



# 4D-Nucleofector™ System Manual

For Research Use Only



The purchase of the 4D-Nucleofector™ System conveys to the buyer the non-transferable right to use the system as well as Lonza's proprietary Nucleofector™ Technology for research conducted by the buyer (whether the buyer is an academic or for-profit entity). For further details about the license please refer to chapter 5.

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# 1 The Nucleofector™ Technology

Since its introduction in 2001, Nucleofector™ Technology has transformed transfection. Nucleofection has proven to be a reliable and reproducible easy-to-use technology, suited to a wide variety of applications. An ever increasing number of publications in a wide range of research areas reflect how Nucleofector™ Technology is driving research in numerous cell types and applications. Nucleofector™ Technology provides an answer to almost every transfection challenge.

## How it Works

Nucleofector™ Technology is based on two unique components, the Nucleofector™ System that delivers the specifically optimized electrical parameters and Nucleofector™ Kits, which contain specific Nucleofector™ Solutions. As part of Nucleofector™ Technology, Lonza provides cell-type specific optimized protocols for many different cell lines and primary cells.

## Transfection of Any Cell

With over 160 protocols optimized by Lonza's R&D Team for cell lines and primary cells and more than 680 entries in the online Cell Transfection Database, Nucleofector™ Technology is the transfection method of choice for difficult-to-transfect cell types. Moreover, Nucleofection is the only electroporation-based technology allowing reliable transfection of adherent cells and cells in suspension.

## Transfection of Any Substrate

Nucleofector™ Technology offers high flexibility within applications, since the same transfection parameters apply for almost all substrates. DNA vectors, RNA duplexes, and peptides can be transfected using our sophisticated transfection protocols. This makes Nucleofection an ideal tool for providing answers to scientific questions in over-expression studies, gene silencing approaches, protein expression, generation of stable clones and many more applications besides.

## Moving Transfection into the Forth Dimension

At Lonza, we believe that listening to our customers is the best way to develop innovative products that meet your needs. In line with this customer-oriented spirit, the 4D-Nucleofector™ System was developed to offer advanced performance, flexibility and convenience. The system is controlled by intuitive software on an up-to-date touch screen user interface. Its modular architecture allows seamless expansion of the system for new applications.

Experience the Advanced Nucleofector™ Platform:

- A novel conductive polymer based cuvette system completely free of toxic metal ions
- Transfection of different cell numbers using the same conditions
- Nucleofection of cells in adherence
- Closed, scalable transfection of larger cell numbers in the range of  $1 \times 10^7$  to  $1 \times 10^9$



[www.lonza.com/cell-database](http://www.lonza.com/cell-database)

# The 4D-Nucleofector™ System



## Support Teams

### Europe

Scientific Support: +32 87 321 611  
scientific.support.eu@lonza.com

### North America

Scientific Support: +1 800 521 0390 (toll free)  
scientific.support@lonza.com

# 2 Operating Instructions

## 2.1 Restrictions


### Medical use restrictions

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

### License statement

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

Purchasers are granted a non-exclusive, non-transferable license for a limited use of Lonza's Nucleofector™ Technology and related software for research and development purposes, the terms of which are disclosed in detail in **chapter 5**. Certain commercial application is allowed under Lonza's license for for-profit-entities, as set forth in the license.

 For license information contact Lonza Cologne GmbH:  
Phone +32 87 321 611  
E-mail [ip.cologne@lonza.com](mailto:ip.cologne@lonza.com)

## 2.2 Maintenance

The 4D-Nucleofector™ System requires minimal maintenance for reliable operation. Unplug the power supply before cleaning and disinfecting the case. Use a damp cloth to wipe down the outer case with water or 70–80% ethanol. Do not use any aerosols for cleaning. Avoid wetting the cuvette holder within the cuvette tray and the connectors located on the rear of the system.

The 4D-Nucleofector™ System is designed for use under a sterile hood either with or without a UV radiation source. Prolonged exposure of the outer casing to UV light may lead to discoloration but not functional impairment of the Nucleofector™ System. However, the system should be removed from the hood during extended UV irradiation.

The 4D-Nucleofector™ System is protected by two main fuses. Both are inside a receptacle incorporated in the power socket of the Core Unit (see figure 2.0). Blown fuses can easily be replaced. Disconnect the 4D-Nucleofector™ System from the power supply and insert a small flat screwdriver into the slot on top of the power plug housing to lever it open. The fuse receptacles (each marked with an arrow) are then also levered open using the screwdriver.

Figure 2.0: Opened fuse receptacles



The 4D-Nucleofector™ System will not function unless both the left and right receptacle contain intact fuses. Blown fuses can usually be identified by molten broken wires inside the glass tube. Only use T2.5A, L fuses to replace 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 4D-Nucleofector™ System has been certified by international safety standards and is safe to use when operated in accordance with this manual.

This system is designed to deliver variable high voltage electrical impulses for the purpose of introducing substrates into eukaryotic cells.

**These electrical impulses can be deadly!**

**Therefore, use this system with care and take the following precautions:**

- Only use the system once you have read the 4D-Nucleofector™ Manual. The manual should be accessible for all users. Make sure that each potential user reads it.
- Do **not** open the system. The system does not contain user-serviceable parts. Under **no** circumstances should circuit components be interfered with, as they can deliver an electric shock even when system is not in operation.
- Do **not** alter the system in any manner.
- Do not obstruct the movement of the drawer holding the Nucleofection vessels or culture plates.
- Do **not** plug any non-Lonza-certified connectors into the external high voltage electrode sockets at the rear side of the 4D-Nucleofector™ System. They are designed to connect certified add-on systems to the 4D-Nucleofector™ System, e.g., the 96-well Shuttle™ Device.
- Only use the system when it is set on top of a safe, leveled and stable table or bench.
- Set up the system in such a way that easy removal of the power cord is possible at any time.
- Do not expose the system to a humid environment.
- The system must not be exposed to direct sunlight nor be placed in a hot environment.
- The system is not approved for use in fire- or explosion-endangered areas, nor for use with inflammable or explosive media.
- Take precautions against severe impacts and vibration while moving and transporting the 4D-Nucleofector™ System.
- Only use the system with Lonza-certified Nucleofector™ Solutions and Lonza-certified Nucleofection vessels (100 µL Nucleocuvette™ Vessel or 20 µL Nucleocuvette™ Strip for X Unit; 1 mL or LV Nucleocuvette™ Cartridge for LV Unit; 24-well Dipping Electrode Array for Y Unit). Using consumables from any other source than Lonza will preclude all warranty and liability claims.
- Do not alter the setup of the pre-assembled LV Nucleocuvette™ Cartridge.
- Standard Nucleofector™ Solutions are not compatible with the 4D-Nucleofector™ System.

- Never unplug high voltage cable while system is running.
- Unpack the Nucleocuvette™ Vessels or 24-well Dipping Electrode Arrays immediately before the experiment. Make sure that the outer contact areas are dry.
- Safety may be compromised if any fluid has been spilled in the close vicinity of the 4D-Nucleofector™ System. Ensure that no fluid is in contact with or has entered the system.
- System safety may be compromised if any fluid enters the system. To ensure that the system is safe to use, contact Lonza Scientific Support for advice on actions or precautions that should be taken.
- Do not insert or place foreign objects in the electrical drawers of the 4D-Nucleofector™ System.
- Safety may be compromised if any foreign object has entered the 4D-Nucleofector™ System. To ensure that the system is safe to use, contact Lonza Scientific Support for advice on actions or precautions that should be taken.
- If the 4D-Nucleofector™ System has been damaged, ensure that the system can not be used by any personnel and contact Lonza Scientific Support for assistance.
- All service shall be performed by Lonza authorized personnel only.
- Handling of system parts that may be contaminated with sample should always be performed using protective gloves and any disposal of such parts must be according to federal, state or local procedures for clinical waste handling and disposal. Use secure leakproof 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.

## 2.4 Waste Disposal

### Disposal of used consumables from Nucleofector™ Kits

Dispose used cuvettes, pipettes and Nucleofector™ Solutions in a biohazard container. Refer to your local waste management organization and to the relevant laboratory safety instructions for proper disposal practices.

