological order. Additionally, for faster access the programs are listed separately in the "Custom Programs" mode.

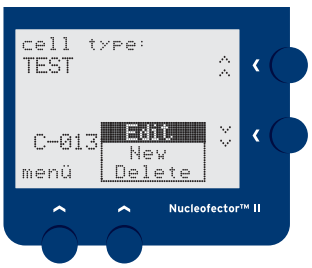

Fig. 6.

6. In order to modify or delete a saved custom program, navigate to the corresponding program in the "Cell Type List" or "Custom Program" mode using the "Up" and "Down" buttons (3, 4) and subsequently press the functional button "options" (6). Move to "Edit" or "Delete" in the popup menu (Fig. 6) using the "Up" and "Down" buttons (3, 4) and select it with the "X" button (7). In case you want to edit your program, you may now modify any position of the custom program entry by the procedure described above for custom program entry.
7. Leave the edit or delete mode, save the changes, and return to the main menu by pressing the "X" button (7).
8. Your Nucleofector™ II Device is now ready for program execution (see 2.6.5).

2.6.5 Program execution

1. After you have chosen the correct program, place the closed cuvette filled with 100 µl nucleofection sample in the cuvette holder (9). For program execution press the "X" button (7). The cuvette carousel (8) closes automatically and the program is executed. Subsequently, the carousel automatically rotates back

to reveal the cuvette. "OK" will appear on the display if the program has been successfully completed. After addition of 500 µl pre-warmed medium (see 4.1 and 4.2) remove the nucleofection sample from the cuvette with the plastic pipette provided in the Nucleofector™ Kit. Discard cuvettes after one use.

› **Note:** Only use fresh amaxa certified cuvettes, which have passed rigorous quality tests. Use of both uncertified cuvettes and re-use of amaxa certified cuvettes (after even one use), impairs experimental results, proper function of the device and risks damaging of the Nucleofector™ II Device itself.

2. Press the "X" button (7) to acknowledge. The device can be re-used immediately after the last program execution.
3. In case the device was unable to execute a program or the program was not completed successfully, the display will indicate the error type and error code. For more information on specific errors, see chapter 6. To continue working, press the "X" button (7) to acknowledge the error message. The device can then be re-used immediately.
4. When you have finished working, please remove the last cuvette from the cuvette holder (9) and switch off the Nucleofector™ II by pressing the power button (1).

› **Note:** The Nucleofector™ programs available correspond to the list of Optimized Protocols that are provided with the Nucleofector™ Kits which can be downloaded at www.amaxa.com/protocols. If the Nucleofector™ program you want to apply is not available on your device, please contact the amaxa Scientific Support Team to receive a chipcard to update the program software.

2.7 Additional Nucleofector™ II settings and options

2.7.1 Change of standard settings

In order to change the settings of your Nucleofector™ II Device press the functional button "menu" (5). By repeatedly pressing the button "menu" (5) you will first pass the three program mode options "Go to Free Program Choice", "Go to Cell Type List" and "Go to Custom Programs" and then be guided through the settings. To return from any sub-menu to the main menu press the "X" button (7).

2.7.2 Motor

The cuvette carousel (8) is connected to a motor which enables automatic cuvette contacting by closing and opening the cuvette holder carousel. In the menu settings you can choose between "on" for fully automated turning or "off" for manual opening and closing.

› **Note:** In case you have switched off the motor option in the menu, you will have to rotate the carousel manually by 180° clockwise. The carousel must be turned completely to the blocked position so that the cuvette contacts the electrodes.

For safety reasons the motor of the Nucleofector™ II Device is equipped with a sensitive blockage detection. In case closing of the carousel is hindered, e.g. by an inserted finger, the motor stops immediately and returns to its starting position. The display shows the corresponding error code (Err2 = Inappropriate or no cuvette). Continue working by pressing the "X" button (7) to exit the error mode.

2.7.3 Display contrast

In order to optimize readability of the display, change of the display contrast is possible by pressing the "Up" and "Down" buttons (3,4). The change has to be confirmed by pressing the functional button "set" (5). By leaving the submenu without pressing "set", the change is reversed in order to reduce the risk of modifying the contrast in such way that you may have difficulties reading the display.

2.7.4 Beep on/off

The beep following the switch-on of the device and erroneous program execution can be inactivated by choosing "off" with pressing the "Up" and "Down" buttons (3,4). The default setting is "on".

2.7.5 Change of start mode

The Nucleofector™ II Device can either start in the "Free Program Choice" mode or with the last program used. Choose the appropriate option by pressing the "Up" and "Down" buttons (3,4). The default setting is "Free Program Choice".

2.7.6 Software version

Press the functional button "menu" (6) to view the current software version of the Nucleofector™ II Device that comprise the operating programs of your Nucleofector™ II Device (For updating the program software see 2.7.7).

2.7.7 Upgrading program software

Fig. 7.

