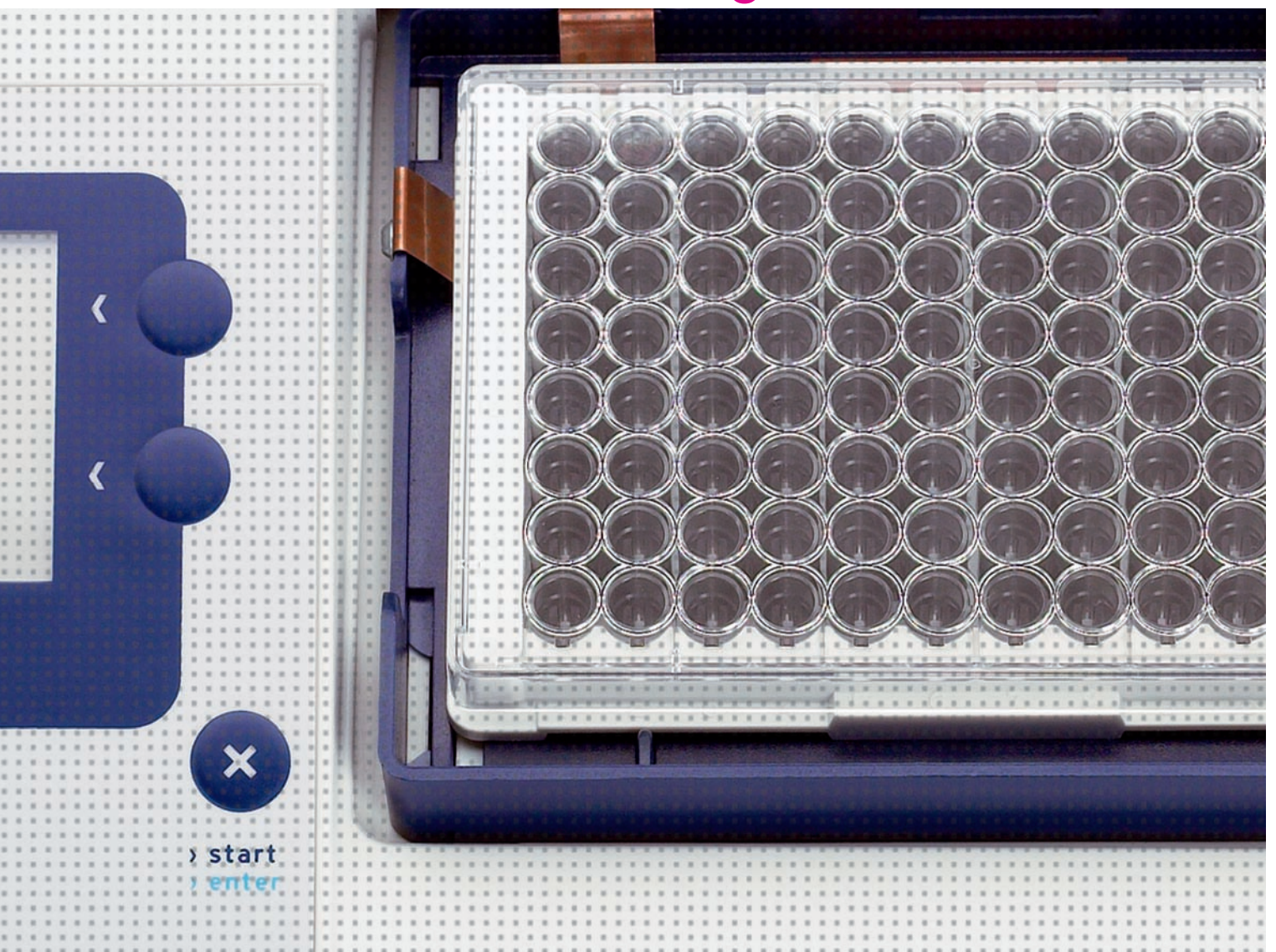


Amaxa™ Nucleofector™ 96-well Shuttle™ System Manual



For future protocol updates, also check www.lonza.com/cell-database

96-well Shuttle™ CD-Rom

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1.0 The Amaxa™ Nucleofector™ Technology

Lonza has developed the Nucleofector™ 96-well Shuttle™ System in response to the increased demand to expand the Amaxa™ Nucleofector™ Technology to high throughput applications and to the research need to transfect difficult-to-transfect cell lines and primary cells in the 96-well format.

Featuring the same unique benefits as the Nucleofector™ Technology, the Nucleofector™ 96-well Shuttle™ System offers an efficient, reproducible and highly flexible high-throughput transfection platform; supporting your drug discovery process such as target identification and validation.