## 2.5 4D-Nucleofector™ System Components

By definition the 4D-Nucleofector™ System is a modular system offering maximum flexibility. Therefore, a complete system may vary in the number and type of components it contains. Furthermore, a system may consist of minor model variants of the units. These variants can be discriminated by their part codes and serial numbers (table 2.1).

Table 2.1: Overview of model variants

Model	Variant A	Variant B
Years	2010 – 2013	2013 – Now
Part codes	Core Unit: AAF-1001B X Unit: AAF-1001X Y Unit: AAF-1001Y	Core Unit: AAF-1002B X Unit: AAF-1002X Y Unit: AAF-1002Y LV Unit: AAF-1002L
Serial numbers	Core Unit: 5**B**** X Unit: 5**X**** Y Unit: 5**Y****	Core Unit: 8**B**** X Unit: 8**X**** Y Unit: 8**Y**** LV Unit: 9**LV****
Unit arrangement	horizontal or vertical (via demountable side panels)	vertical only (fixed side panels)
Core Unit – display frame	silver	blue
X/Y Unit – sample rescue (also see 3.2)	using wheel accessible after removal of side panel	via screw behind termination plug

A typical 4D-Nucleofector™ System includes:

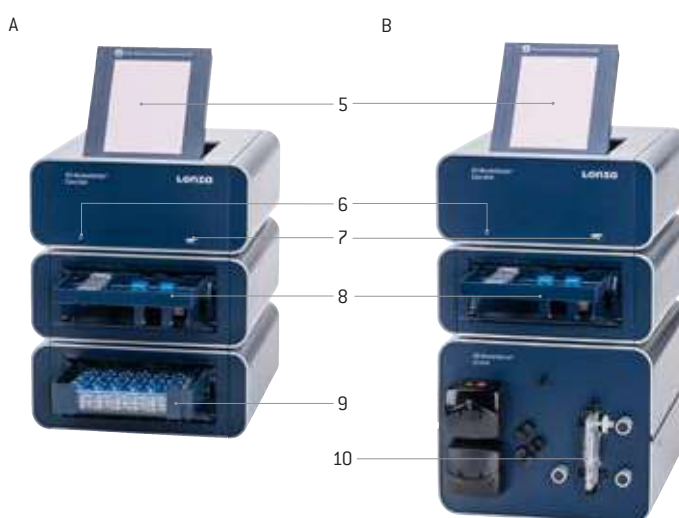
1. One 4D-Nucleofector™ Core Unit
2. At least one functional unit
  - **X Unit:** comprising two retainers for 100 µL Nucleocuvette™ Vessels and one retainer for 16-well Nucleocuvette™ Strips
  - **Y Unit:** enabling transfection of cells in adherence using a 24-well Dipping Electrode Array
  - **LV Unit:** for large volume transfection of up to 10<sup>9</sup> cells
3. Power cord
4. Interface cable for high voltage and data connection (plus termination plug cap, not shown)

Figure 2.1: 4D-Nucleofector™ System before assembly



5. Touch screen (graphical user interface of the system)
6. Operation LED
7. USB port (for USB stick)
8. X Unit with tray holding retainers for two 100 µL Nucleocuvette™ Vessel and one 16-well Nucleocuvette™ Strip
9. Y Unit with tray holding 24-well Dipping Electrode Array for adherent transfection
10. LV Unit with slot for 1 mL or LV Nucleocuvette™ Cartridge

Figure 2.2: 4D-Nucleofector™ System comprising Core, X and Y Unit (A) or Core, X and LV Unit (B)





11. Power cord socket with main power switch and fuses
12. USB port (for PC connection)
13. Serial port for shuttle connection
14. Lonza interface outlet port to connect functional units
15. Lonza interface inlet port to connect functional units
16. Lonza interface inlet port of last functional unit with termination plug
17. HV outlets for the 96-well Shuttle™ Add-on

Figure 2.3: 4D-Nucleofactor™ System comprising Core, X and Y Unit (rear view)



The 4D-Nucleofactor™ System comes with default programs and includes a USB port at the front for software updates.

## 2.6 Set-up Instructions

1. Unpack the components of the 4D-Nucleofactor™ System and check for completeness.
2. Stack the units with the Core Unit (containing the touch screen) on top (figure 2.4)
3. Connect the units (figure 2.5) by using the interface cables:
  - Connect the interface outlet port of the Core Unit (A) with the interface inlet port of the first functional unit (X or Y Unit; B). Make sure that the cable is securely attached and that the retaining screws on the cable housing are screwed tightly into the port.
  - Further functional units are added by connecting the outlet (C) and the inlet port (D) of adjacent units as described above.
  - **Important:** Plug in the interface terminator cap into the outlet of the last unit (C or E).
4. Attach the power cord to the power cord socket at the rear side of the Core Unit and plug it into an appropriate power outlet.
5. Check all connections before turning on the system for the first time.

Figure 2.4: Stacked units



Figure 2.5: Inlets and outlet ports for unit connection



## 2.7 General Use Instructions

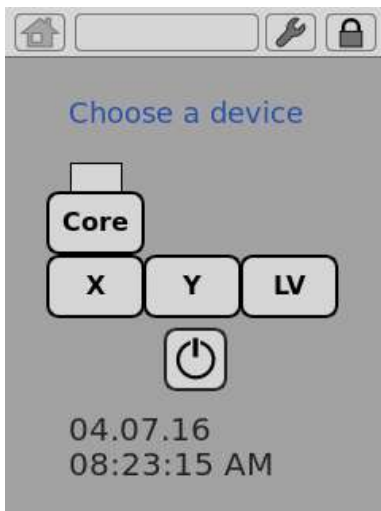
This section gives an overview of the 4D-Nucleofector™ System operating software. Details of the Nucleofection process can be found in the cell-type specific Optimized Protocols ([www.lonza.com/optimized-protocols](http://www.lonza.com/optimized-protocols)).

**NOTE:** All screenshots shown in this section refer to software version 4.0.

### 2.7.1 Turning on the 4D-Nucleofector™ System

Turn on the system using the main power switch at the rear of the Core Unit. The system will boot up — this process may take a few moments — and the blue LED at the front of the Core Unit will be lit. Once the start-up procedure is complete, the 4D-Nucleofector™ graphical user interface (touch screen) will display the software main screen. The main screen (figure 2.6) displays the current configuration of the 4D-Nucleofector™ System (Core Unit and functional units).

Figure 2.6: Main screen



### 2.7.2 Adjusting the Position of the Touch Screen

The touch screen of the 4D-Nucleofector™ System can be set at four angles (0°, 30°, 45°, and 60°), providing convenient access for the user. Press the label on top of the display frame to unlock the display from its home position. From this starting position, the user can adjust it to the most convenient angle by simply pulling it into a more upright position. The screen can be returned to its home position by moving it to the 60° position and then pulling it gently forward towards the front of the Core Unit. A switch will be activated, and the screen will drop automatically back into the starting position. Press down the screen to lock it in its home position.

**NOTE:** Do not try to close the display when the system is switched off. If there is a need to close it during OFF status or in case the display gets stuck, use the release knob shown in figure 2.7 (red circle).