1. In order to upgrade the program list on your Nucleofector™ II, insert the chipcard with the novel software version provided by amaxa into the chipcard reader slot (10) (Fig. 7).
2. After the Nucleofector™ II Device recognizes the chip card, confirm loading of the novel Nucleofector™ software by pressing the "X" button (7).
3. The upgrade starts with a program table. Confirm by pressing the "X" button.
4. Secondly, the list of optimized programs is loaded. Confirm again with the "X" button (1). The stored custom programs will be neither deleted nor overwritten by upgrading novel non-custom optimized programs.
5. Confirm the software upgrade and return to the main menu by pressing the "X" button (1) and remove the chip card from the chipcard reader slot.

3. Essentials for preparing a nucleofection experiment

The efficiency of nucleofection and the cell viability after nucleofection is drastically influenced by cell culture conditions and DNA quality and quantity. Therefore, we recommend the following procedures to prepare a nucleofection experiment.

3.1 Cell culture

Cell culture conditions are crucial for successful nucleofection. Detailed information about media, serum, supplements, confluence/density and recommended passage number for primary cell types and cell lines is included in our cell-type specific Optimized Protocols (available at www.amaxa.com/protocols).

3.2 DNA quality

The quality 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, e.g. QIAGEN® Plasmid or EndoFree® Plasmid Kits.
The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) before use. DNA purity should be measured by the ratio of absorbance (A) at 260 to 280 nm. The A260:A280 ratio should be at or above 1.8 for nucleofection. Gene transfer efficiency can also be affected by the amounts of DNA. For best results, we recommend plasmid amounts between 1–2 µg per nucleofection sample (100 µl). The plasmid amounts can be increased up to 10 µg per sample. This may lead to higher transfection efficiencies, but at the same time may increase mortality of the cells.

3.3 Temperature

Prior to nucleofection, the apppropriate Nucleofector™ Solution should be prewarmed to room temperature. Keep cells resuspended in prewarmed Nucleofector™ Solution at

room temperature. Cooling should be avoided because certain cell types, especially primary cells, are extremely sensitive to low temperatures that may cause irreversible damage.

3.4 Cell number per nucleofection sample

We recommend a number of $1x10^6$ - $5x10^6$ cells (for suspension and adherent cell lines) and of $2x10^5$ - $5x10^6$ cells (for primary cells) per nucleofection sample. Cell numbers of < $2x10^5$ cells per sample are not advisable because gene transfer efficiency may decrease and mortality may increase. According to our experience, using average to high cell numbers for nucleofection improves cell survival.

3.5 Important controls

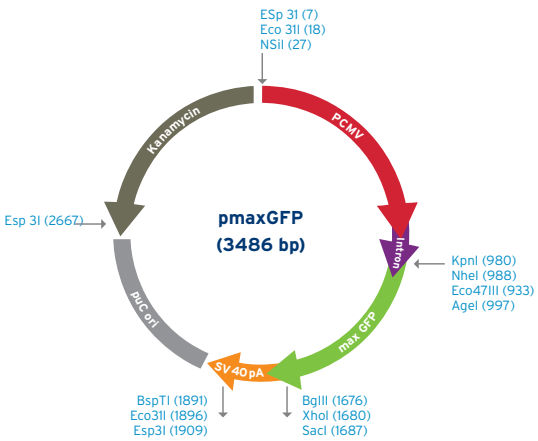

Fig. 8.

Positive control

We strongly recommend establishing the Nucleofector™ technology with the positive control vector pmaxGFP* (Fig. 8) as provided in each Nucleofector™ Kit. pmaxGFP encodes the green fluorescent protein (GFP) from copepod *Potellina sp.* maxGFP expression can be easily analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.

Negative control

To assess the intial quality of cell culture and the potential influence of amount and purity of the transfected DNA on cell viability, we recommend that the following two control samples should be always performed.

Control 1:

Recommended amount of cells in Nucleofector™ Solution with DNA but without application of the program (alternatively: untreated cells)
(Cells + DNA + Solution – program)

Control 2:

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

3.6 General cell culture protocols

3.6.1 Trypsin treatment of adherent cells

1. Aspirate medium from the tissue culture dishes/flasks and discard.
2. Wash cells once with Phosphate Buffered Saline (PBS). Use at least the same volume of PBS as medium used for culturing the cells. Aspirate and discard.
3. Add trypsin-EDTA to cover the cell layer and gently rock the dishes/flasks. Please check the detailed instructions in our Optimized Protocols (www.amaxa.com/protocols).
4. Place the dishes/flasks at room temperature (if necessary place in an incubator at 37°C) until the cells start to detach (usually 2 - 5 minutes).
5. Remove the dishes/flasks from the incubator. Tap against the sides of the dishes/flasks to improve detachment. Check detachment of cells under a microscope. If necessary, continue incubation at 37°C, but avoid overexposure to trypsin.
6. Once the vast majority of cells have dislodged, add culture medium containing serum and supplements to inactivate trypsin. Gently rinse the cells from the dishes/flasks, take an aliquot and count. Centrifuge at 90xg for 10 minutes and resuspend in medium to re-seed or expand cells or in appropriate Nucleofector™ Solution to perform nucleofection experiments.

3.6.2 Freezing cells

1. Cells must be viable, uncontaminated and show the typical morphological characteristics. Furthermore, it is important that the cells are in the logarithmic growth phase.