Library screening without limits

The Nucleofector™ 96-well Shuttle™ System offers transfection efficiencies up to 95% and has the ability to deliver siRNA duplexes, shRNA or reporter gene vectors equally well into virtually any cell type—whether its a suspension or adherent cell line or even a primary cell.

Consistent and reproducible results

The Nucleofector 96-well Shuttle™ System shows low intra- and inter-plate variance, allowing for lot-independent, and consistent, reproducible transfection results.

Miniaturization reduces cell numbers

The adaptation of the Nucleofector™ Technology to the 96-well format is accompanied by a reduced transfection volume of 20 µl, and hence by low cell numbers. This makes the advantages of Nucleofection™ available for cell types that are hard to supply, such as primary neurons or mouse T cells.

Automation friendly

The Nucleofector™ 96-well Shuttle™ System meets the prerequisites for integration into Liquid Handling Systems: disposable 96-well Nucleocuvette™ Plates, automatic processing of a 96-well plate in 3 – 4 minutes, and an HTS-compatible software interface.

Lonza is pleased to expand the possibilities of transfection and help you accelerate your research and future discoveries with the introduction of the Nucleofector™ 96-well Shuttle™ System.

2.0 The Nucleofector™ 96-well Shuttle™ System

The Nucleofector™ 96-well Shuttle™ System consists of three device components and cell type-specific 96-well Nucleofector™ Kits:

The Nucleofector™ II Device



The Nucleofector™ II Device delivers the electrical programs to the 96-well Shuttle™ Device and mediates the data transfer between the laptop and the 96-well Shuttle™ Device. The Nucleofector™ II Device is connected to the 96-well Shuttle™ Device via a high voltage cable from its external electrode sockets. (For use of the Nucleofector™ II Device as a stand alone device, please refer to the Nucleofector™ II Device Manual).

96-well Shuttle™ Device

The 96-well Shuttle™ Device serves as the contacting unit of the system. Nucleofection™ of the transfection samples occurs in 96-well Nucleocuvette™ Plates (see next page) which are processed in the 96-well Shuttle™ Device. The 96-well Shuttle™ Device has been designed to allow for the application of up to 96 different programs per Nucleocuvette™ Plate.

Nucleofector™ 96-well Shuttle™ Software (on a Windows® based laptop)

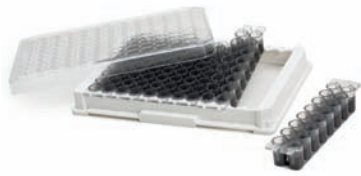


The Nucleofector™ 96-well Shuttle™ Software is the user interface and controls all processes during an experimental run (for details, please refer to Nucleofector™ 96-well Shuttle™ Software Manual).

96-well Nucleofector™ Kits

The Nucleofector™ 96-well Shuttle™ System works in conjunction with cell type-specific 96-well Nucleofector™ Kits containing 96-well Nucleofector™ Solution, Supplement and a 96-well Nucleocuvette™ Plate (different kit sizes are available).

96-well Nucleocuvette™ Plates and Modules (2 x 8)



96-well Nucleofection™ occurs in the newly developed disposable 2 x 8 Nucleocuvette™ Modules with electrodes consisting of an innovative conductive polymer material. Hence, no metal ions are released into the cell suspension during Nucleofection™. Six such modules are combined to form a complete 96-well Nucleocuvette™ Plate (SBS standard). The transfection volume per well is 20 µl. To avoid cross contamination, the plates are disposable and are provided in a sterilized package together with the appropriate lid. The lid is mandatory for processing the plates in the 96-well Shuttle™ Device.

Cell Type-Specific Amaxa™ Optimized Protocols

Lonza provides cell type-specific 96-well Amaxa™ Optimized Protocols for many different cell lines and primary cells. An up-to-date list of the continuously extended range of Amaxa™ Optimized Protocols for the standard Nucleofector™ Device or the 96-well Shuttle™ Device can be found on our online cell database at www.lonza.com/cell-database. If the Amaxa™ Optimized Protocol for the cell line of interest is unavailable, we also offer a Cell Line Optimization 96-well Nucleofector™ Kit enabling you to easily establish 96-well Nucleofection™ Conditions for your specific cell line. For fine-tuning after the optimization process, please contact our Scientific Support Teams:

Phone **Europe:** +49 221 99199 400
North America: 800 521 0390 (toll free)

E-mail **Europe:** scientific.support.eu@lonza.com
North America: scientific.support@lonza.com

3.0 General Instructions

3.1 Restrictions

Medical use restrictions

The Nucleofactor™ Technology is intended for research and investigational use by professionals only. Please note that Lonza's Nucleofactor™ Technology is not intended to be used for diagnostic purposes, for testing or treatment in humans.