Figure 2.7: Releasing the touch screen



Emergency release

Figure 2.8: Software screen elements

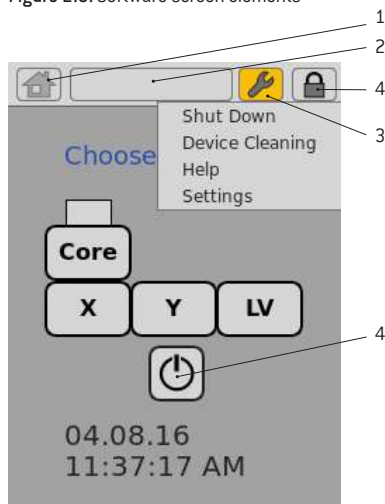
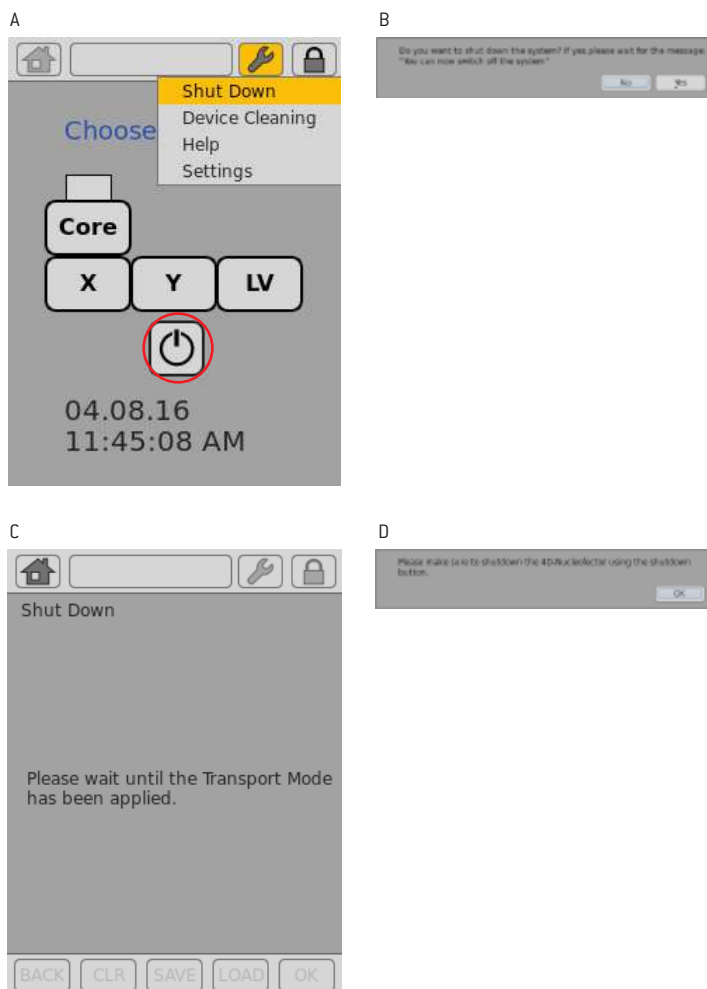


Figure 2.9: System shut down



## 2.7.3 Operating Software

The 4D-Nucleofector™ System is operated via a 5.7 inch touch screen display and controlled by intuitive software. Table 2.2 will familiarize you with the functions of the touch fields and the basic features of the operating software.

Table 2.2: Main menu and general commands or icons

Home icon (1)	Return to the main menu
Top bar (2)	Return to the "Choose a vessel" screen
Wrench icon (3)	<ul style="list-style-type: none"> <li>– <b>Shut Down</b> – activates transport lock (for X and Y Unit) and shuts down 4D-Nucleofector™ System (software version 4.0 or later, in older versions it may not be available or only activate the transport mode; see chapter 2.7.4 for further details)</li> <li>– <b>Device Cleaning</b> – allows convenient cleaning of the vessel tray (see chapter 2.12.5 for details)</li> <li>– <b>Help</b></li> <li>– <b>Settings</b> – opens the settings menu (see chapter 2.12 for details)</li> <li>– <b>Status*</b> – shows device status</li> <li>– <b>Open*</b> – opens the tray of the units</li> <li>– <b>Close*</b> – close the tray of the units</li> </ul>
Lock icon (4)	Activates transport lock (for X and Y Unit) and shuts down the system (software version 4.0 or later, in older versions it may not be available or only activate the transport mode)
BACK	Return to previous screen
CLR	Reset settings for an experiment or a Nucleofection vessel
SAVE	Save experiment or results file
LOAD	Load predefined experiments
OK/START	Confirm selection or execute a program
A-Z, Z-A, Top 10, Last 10	Sort a list alphabetically, display the most frequent 10 items or the most recent 10 items
Magnifier symbol	Activate search functions
i	Display additional information (text)
<, >	Arrow keys to switch between experimental setup screens

## 2.7.4 Turning off the 4D-Nucleofector™ System

This chapter explains how to turn off the 4D-Nucleofector™ System properly. Following this procedure will guarantee a safe shut down of the system and protection against damage of interior fragile parts. The described procedure is valid for software version 4.0 or newer. Older versions may not provide a software shut down (version 2.12 - 2.15) or only offer the transport mode (version 2.16). It is strongly recommended to upgrade the system to the newest software version (chapter 2.12.7).

- Press the "Lock" icon (figure 2.9, A, circle). Alternatively you can press the "Wrench" icon and select "Shut Down".
- A message will appear asking whether you want to shut down the system (figure 2.9, B). Upon pressing "YES", first the transport lock will be applied (for X or Y Unit) (figure 2.9, C).
- Wait until you receive the message "You can now switch off the system"

**NOTE:** By pressing "BACK" you would release the transport lock again.

- Finally, switch off the device via the main power switch located at the rear of the Core Unit. If the system was turned off via the power switch directly (without previous software shut down), a reminder will appear once you turn the system on again next time (figure 2.9, D).

## 2.8 General Instructions for Running a Nucleofection Experiment

This chapter explains some general features and steps required to perform a Nucleofection experiment independent of the different 4D-Nucleofector™ Units. The detailed handling of each unit is described in subsequent chapters 2.9 – 2.11.

### 2.8.1 Overview

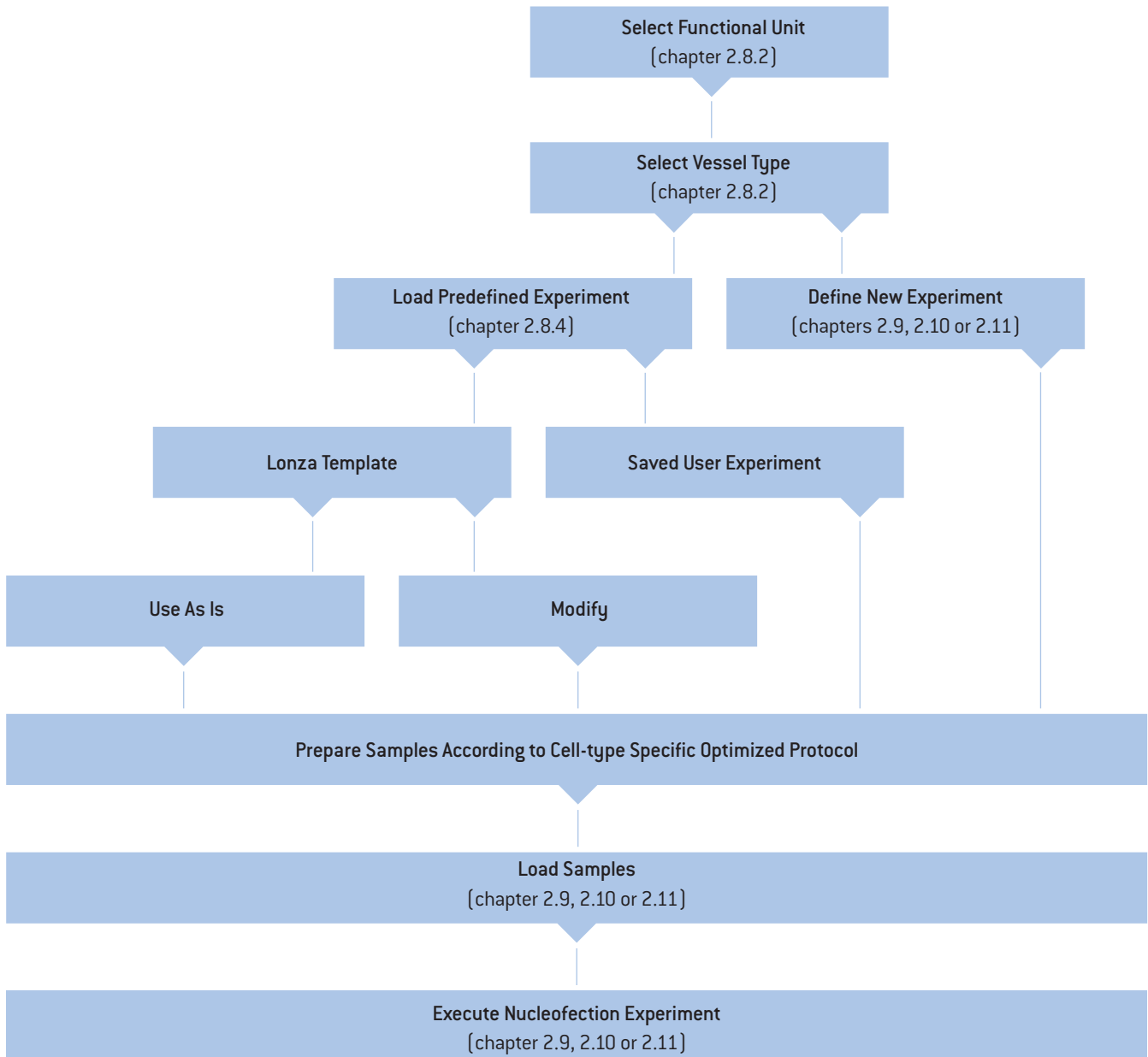
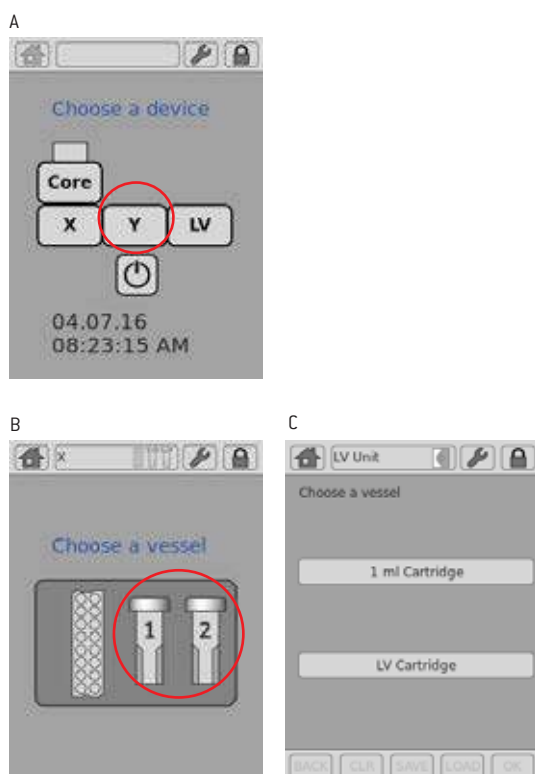