2a. Adherent cells: Trypsinize cells, stop trypsinization and count. Pellet the cells by centrifugation at 90xg for 10 minutes and resuspend at a concentration of 1 – 5x10^6 cells per ml culture medium with 7-10% dimethylsulph-oxide (DMSO).

2b. Suspension cells: Count and pellet cells by centrifugation at 90xg for 10 minutes. Resuspend at a concentration of 1 – 5x10^6 cells per ml culture medium with 7-10% DMSO.

3. Transfer 1 ml cell suspension into a labeled freezing vial. It is recommended that cell type, date, cell density, growth medium and passage number are noted on the label.
4. Freeze the cells by continually decreasing the temperature up to the final storage temperature of –196°C (liquid nitrogen) is reached (For details, please refer to common literature).

3.6.3 Thawing cells

1. Pre-warm the appropriate medium to 37°C in a dish/flask.
2. Place a vial of frozen cells in a 37°C water bath until the cells are just fully thawed. Make sure that cells are processed immediately because DMSO may damage cell membranes after thawing.
3. Gently pipet thawed cells into 20-30 ml pre-warmed medium contained in a dish/flask.
4. Place the dish/flask in an incubator at 37°C.
5. Change medium the following day to eliminate remaining DMSO and dead cells.

3.7 Transient versus stable gene transfer

Gene transfer into mammalian cells has become a powerful tool for the study of gene regulation, analysis of gene expression and biochemical characterization of proteins. Gene transfer can either be transient or stable.

Gene transfer is transient if the DNA is introduced into the nucleus without integrating into the chromosome. This means that transcription of the gene of interest is time-limited. Transient gene transfer is most efficient when circular plasmid DNA is used.

Gene transfer is stable if the DNA is integrated into the chromosome. For stable transfection, linearized DNA should be used.

4. Nucleofection procedures

The following procedures are general guidelines for nucleofection of adherent and suspension cells. In order to select the appropriate Nucleofector™ program in combination with the Nucleofector™ Solution for your specific cell type, refer to the Optimized Protocol included in the cell-type specific Nucleofector™ Kits for primary cells or download the appropriate Optimized Protocol for a cell line from our webpage at www.amaxa.com/protocols.

The list of Optimized Protocols is updated regularly. If no protocol is available for your cell type of interest, please do not hesitate to call our Scientific Support Team (Europe/World: +49 (0)221-991 99-400; USA: 240-632-9110) and ask for the latest developments and amaxa's experimental support. Alternatively, use our online and e-mail based scientific support at www.amaxa.com/supportandinfo.

Materials

Materials provided in amaxa's Standard Nucleofector™ Kits:

- 2.25 ml Nucleofector™ Solution
- 0.5 ml Supplement
- 10-30 µg pmaxGFP (0.5µg/µl in 10 mM Tris pH 8.0) depending on type of kit
- 25 amaxa-certified cuvettes
- 25 plastic pipettes

Required materials not provided in amaxa's Nucleofector™ Kits:

- PBS
- Trypsin-EDTA for adherent cells
- Tissue culture medium containing serum/supplements (freshly prepared).
  For detailed recommendations please refer to the cell-type specific Optimized Protocol.
- 1.5 ml reaction tubes
- 12-well plates (suspension cells) or 6-well plates (adherent cells) in tissue culture quality.

4.1 Nucleofection of suspension cells

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 date of addition on the vial.

One nucleofection sample contains:
$1x10^6$ – $5x10^6$ cells for cell lines
$2x10^5$ – $5x10^6$ cells for primary cells
2 µg pmaxGFP or 1-5 µg DNA (in 1-5 µl H_2O or TE)
or 0.5–3 µg siRNA
100 µl Nucleofector™ Solution

Experimental procedure

1. Cultivate the required number of cells.
2. Prepare 2 µg pmaxGFP or 1-5 µg DNA or 0.5–3 µg siRNA for each sample in reaction tubes.
3. Pre-warm the supplemented Nucleofector™ Solution recommended by amaxa to room temperature. For the transfer of the cells from the cuvette into the culture plates (see step 11) pre-warm an aliquot (500 µl per sample) of culture medium* containing serum and supplements in a 50 ml tube at 37°C.
4. Prepare 12-well plates by filling the appropriate number of wells with 1.0 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO_2 incubator.
5. Take an aliquot of the cell suspension and count the cells to determine the cell density.
6. Centrifuge the required number of cells ($1x10^6$ – $5x10^6$ for cell lines or $2x10^5$ – $5x10^6$ for primary cells per nucleofection sample) at 90xg for 10 min. Discard supernatant completely so that no residual medium covers the cell pellet.
7. Resuspend the pellet in room temperature Nucleofector™ Solution to a final concentration of $1x10^6$ – $5x10^6$ cells/100 µl for cell lines or $2x10^5$ – $5x10^6$ cells /100 µl for primary cells. Avoid storing the cell suspension longer than **15 min** in Nucleofector™ Solution, as this reduces cell viability and gene transfer efficiency (for details, see individual Optimized Protocol).
   Important: Steps 8-12 should be performed for each sample separately.
8. Mix 100 µl of cell suspension with 2 µg pmaxGFP or 1-5 µg DNA or 0.5–3 µg siRNA.
9. Transfer the sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette and avoid air bubbles while pipetting. Close the cuvette with the blue cap.
10. Select the appropriate Nucleofector™ program (for details see chapter 2). Insert the cuvette into the cuvette holder and press the "X" button (7) to start the program.
11. To avoid damage to the cells, remove the sample from the cuvette immediately after the program has finished. Take the cuvette out of the holder. To transfer the cells from the cuvette, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells. Add 500 µl of pre-warmed culture medium* containing serum and supplements (see step 3) to the cuvette and transfer the sample without any further resuspension into the prepared 12-well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heating block.
12. Press the "X" button (7) to confirm the notification by the Nucleofector™ II Device.
13. Repeat steps 8–12 for the remaining samples.

14. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 12-well plates.
15. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following nucleofection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 3 – 8 hours. If this is not the case, the incubation period may be prolonged.

› **Note:** It might be convenient to prepare a master mix instead of single samples. In this case, first mix the appropriate Nucleofector™ Solution, cells and DNA at the recommended ratios. Use 100 µl of master mix per cuvette. For many cell types the master mix can be kept at room temperature for up to 15 min without influencing cell viability and gene transfer efficiency.

* If the cells grow in Dulbecco´s modified Eagle medium (DMEM) or Minimum Essential Medium (MEM), we recommend using RPMI containing serum/supplements, in the steps marked with asterisk.

4.2 Nucleofection of adherent cells

Preparation of the 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 date of addition on the vial.

One sample contains:
$1x10^6$ – $5x10^6$ cells for cell lines
$2x10^5$ – $5x10^6$ cells for primary cells
2 µg pmaxGFP or 1-5 µg DNA (in 1-5 µl H_2O or TE)
or 0.5–3 µg siRNA
100 µl Nucleofector™ Solution

Experimental procedure

1. Cultivate the required number of cells.
2. Prepare 2 µg pmaxGFP or 1-5 µg DNA or 0.5–3 µg siRNA for each sample in Eppendorf tubes.
3. Pre-warm the supplemented Nucleofector™ Solution recommended by amaxa to room temperature. For the transfer of the cells from the cuvette into the culture plates (see step 14) pre-warm an aliquot (500 µl per sample) of culture medium containing serum and supplements in a 50 ml tube at 37°C.
4. Prepare 6-well plates by filling the appropriate number of wells with 1 ml of culture medium* containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO_2 incubator.

5. Remove the medium from the cell layer. Wash cells once with PBS.
6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with culture medium containing serum and supplements (see section 3.6.1).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells at 90xg for 10 min. Discard supernatant completely so that no residual medium covers the pellet.
9. Resuspend the pellet in room temperature Nucleofector™ Solution to a final concentration of $1x10^6$ – $5x10^6$ cells/100 µl for cell lines and $2x10^5$ – $5x10^6$ for primary cells. Avoid storing the cell suspension longer than **15 min** in Nucleofector™ Solution, as this reduces cell viability and gene transfer efficiency (for details, see Optimized Protocol for the specific cell type).
   Important: Steps 10 – 14 should be performed for each sample separately.
10. Mix 100 µl of cell suspension with 2 µg pmaxGFP or 1-5 µg DNA or 0.5–3 µg siRNA.
11. Transfer the sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette and avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Select the appropriate Nucleofector™ program (for details see chapter 2). Insert the cuvette into the cuvette holder and press the "X" button (7) 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"). Remove the cuvette from 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 (see step 3) and transfer the sample without any further resuspension into the prepared 6-well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37° heat block.
14. Press "X" button (7) to confirm the notification of the Nucleofector™ II Device.
15. Repeat steps 10 – 14 for the remaining samples.
16. If you have incubated the samples in 1.5 ml microcentrifuge tubes, transfer all samples into the prepared 6-well plates.
17. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following transfection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 3 – 8 hours. If this is not the case, the incubation period may be prolonged.

› **Note:** It might be convenient to prepare a master mix instead of single samples. In this case, first mix the appropriate Nucleofector™ Solution, cells and DNA at the recommended ratios. Use 100 µl of master mix per cuvette. For many cell types the master mix can be kept at room temperature for up to 15 min without influencing cell viability and gene transfer efficiency.

* If the cells grow in Dulbecco´s modified Eagle medium (DMEM) or Minimum Essential Medium (MEM), we recommend using RPMI containing serum/supplements, in the steps marked with asterisk.

4.3 Analysis of gene transfer efficiency

Gene transfer efficiency can be monitored by different methods such as microscopic observation of reporter gene expression in living cells, by quantification of reporter gene products in cell lysates or by flow cytometry. In the following section, some examples for these different methods are described. Please chose the appropriate methods for your cell type.

4.3.1 Microscopic analysis of gene transfer efficiency

Microscopic analysis of living cells expressing fluorescent proteins

Protocol for suspension cells

1. Resuspend cells and transfer 600 µl cell suspension into a 1.5 ml reaction tube.
2. Centrifuge cells at 90xg, 4ºC for 10 min, discard the supernatant.
3. Resuspend the cell pellet in a small volume of medium or PBS/0.5% BSA and apply to an object slide.
8. Analyze by fluorescence microscopy.