License statement

Lonza Cologne GmbH is holder of various patents, patent applications, copyrights and technical and scientific experience with respect to the Nucleofactor™ Technology. Use of Lonza's Nucleofactor™ Technology and/or related software requires a license from Lonza Cologne GmbH.

Purchasers are granted a non-exclusive, non-transferable license for a limited use of Lonza's Nucleofactor™ Technology and related software for research and development purposes, the terms of which are disclosed in detail in the license agreement accompanying the shipped 96-well Shuttle™ Device. Commercial application is allowed under Lonza's license for for-profit-entities.

Both licenses exclude in particular any right to manufacture, copy, reproduce, transmit, distribute, sell, lease, transfer or sublicense Lonza's Nucleofactor™ Technology and/or related software to any third party. For license information contact Lonza Cologne GmbH by phone +49 221 99199 0 or e-mail ip.cologne@lonza.com.

3.2 Maintenance

The 96-well Shuttle™ Device requires very little maintenance for 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 retainer slot, the cavity of the retainer track and the connectors located on the rear of the device. The 96-well Shuttle™ Device has been designed for use under a sterile hood with or without UV radiation source. Prolonged exposure of the outer casing to UV light may lead to discoloration with no functional impairment of the 96-well Shuttle™ Device. 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 exposure.

3.3 Safety Instructions— Please Read Carefully



High voltage

This symbol indicates high voltage. It calls your attention to items or operations that could be dangerous to you and other persons operating this equipment. An electric shock could cause death or personal injury.

The 96-well Shuttle™ Device has been certified by international safety standards and is safe to use when operated in accordance with this manual.

Only use the 96-well Shuttle™ Device in connection with the Nucleofector™ II Device. Under NO circumstances should it be connected to any other device which delivers high voltage electrical impulses. While connected to the Nucleofector™ II Device, it is designed to mediate variable high voltage electrical impulses which were delivered from the Nucleofector™ Device 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:

- Only use the device once you have read and understood the 96-well Shuttle™ Manual. The manual must be accessible for all users. Make sure that each potential user reads and understands it.
- Do NOT open the device housing. 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 system is not in operation.
- Do NOT alter the device in any manner.
- Only use the approved Lonza power supply (AC/DC adapter part no. FW7555M/12).
- Do not use the device if the insulation of the high voltage cable is damaged.
- The power condition is 12DC, 20VA. Do not use 230V directly, this will damage the device.
- Only use the device when it is set on top of a safe, level and stable table or bench.
- Place the device such that easy removal of the power cord and the high voltage cables from the Nucleofector™ Device is possible at any time.
- Do not expose the device to a humid environment.
- Set up the device in a dry place. Avoid spilling liquid onto or into the 96-well Shuttle™ Device. Do not use the device if it is wet.
- The instrument should not be exposed to direct sunlight nor be placed in a hot environment.
- The device is not approved for use in fire or explosion endangered areas, nor for use with flammable or explosive media.
- Employ precautions against great impact and vibration in moving and transporting the 96-well Shuttle™ Device.
- If one of the high voltage connectors for some reason has been disconnected from the Nucleofector™ II Device, disconnect the power cables from the Nucleofector™ II Device and reconnect the cables according to the system setup diagram (consider high voltage cables as being “hazardous”).
- Under **no circumstances** should the high voltage cables of the 96-well Shuttle™ Device be plugged into external electrode sockets of any other device than Lonza’s Nucleofector™ II Device. This may damage the 96-well Shuttle™ Device and may cause personal injury. Use of any other device from any other source than Lonza will preclude all warranty and liability claims.
- Only use the 96-well Shuttle™ Device in connection with the Nucleofector™ 96-well Shuttle™ Software which is pre-installed on the laptop provided with the 96-well Shuttle™ Device. Apart from the pre-installed software, do not install any additional programs on the laptop to avoid any interference with or damage of the Nucleofector™ 96-well Shuttle™ Software.
- Use the device with Lonza certified 96-well Nucleofector™ Solutions and Lonza certified 96-well Nucleocuvette™ Plates and Modules only. Use of any other solution or plate from any other source than Lonza will preclude all warranty and liability claims.
- 96-well Nucleofector™ Solutions and standard Nucleofector™ Solutions (used for the 100 µl cuvette) are different and not interchangeable. Please do not use the 96-well Shuttle™ Device with the standard Nucleofector™ Solutions as this may lead to fluid splashes and contamination of the samples or device, and may even damage the device.
- Unpack the Nucleocuvette™ Plates just prior to the experiment. Make sure that the outer contact areas are dry.
- Do not dispense any liquid into the 96-well Nucleocuvette™ Plate when inside of the 96-well Shuttle™ Device.
- Always place the lid onto the 96-well Nucleocuvette™ Plate before transferring it in the 96-well Shuttle™ Device.
- Do not use other lids than those provided with the 96-well Nucleofector™ Kits as the system is calibrated to them. Other lids may cause damage of the 96-well Shuttle™ Device.
- Do not obstruct any moving parts, i.e., the retainer of the 96-well Shuttle™ Device.
- Do not move the 96-well Shuttle™ Device at any time if a 96-well Nucleocuvette™ Plate is inside the device as the sample fluid from the plate may enter the device.
- Confirm that no fluid gets in contact with or enters the device. If any fluid has been spilled in the close vicinity of or onto the 96-well Shuttle™ Device the safety may be compromised. To confirm that the device use is safe, contact Lonza Scientific Support for actions or precautions.
- Never place any foreign object on the device or onto the retainer or its bay area to avoid the risk of equipment damage.