Figure 2.10: Unit and vessel selection



## 2.8.2 Unit and Vessel Selection

1. Select the functional unit (X, Y or LV) you wish to work with by pressing the appropriate icon (figure 2.10, A).
2. For X and LV Unit: A screen appears displaying the Nucleofection vessels available for the selected functional unit (figure 2.10, B, C). Press the icon displaying the vessel type you wish to use.

## 2.8.3 Experiment Parameters - Overview

An experiment comprises a selected set of vessels or wells that should be processed with defined Nucleofection parameters. Table 2.3 describes the parameters that can be defined.

Table 2.3: Unit and vessel selection

Parameter	Description
Cell Type Program	Using the “Cell Type Program” option is the easiest way to select parameters for a vessel or well or group of those. Each “Cell Type Program” is assigned to the optimal program for the certain cell type as defined in the Optimized Protocol (e.g., “T cells, human, unstim.” is affiliated to the optimal Nucleofector™ Program and Solution for unstimulated human T cells). The “Cell Type Program” list can be extended by the user with customized cell-type codes (e.g., after a cell line optimization; 2.12.3). Lonza defined programs are highlighted in blue while custom program codes will be highlighted in black.
Solution	In case the recommended Nucleofector™ Solution is not selected automatically via the “Cell Type Program” option, the “Solution” can be selected manually.
Pulse Code	In case the recommended Nucleofector™ Program is not selected automatically via the “Cell Type Program” option, a “Pulse Code” (e.g. FI-115) can be entered manually. <b>Note:</b> For manual “Pulse Code” selection, the “Solution” has to be selected first (see below).
Control (X and Y Unit)	<ol style="list-style-type: none"> <li>1. Sample: This is the default setting and defines a normal sample containing cells and substrate. The selected Nucleofection program will be applied to this position.</li> <li>2. No DNA: Negative control. Nucleofection program applied to vessel with cells but without substrate</li> <li>3. No program: Negative control. No Nucleofection program applied to a vessel containing cells and substrate</li> </ol> <p>In case of “Sample” or “No DNA” the selection has only an informative purpose. The selection of “No program” has a functional effect, i.e. no pulse will be applied.</p>
Volume [LV Unit with LV Nucleocuvette™ Cartridge]	When using the scalable cuvette version, the LV Nucleocuvette™ Cartridge, the volume that should be processed has to be defined. Depending on the number of reservoirs used, one or two volume parameters must be specified. The maximum total volume that can be entered is 20 mL.

## 2.8.4 Using a Predefined Experiment

A predefined experiment can either be a ready-to-use template pre-installed by Lonza (for X and Y Unit only) or a previous experiment that was saved by the user.

The use of predefined experiments is described showing the X Unit as an example, but the procedure would be the same for Y or LV Unit.

1. After unit and vessel type selection (see 2.8.2) a screen appears with a field showing “Choose Experiment or Position” (figure 2.11, A).
2. Press on the touch field labeled “Choose Experiment”
3. A list of predefined experiments will appear (figure 2.11, B). The list comprises experiments pre-defined by Lonza (template files; blue) and all experiments saved by the user (black).
4. Select the desired experiment by tapping on the appropriate line of the list. The experiment selected will be highlighted. Confirm your selection by pressing “OK”.
5. By pressing on the icons displaying the Nucleofection vessels or wells you can check the settings (“Cell Type Program,” “Pulse Code,” etc.) for the selected well (figure 2.11, C).
6. In case you want to modify the settings of a predefined experiment, click on the well(s) you want to change and adapt the settings (for unit-specific details see chapters 2.9, 2.10 or 2.11). An asterisk (\*) will be added to the experiment file name to indicate the deviation from the original file. You can save the changed experiments under a new name by pressing “SAVE”. Alternatively you may review the summary first (see next step) and then save it.
7. To accept all settings, press “OK”.
8. A summary of the experimental settings will appear (figure 2.11, D). If the experimental setup is as desired, load the samples (for unit-specific details see chapters 2.9.1, 2.10 or 2.11).

Figure 2.11: Using a predefined experiment (example: X Unit)

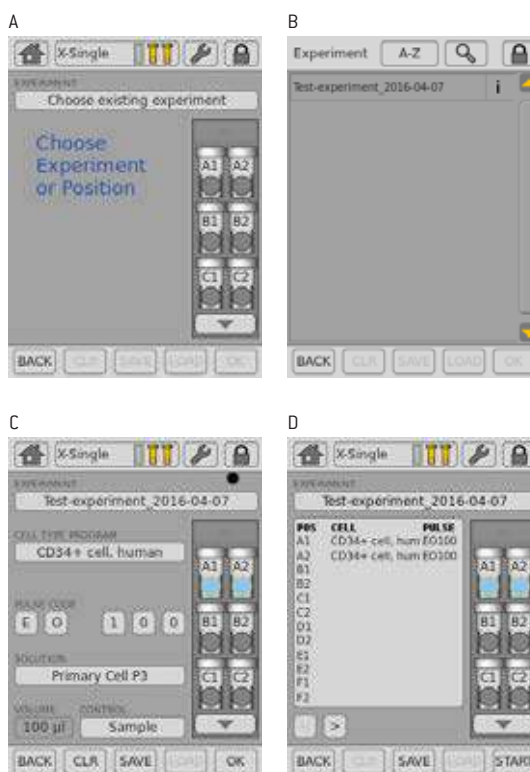




Figure 2.12: Vessel types for 4D-Nucleofector™ X Unit



## 2.9 4D-Nucleofector™ X Unit

The 4D-Nucleofector™ X Unit can handle two types of vessels:

- 16-well 20 µL Nucleocuvette™ Strips for cell numbers from  $2 \times 10^4$  to  $1 \times 10^6$  cells
- Single 100 µL Nucleocuvette™ Vessels for cell numbers from  $2 \times 10^5$  to  $2 \times 10^7$  cells

Figure 2.13: Experiment definition (X Unit; example: 16-well Nucleocuvette™ Strips)



### 2.9.1 Defining a New Experiment

1. After unit and vessel type selection (see chapter 2.8.2) a screen appears with the message “Choose Experiment or Position” (figure 2.13, A). You can now either select a predefined experiment (see chapter 2.8.4) or define an experiment from the beginning. By tapping on a position you can select one or multiple samples/wells (e.g. A1 and A2) that should be defined with the same parameters. The selected positions are highlighted with an orange frame (figure 2.13, B).