Protocol for adherent cells

1. Aspirate and discard the medium.
2. In each well, wash the adherent cells with 1 ml cell culture medium or PBS.
3. Cover the cells with cell culture medium or with PBS. Carefully observe the cells by microscopic examination, as some cell types change their shape or even detach when treated with PBS.
4. Analyze by fluorescence microscopy.

Alternatively:

Protocol for fixed adherent cells

Adherent cells can be fixed by many different methods (e.g. with 3.5-4% paraformaldehyde in PBS, with 70% ethanol/50 mM glycine, pH 2.2, with ice-cold methanol. For intracellular antibody staining subsequent permeabilization with e.g. 0.25% Triton X-100 is necessary.

Depending on the reporter gene used, expression can be directly observed by fluorescence microscopy (fluorescent proteins), by enzyme-linked color reactions (e.g. X-Gal staining), by antibody staining of surface markers or antibody staining of intracellular proteins (immunofluorescence or immunohistochemistry).

In this section, only one commonly used staining method
(X-Gal staining of cells expressing β-galactosidase as reporter) is described.

1. Aspirate and discard the medium.
2. In each well, wash the adherent cells with 1 ml PBS.
3. To a 6-well plate, add 1 or 2 ml fixative (2% formaldehyde/0.2% glutaraldehyde in PBS) per well and incubate 10 to 15 min at room temperature.
4. Aspirate and discard the fixative.
5. Wash the cells three times with PBS. If desired, fixed cells can be stored in PBS for several days at 2 to 8°C.
6. Prepare a stock solution of 40 mg/ml X-Gal in DMSO.
7. To a 6-well plate, add 1 ml freshly prepared staining solution (1 mg/ml X-Gal, 5 mM potassium ferricyanide $K_3Fe(CN)_6$, 5 mM potassium ferrocyanide $K_4Fe(CN)_6$, 2 mM $MgCl_2$, in PBS) per well and incubate for 0.5 to 2 hours at 37°C until cells expressing β-galactosidase stain blue.
8. Wash the cells three times with PBS.
9. Add 2 ml PBS and analyze the cells using a light microscope.
10. For longer storage, replace PBS by glycerol and cover the preparation with a cover slip.

4.3.2 Antibody staining of living cells expressing a selected surface marker

Protocol for suspension cells

1. In each well, resuspend cells and transfer 600 µl cell suspension into a 1.5 ml reaction tube.
2. Centrifuge cells at 90xg, 4°C for 10 min, discard the supernatant.
3. Resuspend the cell pellet in 100 µl PBS/0.5% BSA and add a fluorescent dye-conjugated antibody directed against the surface marker you are using (for examples, see chapter 7).
4. Incubate for 10 min on ice in the dark.
5. Add 1 ml PBS/0.5% BSA.
6. Centrifuge cells at 90xg, 4°C for 10 min, discard the supernatant.
7. If you need to stain with a secondary antibody, repeat steps 3 – 7.
8. Resuspend the cell pellet in a small volume PBS/0.5% BSA and apply to an object slide.
9. Analyze by fluorescence microscopy.

Protocol for adherent cells

1. Aspirate and discard medium.
2. In each well, wash the adherent cells with 1 ml PBS.
3. Add 300 µl trypsin/EDTA and wait until the cells detach.
4. Add 400 µl PBS/0.5% BSA per well to stop the trypsinization. Rinse the cells from the wells and transfer into a 1.5 ml reaction tube.
5. Centrifuge the cell suspension at 90xg, 4°C for 10 min and discard the supernatant.
6. Resuspend the pellet in 100 µl PBS/0.5% BSA and add a fluorescent dye-conjugated antibody directed against the surface marker you are using (for examples, see chapter 7).
7. Incubate for 10 min on ice in the dark.
8. Add 1 ml PBS/0.5% BSA.
9. Centrifuge cells at 90xg, 4°C for 10 min, discard the supernatant.
10. If you need to stain with a secondary antibody repeat steps 6 – 10.
11. Resuspend the cell pellet in 400 µl PBS/0.5% BSA per sample.
12. Add 1 µl propidium iodide to stain dead cells and mix.
13. Analyze samples by flow cytometry.

› **Note:** For analysis of all cells including the ones not yet attached, collect the supernatant of each well and pool with the trypsinized cells.

4.3.3 Analysis of gene transfer efficiency using enzyme-linked assays

Chloramphenicol acetyltransferase (CAT), β-galactosidase and firefly luciferase are enzymes commonly used as reporter systems (see chapter 7). They catalyze biochemical reactions where the reaction product or the reaction itself can be quantified by measurement of color or fluorescence intensity (colorimetry, fluorometry), light emission (chemiluminescence) or turnover of a radioactively labeled substrate. Many different substrates and detection kits are available, e.g. from Tropix, Promega, Stratagene, Roche or Molecular Probes.

Protocol for suspension cells

1. In each well, resuspend cells and transfer 600 µl cell suspension into a 1.5 ml reaction tube.
2. Centrifuge cells at 90xg, 4°C for 10 min, discard the supernatant.
3. Process the cells according to the procedures outlined in the detection kit you are using.
4. Analyze samples using a spectrophotometer, an ELISA reader, a luminometer, flow cytometry, scintillation counting or X-ray film exposure.