- If any foreign object has entered the 96-well Shuttle™ Device, the safety may be compromised. To confirm that the device use is safe, contact Lonza Scientific Support for actions or precautions.
- If the 96-well Shuttle™ Device has been damaged, confirm that the device cannot be used by any personnel and contact Lonza Scientific Support for assistance.
- All service shall only be performed by Lonza authorized personnel.
- Handling of device parts that have the possible risk of sample contamination shall always be performed with protective gloves and any disposal of such parts must be according to Federal, state and local procedures for clinical waste handling and disposal. Use secure leak-proof containers and avoid unprotected handling of such parts.

Lonza 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.

3.4 Waste Disposal

Disposal of consumables from 96-well Nucleofector™ Kits (96-well Nucleocuvette™ Plates or Modules and 96-well Nucleofector™ Solutions)

- Discard plates, modules and expired Nucleofector™ Solution residuals in a typical biohazard container.
- Refer to your local waste management organization and to the relevant laboratory safety instructions for proper disposal practices.

4.0 Overview— Main Steps to Perform a 96-well Nucleofection™ Experiment

Step	Description	For details see
1	Connect Nucleofector™ II Device, 96-well Shuttle™ Device and Laptop.	Chapter 5.4
2	Switch on all 3 components.	
3	Start the 96-well Shuttle™ Software on the laptop and login.	Software Manual
4	Check your user settings (optional).	Software Manual
5	Check if USB connection between laptop and Nucleofector™ II Device is active or re-connect.	Software Manual
6	Define your experiment by opening an existing Parameter File or creating a new one.	Software Manual
7	Prepare samples and transfer them in the 96-well Nucleocuvette™ Plate.	Amata™ Optimized Protocol
8	Insert the 96-well Nucleocuvette™ Plate into 96-well Shuttle™ Device.	Chapter 5.5
9	Start Nucleofection™ Process from a) the laptop or b) the 96-well Shuttle™ Device.	Software Manual Chapter 6.3
10	Monitor Nucleofection™ Process during program execution a) either via the 96-well Shuttle™ Software b) or on the display of the 96-well Shuttle™ Device.	Software Manual Chapter 6.4
11	After run completion, remove 96-well Nucleocuvette™ Plate from the 96-well Shuttle™ Device and transfer samples into culture plates.	
12	View results on the laptop to check if errors occurred.	Software Manual
13	Close files and exit 96-well Shuttle™ Software and switch off all components.	Software Manual

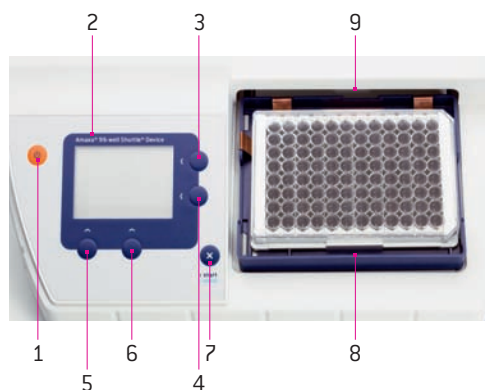
5.0 Set-up Instructions

5.1 System Components

The 96-well Shuttle™ Device is delivered with the following components:

- One 96-well Shuttle™ Device with integrated high voltage cables
- One external power supply unit with power cord (AC/DC adapter part no. FW7555M/12)
- One serial data cable
- One USB cable
- One 96-well Shuttle™ Manual
- One laptop (e.g., from Dell™) preinstalled with
 - Microsoft® Windows® XP Professional (plus Installation CD)
 - Microsoft® Office Basic Edition 2003 (Word, Excel®, Outlook®)
 - Nucleofactor™ 96-well Shuttle™ Software Accessories of the laptop
- One Nucleofactor™ 96-well Shuttle™ Software Manual
- Installation CD for Nucleofactor™ 96-well Shuttle™ Software