**NOTE:** For the 16-well Nucleocuvette™ Strips, you can select a whole column by double clicking on the top or bottom well. Wells can be de-selected by tapping on the position again. The orange frame will disappear.

2. Upon well selection the fields “CELL TYPE PROGRAM”, “CUSTOM PROGRAM”, “PULSE CODE”, “SOLUTION” and “CONTROL” will be activated (figure 2.13, B). For further explanation about parameters, please refer to chapter 2.8.3.
3. Press the field “CELL TYPE PROGRAM” to choose predefined Nucleofection conditions from a cell type list (figure 2.13, C). Use the search (magnifying glass symbol) or the sort list functions (A-Z) to find conditions more quickly.
  - a. Select the desired cell type code by tapping on the appropriate line of the cell list. The cell type selected will be highlighted. For additional information about the cell type selected press “i”.
  - b. To confirm your selection press “OK”.
  - c. If required, modify pulse code by pressing the letter or number code fields. A keyboard will appear, enabling you to change settings. The solution code can be modified via a selection list.

**NOTE:** Instead of defining solution and program code via the CELL TYPE PROGRAM, both parameters can also be selected manually, e.g. in case no predefined cell type program is available. For adding new cell type programs, please refer to chapter 2.7.4.

4. Define control options for the selected vessel by choosing (figure 2.13, D)
5. Optional: At this stage (or at step ?) you can save your defined experiment for future use by pressing the “SAVE” button. A keyboard will appear allowing you to define a name (max. length: 26 characters). You may enter further information about your experiment by touching the “Info” field and typing in your text (figure 2.13, E).
6. Confirm and save the experiment parameters by pressing “OK” (saves the experiment and opens the drawer) or “SAVE” (saves the experiment for later use).
7. A summary of the defined settings will be displayed (figure 2.13, F). Please check for correctness before loading samples (see chapter 2.9.2) and starting the experiments by pressing “START” (see chapter 2.9.3)

## 2.9.2 Loading Samples

8. Load the 16-well Nucleocuvette™ Strip (figure 2.14, A, B) or one or two 100 µL Nucleocuvette™ Vessels into the sample tray (figure 2.14, E, F)
9. Make sure that the strip is oriented properly:
  - For the 16-well Nucleocuvette™ Strip, the yellow pin at the rear end of the strip must be visible (figure 2.14, C). If the strip is mounted in the wrong orientation its rear end will stay above the strip holder and the yellow pin is hardly visible (figure 2.14, D).
  - For the 100 µL Nucleocuvette™ Vessel, the cuvettes should be mounted such that the label LONZA on the lid is readable and the opening clip is at the front (figure 2.14, G, H).

Figure 2.14: Loading of samples

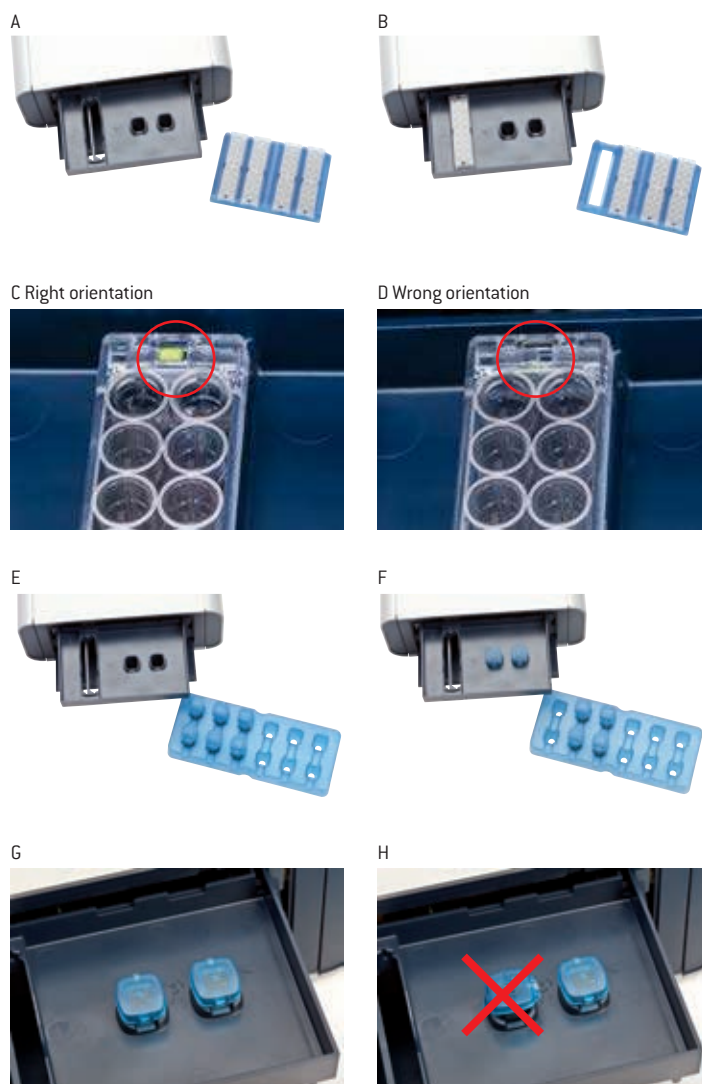
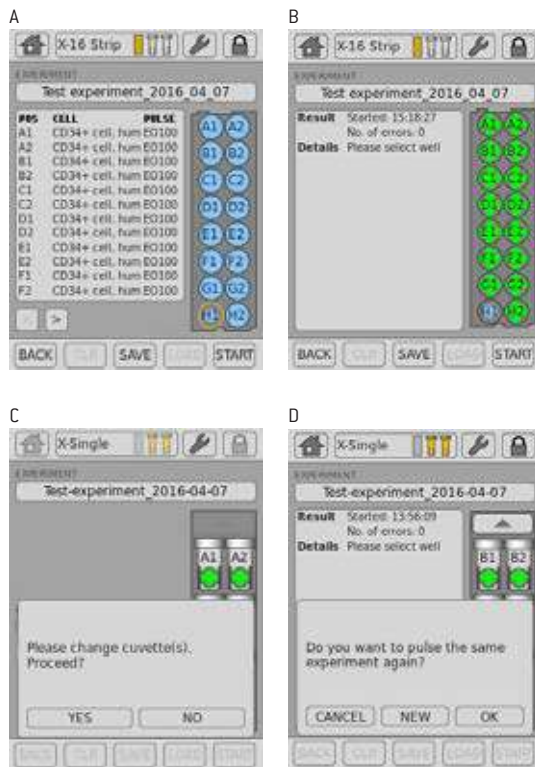




Figure 2.15: Experiment progress (X Unit)



### 2.9.3 Running the Experiment

10. After loading the samples press “START” to run the experiment (figure 2.15, A).
11. The progress of the experiment is indicated by changing the color of the cuvette or well positions (figure 2.15, B) (for color codes see chapter 4).

**NOTE:** When working with the single 100  $\mu$ L Nucleocuvette™ Vessels you can process two cuvettes at once. If more than two cuvettes have been defined the drawer opens automatically after each set of two and a message “Please change cuvette(s). Proceed?” will be displayed (figure 2.15, C). Load next samples and press “YES” to continue or press “NO” to interrupt the experiment.

12. When the experiment is complete, a result file summarizing the Nucleofection process will be displayed (figure 2.15, B).
13. The result file will be saved automatically by the system and can be reopened as described in chapter 2.12.2.
14. You can repeat the same experiment by pressing “START” again. A message will appear asking “Do you want to pulse the same experiment again?” (figure 2.15, D).
15. Press “OK” to start the experiment, “NEW” to return to the “Experiment” screen and define a new experiment or “CANCEL” to return to the “Results” screen.

## 2.10 4D-Nucleofector™ Y Unit

### 2.10.1 Defining a New Experiment

1. After unit selection (see chapter 2.8.2) a screen appears that allows you to define a new experiment or select a previously saved experiment by pressing “Choose existing experiment” (figure 2.16, A).
2. When defining a new experiment, by tapping on a position you can select one or multiple samples/wells (e.g. A1 and A2) that should be defined with the same parameters. The selected positions are highlighted with an orange frame (figure 2.16, B).