Protocol for adherent cells

1. Aspirate and discard the medium.
2. In each well, wash the adherent cells with 1 ml PBS.
3. Process the cells according to the procedures outlined in the detection kit you are using.
4. Analyze samples using a spectrophotometer, an ELISA reader, a luminometer, flow cytometry, scintillation counting or X-ray film exposure.

› **Note:** Due to the direct transport of DNA into the nucleus, gene expression can often be detected much earlier with nucleofection as compared to other non-viral transfection methods. E.g. when using luciferase as a reporter system, gene expression should be monitored after 5-7 hours.

4.3.4 Analysis of gene transfer efficiency using flow cytometry

Flow cytometric analysis of living cells expressing fluorescent proteins.

Protocol for suspension cells

1. In each well, resuspend cells and transfer 600 µl cell suspension into a 1.5 ml reaction tube.
2. Centrifuge cells at 90xg, 4°C for 10 min, discard the supernatant.
3. Resuspend the cell pellet in 400 µl PBS/0.5% BSA per sample.
4. Add 1 µl propidium iodide (10 µg/ml) to stain dead cells and mix.
5. Analyze samples by flow cytometry.

Protocol for adherent cells

1. Aspirate and discard medium.
2. In each well, wash the adherent cells with 1 ml PBS.
3. Add 300 µl trypsin/EDTA and wait until the cells detach.
4. Add 400 µl PBS/0.5% BSA per well to stop trypsinization. Rinse the cells from the wells and transfer into a 1.5 ml reaction tube.
5. Centrifuge the cell suspension at 90xg, 4°C for 10 min and discard the supernatant.
6. Resuspend the cell pellet in 400 µl PBS/BSA.
7. Add 1 µl propidium iodide to stain dead cells and mix.
8. Analyze samples by flow cytometry.

› **Note:** For analysis of all cells including the ones not yet attached, collect the supernatant of each well and pool with the trypsinized cells.

5. Troubleshooting

The following troubleshooting guide may be helpful if experiments using the Nucleofector™ II Device do not work as expected. The listed comments are intended to help optimize experimental conditions.

Should you have any questions regarding the Nucleofector™ II or protocols in this instruction manual, please do not hesitate to contact amaxa's Scientific Support Team.

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Problem	Likely cause	Comments
Low survival rate	Cells remained too long in the cuvette	To avoid damage to the cells, transfer cells immediately into pre-warmed medium (37°C). We recommend removing the sample from the cuvette directly after nucleofection.
	Cells are damaged by harvesting or through handling	Avoid severe conditions during harvesting, especially centrifugation at higher speed and overexposure to trypsin. Pipette cells smoothly. Use a plastic pipette provided in the kit for removal of cells from the cuvette. Add 500 µl pre-warmed medium to the cuvette before removal of the cells.
	Cells are stressed by culture conditions	Cells should be viable and in culture for certain passages. Freshly thawed cells should not be used for nucleofection. Avoid excessive cell densities or cell confluencies since this may negatively influence the viability of the cells after nucleofection. For detailed recommendations on passage number, cell density and confluence please refer to the individual Optimized Protocol.

Problem	Likely cause	Comments
Low survival rate	Cells are stressed by centrifugation	Centrifuge at lower speed (90xg).
	Multiple use of cuvettes	We strongly recommend using cuvettes only once, because the electric pulses that are applied drastically reduce their quality and impair their physical integrity.
	Cells are contaminated with Mycoplasma	Test cultures for Mycoplasma contamination. Cure contaminated cells with Plasmocin™ (Cat.No. VZA-1012) and prevent new infections by addition of either Primocin™ for primary cells (Cat. No. VZA-1021) or Normocin™ for cell lines (Cat. No. VZA-1001) to culture medium. For more details got to www.amaxa.com/antibiotics.
	Poor DNA quality	Plasmid DNA used should be of high purity. We strongly recommend the use of high quality products for plasmid purification such as QIAGEN® Endo Free® Plasmid Kits. Do not use procedures involving phenol or chloroform treatment.
Low gene transfer	Plasmid amount is too low	We recommend a plasmid amount between 1 – 5 µg DNA per sample. If both gene transfer efficiency and cell mortality are low the plasmid amount can be increased up to 10 µg per sample. Increasing the DNA amount may lead to higher gene transfer efficiencies but at the same time may result in higher cell mortality.
	High cell confluency/density	Gene transfer efficiency into many cell types is poor if the cells are too dense at the time of harvest. Please follow guidelines in Optimized Protocols.
	Too high or too low cell number in the cuvette	Cell numbers higher than $1x10^7$ or lower than $2x10^5$ cells per sample can reduce the gene transfer efficiency drastically. We recommend using $1x10^6$ – $5x10^6$ cells per sample for cell lines and $2x10^5$ – $5x10^6$ cells per sample for primary cells.
	Poor DNA quality	Plasmid DNA used should be of high purity. We strongly recommend the use of high quality products for plasmid purification such as QIAGEN® Endo Free® Plasmid Kits. Do not use procedures involving phenol or chloroform treatment.