5.2 Front Panel of the 96-well Shuttle™ Device

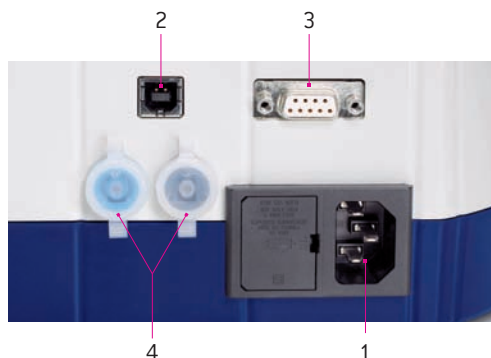


- 1 POWER button
- 2 Graphic display
- 3 UP button
- 4 DOWN button
- 5 Functional button: MENU, BACK
- 6 Functional button: OPEN, CLOSE, PAUSE
- 7 "X" button with "START" or "ENTER" function
- 8 Retainer
- 9 Retainer slot

5.3 Rear Panel of the 96-well Shuttle™ Device

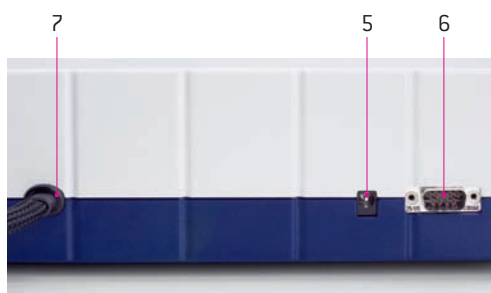
For rear panel refer to 5.4.

5.4 Assembly of the System Components



Rear side of Nucleofactor™ II Device:

- 1 Power cord receptacle
- 2 Receptacle for USB connection to Laptop
- 3 Receptacle for serial connection to 96-well Shuttle™ Device
- 4 Receptacle for high voltage cables from 96-well Shuttle™ Device



Rear side of Nucleofactor™ II Device:

- 5 Low voltage power cord receptacle
- 6 Receptacle for serial connection to Nucleofactor™ II Device
- 7 High voltage cable to Nucleofactor™ II Device



Rear side of Laptop (example):

- 8 USB connection to Nucleofactor™ II Device

1. Remove all packing material.
2. Place the Nucleofactor™ II Device, the 96-well Shuttle™ Device, and the laptop on a safe and plain table or bench and build up the following connections. Assemble all components without direct power (the order of building up the connections is irrelevant):
 - Connect the high voltage cable from the 96-well Shuttle™ Device [7] with the external high voltage electrode sockets on the backside of the Nucleofactor™ II Device. [4]. Important: **The blue cable has to be plugged into the blue socket and the black cable into the black socket.**
 - Connect the data cable between the 96-well Shuttle™ Device [6] and Nucleofactor™ II Device [3].
 - Connect the USB cable between the Nucleofactor™ II Device [2] and the laptop [8].

Connect all three devices to direct power supply (1, 5, laptop not shown) by plugging the power cords into an appropriate electrical outlet. Both, the Nucleofactor™ II Device and the 96-well Shuttle™ Device will automatically be in the standby mode. Turn the devices on by pressing the orange POWER button (1) in the upper left corner of the front panel. The device performs a system check accompanied by a short animation in the front graphic display (2). During this system check, the retainer opens automatically.

5.5 Handling of the 96-well Nucleocuvette™ Plates

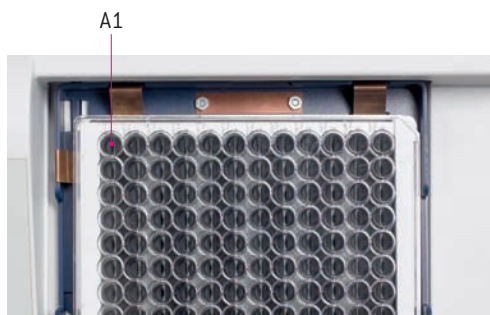
Insertion of a 96-well Nucleocuvette™ Plate into the 96-well Shuttle™ Device

- To maintain correct contact within the 96-well Shuttle™ Device, use of the lid is absolutely necessary.

Note

Do not use other lids than those provided in the 96-well Nucleofactor™ Kits as the system is calibrated to them. Other lids may cause damage of the 96-well Shuttle™ Device.

- Always place the frame with position A1 in the upper left edge of the retainer of the 96-well Shuttle™ Device.



- The samples will be processed by the 96-well Shuttle™ Device in columns, starting with A01, B01, C01 ... until H12.