**NOTE:** You can select a whole column by double clicking on the top or bottom well. Wells can be de-selected by tapping on the position again. The orange frame will disappear.

3. Upon well selection the fields “CELL TYPE PROGRAM”, “CUSTOM PROGRAM”, “PULSE CODE”, “SOLUTION” and “CONTROL” will be activated (2). For further explanation about parameters, please refer to chapter 2.8.3.
4. Press the field “CELL TYPE PROGRAM” to choose predefined Nucleofection conditions from a cell type list (figure 2.16, C). Use the search (magnifying glass symbol) or the sort list functions (A-Z) to find conditions more quickly.
  - a. Select the desired cell type code by tapping on the appropriate line of the cell list. The cell type selected will be highlighted. For additional information about the cell type selected press “i”.
  - b. To confirm your selection press “OK”.
  - c. If required, modify pulse code by pressing the letter or number code fields. A keyboard will appear, enabling you to change settings. The solution code can be modified via a selection list.

**NOTE:** Instead of defining solution and program code via the CELL TYPE PROGRAM, both parameters can also be selected manually, e.g. in case no predefined cell type program is available. For adding new cell type programs, please refer to chapter 2.12.3.

5. Define control options for the selected well by choosing (figure 2.16, E)
6. Optional: At this stage (or at step 8) you can save your defined experiment for future use by pressing the “SAVE” button. A keyboard will appear allowing you to define a name (max. length: 26 characters). You may enter further information about your experiment by touching the “Info” field and typing in your text (figure 2.16, F).
7. Confirm and save the experiment parameters by pressing “OK” (saves the experiment and opens the drawer) or “SAVE” (saves the experiment for later use).
8. A summary of the defined settings will be displayed (figure 2.16, G). Please check for correctness before loading samples (see chapter 2.10.2) and starting the experiments by pressing “START” (see chapter 2.10.3).

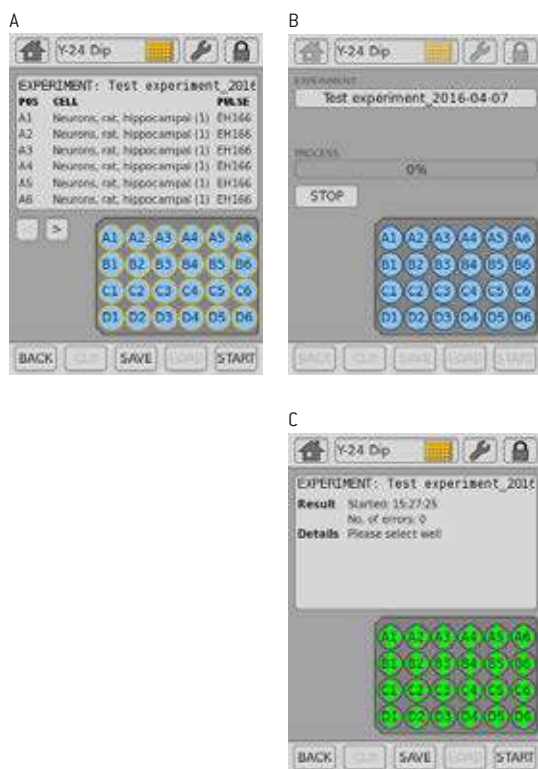
Figure 2.16: Experiment definition (Y Unit)



Figure 2.17: Loading of samples (Y Unit)



Figure 2.18: Experiment progress (Y Unit)



## 2.10.2 Loading Samples

9. Insert the 24-well Dipping Electrode Array into the 24-well culture plate containing your Nucleofection samples. Make sure that the dipping electrode array is inserted in the right orientation.
10. Place 24-well plate with inserted dipping electrode array into the retainer of the 4D-Nucleofector™ Y Unit. Well “A1” must be in upper left position. If the array-plate sandwich was entered in the wrong orientation an error message will appear after pressing “START”.
11. It is not recommended to re-use dipping electrodes as this may lead to suboptimal transfection efficiencies. An RFID chip contained in the dipping electrode lid logs usages and after pressing “START” the software will check for used wells and offer you different options how to continue.

## 2.10.3 Running the Experiment

12. After loading the samples press “START” to run the experiment (figure 2.18, A).
13. The progress of the experiment is indicated by changing the color of the cuvette or well positions (figure 2.18, B) (for color codes see chapter 4).
14. When the experiment is complete, a result file summarizing the Nucleofection process will be displayed (figure 2.18, C).
15. The result file will be saved automatically by the system and can be reopened as described in chapter 2.12.2.
16. You can repeat the same experiment by pressing “START” again. A message will appear asking “Do you want to pulse the same experiment again?”.
17. Press “OK” to start the experiment, “NEW” to return to the “Experiment” screen and define a new experiment or “CANCEL” to return to the “Results” screen.

## 2.11 4D-Nucleofector™ LV Unit

The 4D-Nucleofector™ LV Unit can handle two types of vessels, the fixed volume 1 mL Nucleocuvette™ Cartridge for cell numbers up to  $1 \times 10^8$  cells and the scalable LV Nucleocuvette™ Cartridge for cell numbers up to  $1 \times 10^9$  cells.

### Components of 1 mL Nucleocuvette™ Cartridge (figure 2.19, A)

1. Transparent handle/handle bar
2. Contacting side/part/area
3. Injection port (rear side)
4. Air outlet port with 0.2  $\mu\text{M}$  sterile filter

### Components of the LV Nucleocuvette™ Cartridge (figure 2.19, B, C)

1. Cartridge
2. Venting tube with 0.2  $\mu\text{M}$  sterile filter
3. Inlet port 1 (spinning Spiros with red dust protection cap)
4. Inlet port 2 (spinning Spiros with red dust protection cap)
5. Outlet port (spinning Spiros with red dust protection cap)
6. Reservoirs (swabbable injection port with male Luer cap)

**NOTE:** Please do not alter or disassemble the pre-assembled cartridge or reservoirs.

Figure 2.19: Nucleocuvette™ Cartridge types for LV Unit

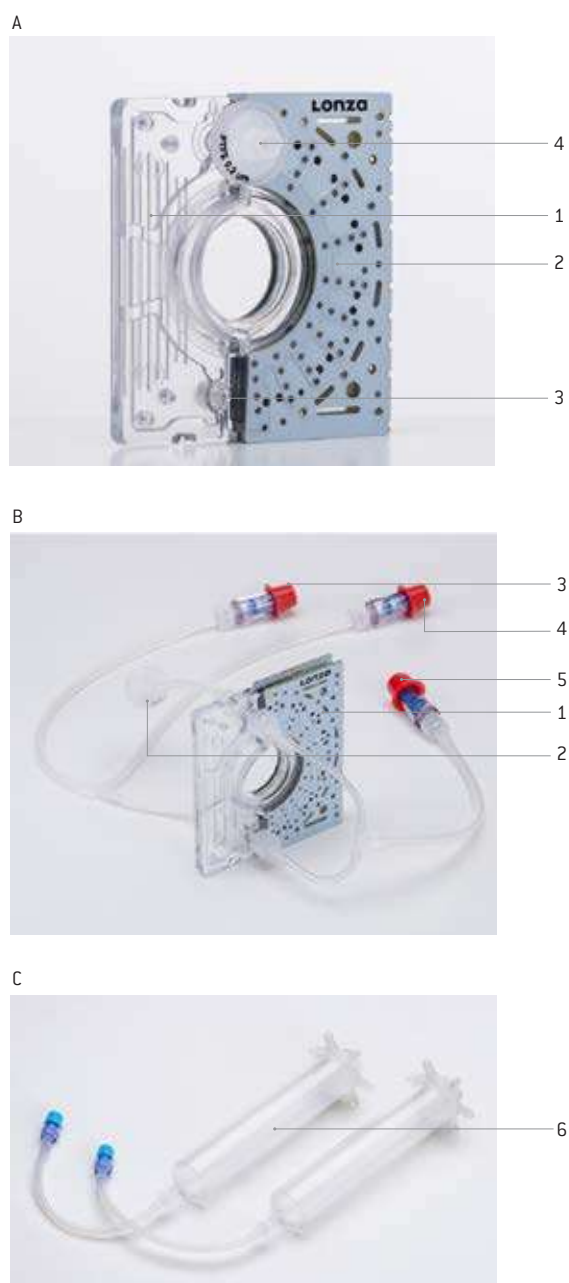


Figure 2.20: Experiment definition (LV Unit, 1 mL Nucleocuvette™ Cartridge)