6. Nucleofector™ error codes

The following guide indicates possible Nucleofector™ error codes and provides suggestions to solve the problem on your own. Should these suggestions not resolve the problem, please call amaxa's Scientific Support Team (Europe/World: +49 (0)221-99199-400; USA 240-632-9110). Alternatively use our internet and e-mail based scientific support at www.amaxa.com/supportandinfo. If the Nucleofector™ II has to be returned for repair, please contact our Scientific Support Team for shipping and warranty instructions.

Arc discharge correction

Arcing is a complete or partial discharge circumventing the sample and is often accompanied by a flash and a noise. This problem is usually caused by imperfect cuvettes or cuvette filling. The Nucleofector™ II Device is equipped with a hardware safety that immediately detects arc formation at its beginning to protect the cells from damage. After the arc interruption the Nucleofector™ II resumes program execution. Normally, the program can be completed successfully ("arc discharge correction 1 or 2") and only limited differences in transfection efficiency can be observed. When repeated arc discharges within one program occurs, it might be impossible for the Nucleofector™ II to complete program execution. In this case ("Err8 - Arc discharge") significant impacts on transfection efficiency might be detected. Discard the possibly damaged cuvette and its contents, reset the device by pressing the "X" button (1), and repeat the experiment with a new cuvette. It is not necessary to switch off the Nucleofector™ II Device.

Code	Possible error	What happened?	Procedure
Arc discharge correction 1 or 2	Inappropriate cuvette or Nucleofector™ Solution or inappropriate volume in the cuvette or device possibly defective	Device possibly generated an arc discharge. Program was automatically resumed and successfully completed. A limited impairment on efficiency and vitality has to be presumed.	Reset device by pressing any button. Utilize sample if maximum efficiency is not essential or try again with a new sample. For further samples check if the volume in the cuvette is 100 µl.
WEAK - Re-used cuvette or inappropriate solution	Re-used cuvette or inappropriate Nucleofector™ Solution	A weak pulse occurred. Only sub-optimal efficiency might be achieved.	Use a fresh cuvette. Check type of Nucleofector™ Solution.
Err1A - Internal communication failure	Internal error or device possibly defective	No pulse generated.	Acknowledge the error message by pressing any button; try to apply program a second time. If Err1A re-occurs, switch off the device, wait for 2 seconds and switch on again.
Err1B - Internal communication failure	Internal error or device possibly defective	Pulse generated with unclear outcome.	Acknowledge the error message by pressing any button; utilize sample if maximum efficiency is not essential. Otherwise, try with a new sample. If Err1B re-occurs, switch off the device, wait for 2 seconds and switch on again.
Err2 - Inappropriate or no cuvette	Inappropriate or no cuvette Inappropriate Nucleofector™ Solution or inappropriate volume	No pulse generated.	Check cuvette. Check type and volume of Nucleofector™ Solution. Cuvette can be re-applied.
Err3 - Device possibly defective	Device overheated or possibly defective	Very weak pulse generated.	Switch off the device. Wait for 10 minutes, switch on again; repeat the program with a new sample.
Err7 - Supply voltage too low	Supply voltage insufficient or device has been switched off for a long time.	No pulse generated.	Acknowledge the error message by pressing any button. Try again with the same cuvette.
Err8 - Arc discharge	Inappropriate cuvette or Nucleofector™ Solution or inappropriate volume in the cuvette or device possibly defective	Device possibly generated an arc discharge leading to incomplete program execution. A substantial impairment on efficiency and viability has to be presumed.	Acknowledge the error message by pressing any button. Check the volume of Nucleofector™ Solution. Try again with a new cuvette.
Err9 - Inappropriate solution, volume or cuvette	Inappropriate cuvette or Nucleofector™ Solution or volume or device possibly defective	Device possibly generated an overcurrent.	Acknowledge the error message by pressing any button. Try again with new cuvette.
Err11 - DSP error	Internal error or device possibly defective	Pulse possibly omitted.	Switch the device off and on to reset. Try again with a new sample.
Err12 - Motor error	Carousel motor failure	Motor control communication or device possibly defective.	Acknowledge the error message by pressing any button. Try again.

7. Genetic reporter systems

Genetic reporter systems are useful biological tools to study eukaryotic gene expression and regulation. Reporter genes are most frequently used as indicators of transcriptional activity in cells. In most cases a reporter gene is cloned behind a constitutive promoter sequence in an expression vector. After gene transfer, the cells are assayed for the presence of the reporter by detecting the reporter mRNA, the reporter protein or by measuring the activity of the reporter enzyme. Ideally, the reporter gene is not endogenously expressed in the cell type of interest and does not influence cellular physiology.

Genetic reporter systems are commonly used to monitor gene transfer efficiencies by assaying reporter gene products.

Green fluorescent protein (GFP)

The Green Fluorescent Protein (GFP) was isolated from different organisms (e.g. the jellyfish Aequoria victoria and the anthozoan Renilla reniformis). When exposed to blue light (450 – 500 nm), GFP emits green light without the need of additional proteins, substrates or co-factors. The GFP gene can be expressed in mammalian cells, and protein expression can be visually monitored in living cells allowing real time observation. Quantification of GFP expression is often performed by flow cytometry.