Note

Do not re-use the Nucleocuvette™ Modules as this will influence biological results and may lead to arc discharges.

Assembly of the 96-well Nucleocuvette™ Modules

The 96-well Nucleocuvette™ Plates provided with the 96-well Nucleofector™ Kits are already assembled. However, in case of re-assembly be aware of the following:

- Handle modules, lid and plate under sterile conditions to prevent the risk of contamination.
- Take care that the 2 x 8 Nucleocuvette™ Modules are correctly inserted ("clicked") into the frame.

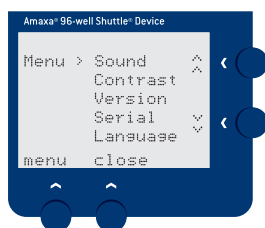
6.0 Operating Instructions for the 96-well Shuttle™ System

The Nucleofector™ 96-well Shuttle™ System offers highly convenient handling due to the ability to control and monitor the Nucleofection™ Experiment either from the laptop or the 96-well Shuttle™ Device. The 96-well Shuttle™ Device provides a graphic user interface reflecting the same functionalities like the Nucleofector™ 96-well Shuttle™ Software in terms of:

- Starting the Nucleofection™ Process (see 6.3)
- Pausing, stopping or resuming the Nucleofection™ Process (see 6.4)
- Monitoring Nucleofection™ Process (see 6.4)

6.1 Main Menu

Via the main menu, the settings for display contrast and sound options can be defined. It can be entered by the functional button MENU (5). This can be done also while the 96-well Shuttle™ Device is performing a Nucleofection™ Experiment.



The main menu comprises the following submenus. To enter any submenu, press the “X” button (ENTER, 7) for leaving the menu, press the functional button BACK (5).

6.1.1 Sound

The 96-well Shuttle™ Device offers the possibility to call the users attention for:

- Completion of the Nucleofection™ Process (Sound–Completed)
- Errors which interrupt the Nucleofection™ Process and require user intervention (Sound–Warning), e.g., in case the lid is missing
- Errors which do not interrupt the Nucleofection™ Process and do not require user intervention (Sound–Error), e.g., a program execution error in a well

Each signal is affiliated to a certain sound. The loudness of each sound can be chosen by pressing the UP and DOWN buttons (3, 4). The default setting is “on”.

6.1.2 Display

In order to optimize readability on the display, regulation of the display contrast is possible by pressing the UP and DOWN buttons (3, 4). The change has to be confirmed by pressing the “ENTER” button (x). By leaving the submenu without pressing “ENTER”, 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 the minimum contrast setting is 10%.

6.1.3 Version

This submenu indicates the software version of the 96-well Shuttle™ Device.

6.1.4 Serial no.

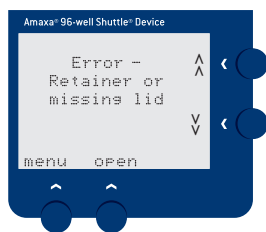
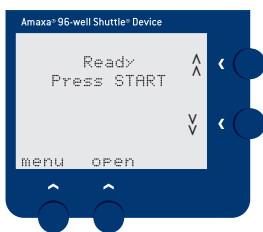
This submenu shows the serial number of the 96-well Shuttle™ Device.

6.1.5 Language

The 96-well Shuttle™ Device display offers a language option. The default setting is English. It can be switched to Japanese (or back to English) by pressing the UP and DOWN buttons (3,4). The change has to be confirmed by pressing the button “ENTER” (6). By leaving the submenu without pressing “ENTER”, the change is reversed in order to reduce the risk of modifying the language by mistake.

6.2 Opening and Closing the Retainer

The retainer of the 96-well Shuttle™ Device can be opened and closed manually by pressing the OPEN/CLOSE button (6). After closing the retainer the system detects if a 96-well Nucleocuvette™ Plate with lid is inserted and indicates “Ready—Press START”. In case no plate is inserted or the lid is missing, the display shows “Error—Retainer or missing lid”.



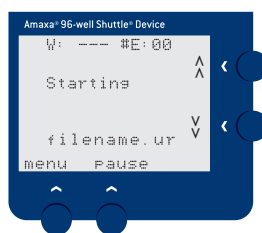
6.3 Starting a 96-well Experiment from the 96-well Shuttle™ Device

For handling convenience, the Nucleofector™ 96-well Shuttle™ System offers the possibility to directly start the Nucleofection™ Process from the 96-well Shuttle™ Device. To use this option, upload the required Parameter File from the laptop to the 96-well Shuttle™ Device (see software manual), insert the prepared 96-well Nucleocuvette™ Plate into the retainer and press the “START” button. Alternatively, the Nucleofection™ Process can be started via the Nucleofector™ 96-well Shuttle™ Software (for details refer to the Amaxa™ Nucleofector™ 96-well Shuttle™ System Software Manual).