Figure 2.21: Loading of sample (LV Unit, 1 mL Nucleocuvette™ Cartridge)



## 2.11.1 Using the 1 mL Nucleocuvette™ Cartridge (Fixed Volume)

### 2.11.1.1 Defining a New Experiment

- After unit and vessel type selection (see chapter 2.8.2) a screen appears which allows you to define experiment parameters select a previously saved experiment by pressing “Choose existing experiment” (figure 2.20, A).
- Press the field “CELL TYPE PROGRAM” to choose predefined Nucleofection conditions from a cell type list (figure 2.20, B). Use the search (magnifying glass symbol) or the sort list functions (A-Z) to find conditions more quickly.
  - Select the desired cell type code by tapping on the appropriate line of the cell list. The cell type selected will be highlighted. For additional information about the cell type selected press “i”.
  - To confirm your selection press “OK”.
  - If required, modify pulse code by pressing the letter or number code fields. A keyboard will appear, enabling you to change settings. The solution code can be modified via a selection list.
- Instead of defining solution and program code via the CELL TYPE PROGRAM, both parameters can also be selected manually, e.g. in case no predefined cell type program is available. For adding new cell type programs, please refer to chapter 2.12.3.
- Optional: At this stage (or at step 8) you can save your defined experiment for future use by pressing the “SAVE” button (figure 2.20, C). A keyboard will appear allowing you to define a name (max. length: 26 characters).
- You may enter further information about your experiment by touching the “Info” field and typing in your text (figure 2.20, D).
- Confirm and save the experiment parameters by pressing “OK” or “SAVE”.
- Press OK to continue (figure 2.20, E)
- A summary of the defined settings will be displayed (figure 2.20, F). Please check for correctness before continuing.

### 2.11.1.2 Loading Samples

- Fill the 1 mL Nucleocuvette™ Cartridge with the cell-substrate solution (figure 2.21, A ). A volume of 1 mL is required to completely fill the cuvette. Using a higher or lower volume may lead to errors.
- Insert the filled cartridge into the slot until it snaps in (figure 2.21, B).



### 2.11.1.3 Running the Experiment

11. When the cartridge is inserted, press “START” to initiate the Nucleofection process (figure 2.22, A).
12. The progress of the experiment is indicated by a progress bar (figure 2.22, B).
13. When the experiment is complete, a result file summarizing the Nucleofection process will be displayed.
14. The result file will be saved automatically by the system and can be reopened as described in chapter 2.12.2. The overall result is indicated by a color code: green for “OK” (figure 2.22, C), yellow in case of few errors and red in case of multiple errors (figure 2.22, D). If errors occurred you can check for more details by pressing “More details”. The result details (figure 2.22, E) will display the type of error and its percent occurrence in the processed volume. For additional information on the error press “i”.
15. Once the process is completed take out the cartridge by pressing the black levers.
16. Aspirate the transfected cell suspension and transfer cells into culture. Optionally you may wash the cartridge with medium to aspirate residual liquid.
17. You can repeat the same experiment by pressing “START” again.

Figure 2.22: Experiment progress (LV Unit, 1 mL Nucleocuvette™ Cartridge)

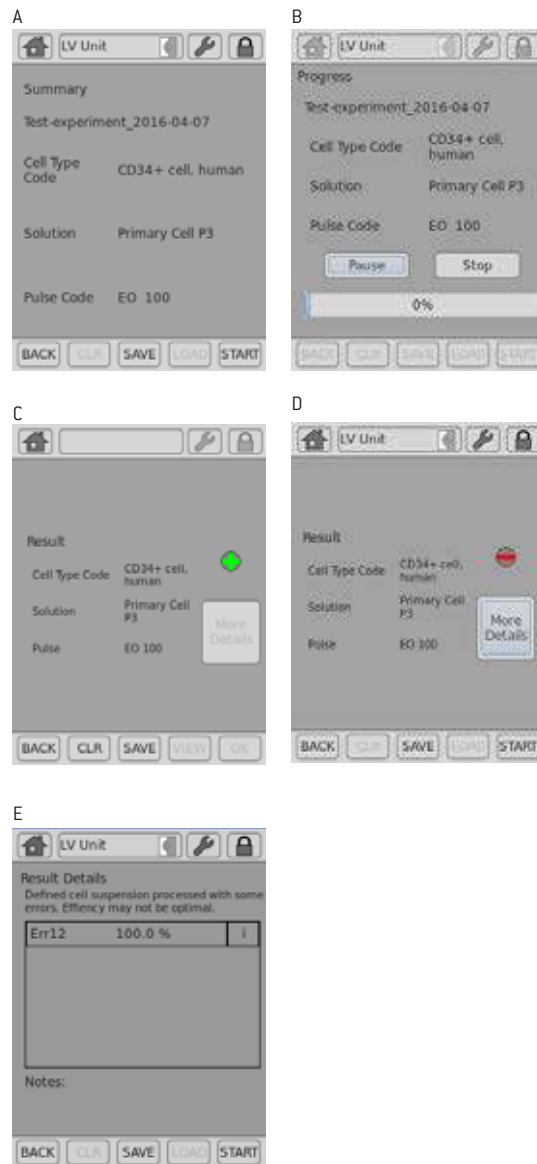
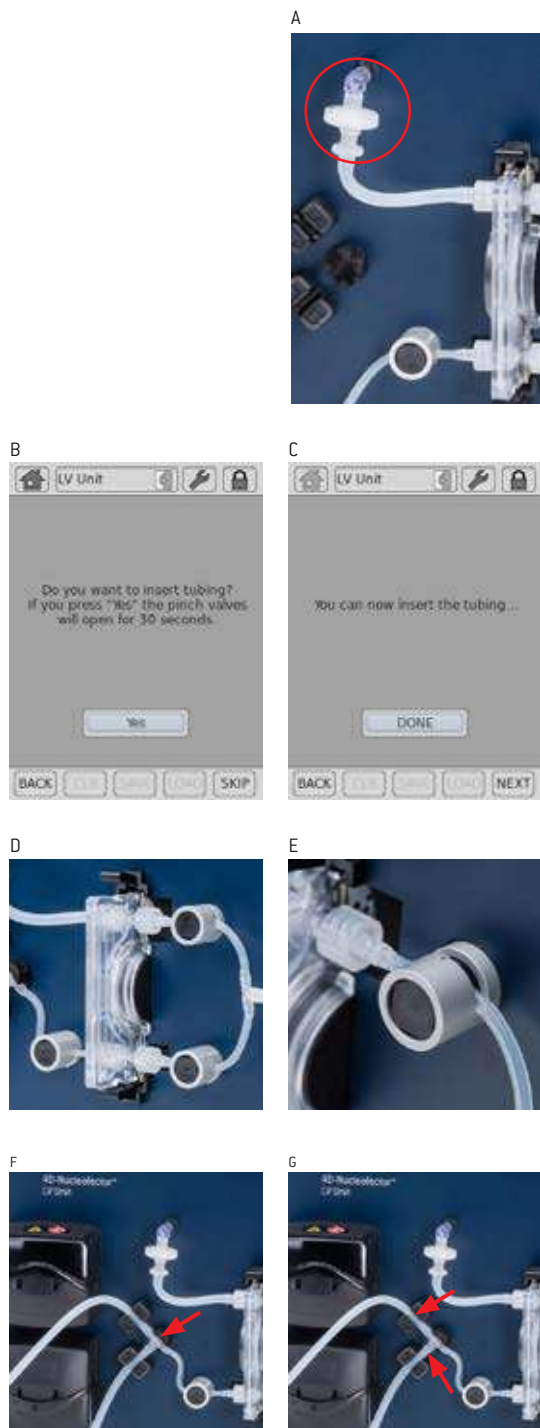


Figure 2.23: Mounting the LV Nucleocuvette™ Cartridge



## 2.11.2 Using the LV Nucleocuvette™ Cartridge (Scalable Volume)

### Before starting to prepare any cells

- Insert LV Nucleocuvette™ Cartridge including all tubes into the 4D-Nucleofector™ LV Unit (see steps 1-10 below)
- Connect outlet reservoir (see steps 11-12 below)
- Define the experiment (chapter 2.11.2.2)

### 2.11.2.1 Mounting an LV Nucleocuvette™ Cartridge

1. Insert the pre-assembled LV Nucleocuvette™ Cartridge into the slot until upper and lower snap mechanism audibly snaps in.
2. Attach the short venting tube with the filter (upper left of the cartridge) to the male Luer connector on the front plate (figure 2.23, A).
3. After unit and vessel type selection (see chapter 2.8.2) a pop up window will appear asking whether you want to insert the pre-assembled LV Nucleocuvette™ Cartridge the device now (figure 2.23, B). If you want to do that at a later time point press “SKIP” and continue with step 14.
4. Upon pressing “YES” the pinch valves will automatically open for 30 seconds allowing to conveniently insert the tubes into the three pinch valves and a new window will appear (figure 2.23, C).
 