Recently, GFP variants have been developed which are designed for more efficient translation in mammalian cells. These improved proteins are human codon-optimized and lead to a higher expression in mammalian systems. The improved variants also include fluorophore mutations that increase their fluorescence intensity as compared to wild-type GFP. For dual- or multiple-labeling experiments GFP variants in different colors are available.

The Yellow Fluorescent Protein (YFP) contains mutations that shift the emission of the GFP fluorophore from green to yellowish-green. The YFP is also optimized for higher expression in mammalian systems; the fluorescence is roughly equivalent to that of GFP.

A new GFP from the copepod *Pontellina sp.*, maxGFP, has recently been discovered. An expression vector encoding maxGFP, pmaxGFP*, is now provided in all Nucleofector™ Kits. maxGFP fluorescence intensity is equally bright or slightly exceeds eGFP (Clontech). Therefore maxGFP is the ideal positive control for your nucleofection experiment. For an easy set-up of siRNA experiments an siRNA Test kit (Cat. No. VSC-1001) is available which contains pmaxGFP and an siRNA directed against maxGFP.

As a positive control and in order to establish the Nucleofector™ technology we recommend the use of pmaxGFP vector provided in each Nucleofector™ Kit. For a detailed recommendation please refer to chapter 4.

Other commonly used reporter systems

For optimization and monitoring of gene transfer efficiencies alternative reporter systems are available. The most commonly used systems are mentioned below. Please note that some reporter genes are not expressed in every cell type.

β-galactosidase

The prokaryotic enzyme β-galactosidase catalyzes the hydrolysis of β-galactoside sugars (e.g. lactose). Its enzymatic activity in cell lysates can be assayed by using special substrates that allow quantification via spectrophotometry (e.g. with o-nitrophenyl-β-D-galactoside = ONPG), fluorometry (e.g. with a 4-methyl-umbelliferyl-β-galactopyranoside compound = MUG) or chemiluminescence (e.g. with 1,2 dioxetan-galactopyranoside derivatives). A major strength of this system is the ability to assay β-galactosidase activity *in situ*. This allows determination of the percentage of nucleofected cells.
The β-galactosidase reporter gene is also frequently used as a control vector for normalizing gene transfer efficiency when co-transferred with other reporter systems.

Firefly luciferase

The bioluminescent reaction catalyzed by firefly luciferase from *Photinus pyralis* is based on the oxidation of beetle luciferin with concomitant production of photons. In the conventional assay, the substrate luciferin, ATP and Mg^{2+} are mixed with cell lysates containing luciferase. In the following reaction, the oxidation of luciferin, a flash of light is emitted which fades rapidly. The light emission can be detected using a luminometer or a liquid scintillation counter. Light emission is proportional to the luciferase activity in the lysate, thus allowing the indirect measurement of luciferase expression. Adding coenzyme A to the reaction mixture enhances the sensitivity of the firefly luciferase assay.

Using the Dual-Luciferase Reporter Assay System (Promega) the activity of two different luciferase reporter enzymes can be measured in one sample by co-transfection of two plasmids one encoding firefly luciferase (mostly used "experimental" reporter) and the

second encoding *Renilla* luciferase from *Renilla reniformis* (mostly used as internal control). Normalizing the activity of firefly luciferase to the activity of the *Renilla* luciferase minimizes experimental variability.

Chloramphenicol acetyltransferase (CAT)

This prokaryotic enzyme catalyzes the transfer of the acetyl group from acetyl-CoA to chloramphenicol. In the common CAT assay, the enzyme reaction can be quantified by incubating cell lysates with 14C-labeled chloramphenicol. The acetylated and non-acetylated forms of chloramphenicol are detected by scintillation counting or physical separation through thin layer chromatography. CAT activity can be estimated by exposing the plates to X-ray film. Another frequently used non-radioactive method is a CAT ELISA, where CAT expression is quantified by immunological detection.

Surface markers

A variety of proteins that are guided to the surface of the cellular membrane have been used for monitoring expression. These surface markers can be detected by staining with corresponding antibodies that are coupled to a fluorescent dye. Subsequently, cells are analyzed by flow cytometry, allowing quantitative analysis. Expression of surface antigens also allows physical selection of nucleofected cells by using immobilized antibodies.

Among the proteins that have been used as surface markers are H-2K^{k} (heavy chain of the MHC I complex from mouse), CD3 or CD4 usually expressed in mouse and human T cells, as well as various viral proteins, such as fragments of the hemagglutinin protein from influenza virus. Another possibility is the use of membrane proteins that have been tagged with a potent epitope. Epitope tagging with virus protein fragments, peptides from c-myc or a histidin hexamer is commonly applied.

Human growth hormone (hGH)

The human growth hormone (hGH) reporter system differs from most other reporter gene systems with respect to one important aspect: the human growth hormone is secreted into the culture medium by nucleofected cells and is measured using samples of culture medium supernatants. This avoids the necessity to lyse cells for quantification of the reporter protein and allows easy monitoring of transient expression kinetics. The secreted protein is usually detected by using specific 125I-labeled antibodies directed against hGH (scintillation counter) or a hGH ELISA.