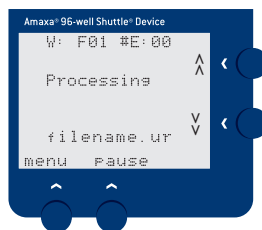
6.4 Display and Actions During the 96-well Nucleofection™

During the execution of a 96-well Nucleofection™ Experiment the display of the 96-well Shuttle™ Device shows the progress of the execution.

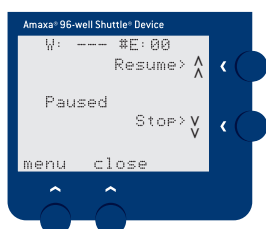
1. After “START” has been pressed (via software or at the 96-well Shuttle™ Device) the display shows the name of the active Result File (*.ur). There is lag phase of a few seconds after pressing the “START” button (indicated by “Starting”). During this time, the process can be stopped before the experiment begins by pressing the functional button PAUSE (6) (see below), e.g., if the wrong Parameter File is loaded or if you recognize any mistake within the programming of the file or pipetting of the wells.



2. After the short lag phase, Nucleofection™ starts. The display shows “Processing” and indicates the currently processed well number (e.g., W: F01) and the number of errors occurred so far (e.g., #E: 00).

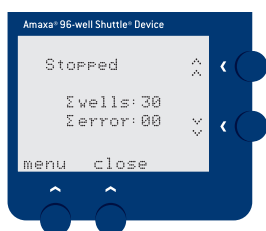


3. The experiment can be **interrupted** at any time by pressing the functional button PAUSE [6]. The retainer will open automatically. If automatic opening of the retainer is disabled via the user settings (see 6.2) the retainer has to be opened by pressing the functional button OPEN [6].

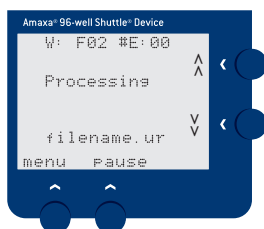
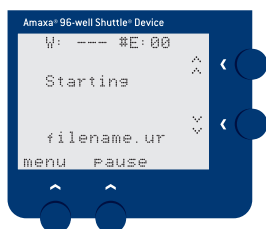


4. Options during the paused status:

- (a) **Abortion of the experiment:** The experiment can be completely aborted by pressing the STOP button (DOWN button, 4). The display indicates the total number of wells processed (e.g., \sum wells: 30) and the number of errors occurred (e.g., \sum error: 00). Results will be downloaded into the Result File of the Nucleofector™ 96-well Shuttle™ Software.

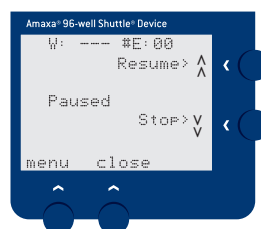


- (b) **Resumption of the experiment:** The experiment can be resumed by pressing the RESUME button (UP button, 3). The 96-well Shuttle™ Device continues with the next well in line and the process can be monitored on its display or in Nucleofector™ 96-well Shuttle™ Software.



5. **Completion of the 96-well Nucleofection™ Experiment:** At the end of a Nucleofection™ Experiment, the retainer opens automatically (if “automatical opening” was chosen in the user settings of Nucleofector 96-well Shuttle™ Software; see

software manual). The display indicates “Nucleofection™ completed” together with the total number of wells processed (e.g., \sum wells: 96) and the total number of errors that occurred (e.g., \sum errors: 01). Results will be downloaded into the Result file of the Nucleofector™ 96-well Shuttle™ Software.



6.5 Repetition of the Experiment

As long as no new Parameter file is uploaded to the 96-well Shuttle™ Device and the components of the Nucleofector™ 96-well Shuttle™ System have not been switched off, the system offers the possibility to repeat the same experiment with a new plate by just pressing the START button at the 96-well Shuttle™ Device again. The Nucleofector™ 96-well Shuttle™ Software will automatically create a new Result file which is stored under the initial Result file name plus an actual time stamp. Please make sure that all required components are still active.

7.0 Troubleshooting

7.1 96-well Nucleofection™ Results

The following troubleshooting guide may be helpful if experiments using the Nucleofector™ 96-well Shuttle™ System do not work as expected. The listed comments are intended to help optimize experimental conditions. Should you have any questions regarding the Nucleofector™ 96-well Shuttle™ System in this instruction manual, please do not hesitate to contact Lonza's Scientific Support Team.

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Problem	Possible error	What happened?
Low survival rate	Cells remained too long in the Nucleocuvette™ Modules.	If a 10 minute post-incubation step in 96-well Nucleofector™ Solution is not recommended (please refer to cell type-specific 96-well protocol), immediately add pre-warmed medium (37°C) to the wells and remove the sample from the modules to avoid damage to the cells once the Nucleocuvette™ Plate has been processed.
	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. After Nucleofection™ add 80 µl pre-warmed medium to the wells and resuspend cells carefully before removal of the cells.
	Cells are stressed by culture conditions.	Cells should be viable and in culture for a certain number of 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 Amaxa™ Optimized Protocol.
	Cells are stressed by centrifugation.	Centrifuge at lower speed (90 xg).
	Multiple use of Nucleocuvette™ Modules.	We strongly recommend using Nucleocuvette™ Modules only once, because the electric pulses that are applied drastically reduce their quality and impair their physical integrity.

Problem	Possible error	What happened?
Low survival rate	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 0.2–1 µg DNA per sample. If both gene transfer efficiency and cell mortality are low, the plasmid amount can be increased up to 2 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. Too high or too low cell number in the cuvette.	Gene transfer efficiency into many cell types is poor if the cells are too dense at the time of harvest. Please follow guidelines in the Amaxa™ Optimized Protocols. We recommend using 1×10^4 – 1×10^6 cells per sample for cell lines and primary cells. Please refer to the Amaxa™ Optimized Protocol for specific details.
	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.

7.2 Handling of 96-well Shuttle™ Device

Problem	Procedure
Retainer sticks within the retainer slot	<ol style="list-style-type: none"> 1. Switch the 96-well Shuttle™ Device off and disconnect it from power, detach the high voltage cables from the Nucleofactor™ Device. 2. Remove the label “Keep area clean and dry” on the white steering plate. 3. Use the provided screwdriver and turn the screw which is visible in the white steering plate counter-clockwise to retract the contacting electrodes. 4. Withdraw the retainer by carefully using the screwdriver as a lever. Remove the inserted Nucleocuvette™ Plate. 5. By switching the 96-well Shuttle™ Device on again it will re-calibrate itself.

7.3 Error Codes of 96-well Shuttle™ Device

The display of the 96-well Shuttle™ Device only indicates communication errors and mechanical errors directly connected to the use of the 96-well Nucleocuvette™ Plates.

All program execution errors in a well during the processing of a Nucleofection™ Experiment will be counted up (as indicated by #E). The respective error code will be stored in the Result File of the Nucleofector™ 96-well Shuttle™ Software (see software manual which provides a detailed description of such errors).

96-well Shuttle™ Device Error Codes and suggestions to solve the problem on your own

Code	Possible error	Procedure
Error - Retainer or missing lid	Motor failure or mechanics possibly blocked or device defective.	Verify loading of appropriate plate with lid and check plate orientation. Check if any foreign object hinders the movement of the retainer. Then press OPEN and CLOSE twice. The retainer should move back into its default position.
	No plate inserted or plate without lid.	Press OPEN and insert plate or add lid.
Not connected	The data cable between the Nucleofector™ II Device and the 96-well Shuttle™ Device is disconnected.	Check serial cable connection.
Error - Module missing	One or more modules in defined positions are missing or not inserted correctly into the frame.	Press OPEN and insert module at defined position or check correct module insertion.

Should these suggestions not resolve your problem, please contact Lonza's Scientific Support Team.

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In case the 96-well Shuttle™ Device has to be returned for repair, please contact our Scientific Support Team for shipping and warranty instructions.

8.0 Helpful Links

Topic	Please refer to
List of all Nucleofector™ References	www.lonza.com/nucleofection-citations
More details about the Nucleofection™ of siRNA	www.lonza.com/RNAi
List of Amaxa™ Optimized Protocols	www.lonza.com/protocols
List of all cell lines for which optimized Nucleofection™ Conditions are already available or for which customer data exists	www.lonza.com/cell-database
Further information about the system and its automation	www.lonza.com/96well
Further information about the 96-well Shuttle™ Software	www.lonza.com/96w-software

9.0 Appendix

	Technical data
Power supply	Input: 100–240 VAC 50–60 Hz 400 mA Output: 12DC, 1.2A Only use power supply Friwo Type FW 7555M/12
Power condition	12DC, 20VA
Operation temperature range	15–40 °C
Altitude	< 2000 m above sea level
Electrical safety class	EN 61010-1 UL 61010A-1 IP 20
Weight	3.0 kg 6.61 lb
Dimensions (w x d x h)	34 x 27 x 10 cm 13.39 x 10.63 x 3.94 in
Manufacturing date	The manufacturing year is encoded by the second and third digit of the serial number, e.g., serial number x06xxxxx was manufactured in 2006.

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