**Note:** The open time of the pinch valves is limited to 30 seconds to avoid overloading of the electronics. Alternatively, you can open the pinch valves manually by pushing the black button on the valves.
5. Insert the tubes into the three pinch valves (figure 2.23, D). Ensure that they are inserted completely (figure 2.23, E). After 30 seconds, the pinch valves will close automatically.
6. Press “DONE” when mounting is completed (figure 2.23, C). In case you did not finalize insertion within 30 seconds, you may re-open the valves by pressing “YES” again or via “Settings” menu or manually (see “Note” above).
7. Insert the T connector on the left hand side of the cartridge into the T connector holder (figure 2.23, F).
8. Insert the two larger diameter tubes into the liquid sensors (figure 2.23, G). Ensure the tubes are inserted completely into the sensors.

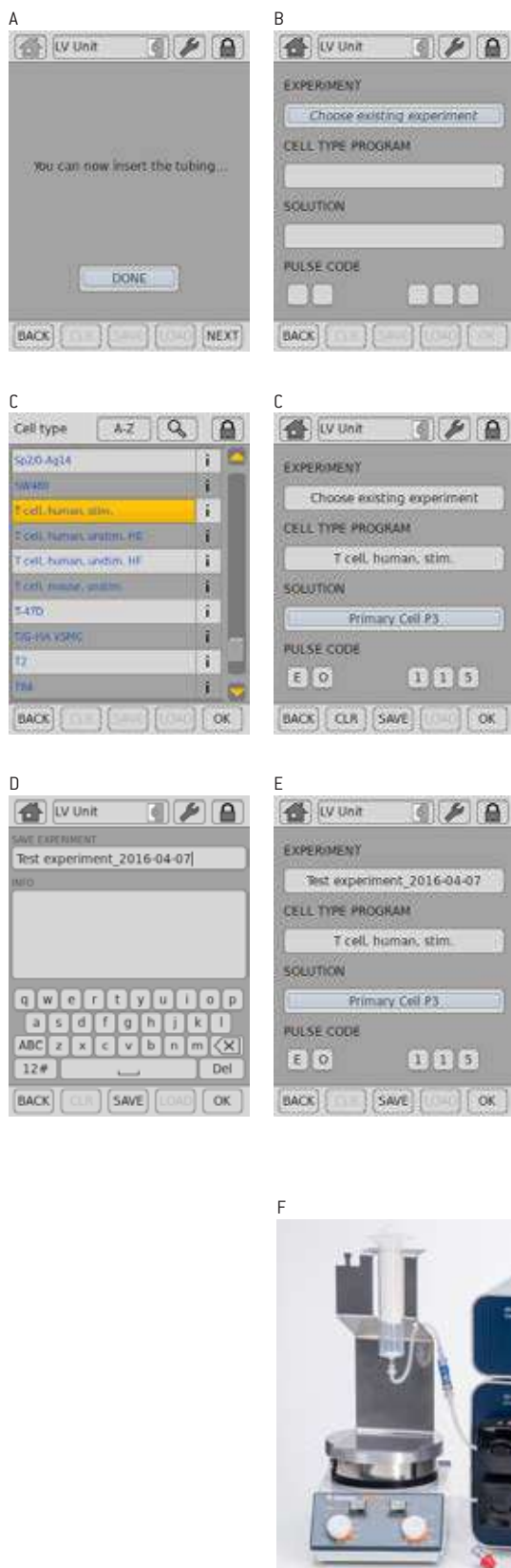
9. Insert the upper tube into the upper pump and close the flap (figure 2.23, H).
10. Repeat with the lower tube and the lower peristaltic pump (optional if working with 2 reservoirs).
11. Fill **outlet reservoir** with medium (for detailed recommendations, please refer to cell type-specific protocol) and mount it into a 4D-Nucleofector™ LV Reservoir Rack (figure 2.23, I).
12. Remove the red cap from the Spiros connector on the outlet tube of the cartridge and connect it to the swabbable injection port of the outlet reservoir (or any other reservoir with female Luer connector, figure 2.23, J).
13. Before continuing with defining the experiment (chapter 2.11.2.2) check setup status. At this point steps 1-6 and 11 of the arrangement shown in figure 2.25 should be done.

Figure 2.23: Continued





Figure 2.24: Experiment definition (LV Unit, LV Nucleocuvette™ Cartridge)



System with one inlet reservoir

### 2.11.2.2 Defining a New Experiment

14. Press “NEXT” (figure 2.24, A) to define experiment parameters or select a previously saved experiment by pressing “Choose existing experiment” (figure 2.24, B).
15. Press the field “CELL TYPE PROGRAM” to choose predefined Nucleofection conditions from a cell type list (figure 2.24, C). Use the search (magnifying glass symbol) or the sort list functions (A-Z) to find conditions more quickly.
  - a. Select the desired cell code by tapping on the appropriate line of the cell list. The cell type selected will be highlighted. For additional information about the cell type selected press “i”.
  - b. To confirm your selection press “OK”.
  - c. If required, modify pulse code by pressing the letter or number code fields. A keyboard will appear, enabling you to change settings. The solution code can be modified via a selection list.
16. Instead of defining solution and program code via the CELL TYPE PROGRAM, both parameters can also be selected manually, e.g. in case no predefined cell type program is available. For adding new cell type programs, please refer to chapter 2.12.3
17. Optional: At this stage (or at step 20) you can save your defined experiment for future use by pressing the “SAVE” button (figure 2.24, D). A keyboard will appear allowing you to define a name (max. length: 26 characters).
18. You may enter further information about your experiment by touching the “Info” field and typing in your text (figure 2.24, E).
19. Confirm and save the experiment parameters by pressing “OK” or “SAVE”
20. On the next screen (figure 2.24, F), press “OK”.

Before continuing, prepare your samples:

21. Prepare cell suspension under the sterile hood (for detailed recommendations, please refer to cell type-specific protocol)
22. Fill a defined volume of cells and substrate into the inlet reservoir(s) mounted on a 4D-Nucleofector™ LV Reservoir Rack.
23. Place the rack with the cell suspension reservoir on a magnetic stirring platform to avoid cell sedimentation when working with larger volumes. Start stirring the cell suspension at ~300 rpm. Ensure that magnet is truly stirring.
24. Remove red caps from the Spiros connectors on the inlet tubes of the cartridge and the blue caps from the swabbable injection port of the outlet reservoirs and connect both (figure 2.24, G).
25. The system is now fully assembled. Check correct assembly as shown in figure 2.25.

Once samples have been prepared finalize experiment definition:

26. Continue with entering the volume(s) that you are going to process (figure 2.24, H).

- a. If you work with **one reservoir (or bag)** containing a premixed suspension of cells and substrate, enter the total volume of the cell suspension you want to process.
- b. In case you work with **two reservoirs (or bags)** to keep cells and substrate separate, tick the box "Substrate volume". Now you can define volumes for cells and substrate independently.

**Note:** The maximum total volume that can be entered is 20 mL.

27. Upon pressing OK a summary of the defined settings will be displayed (figure 2.24, I). Please check for correctness before pressing "START".

Figure 2.24: Continued

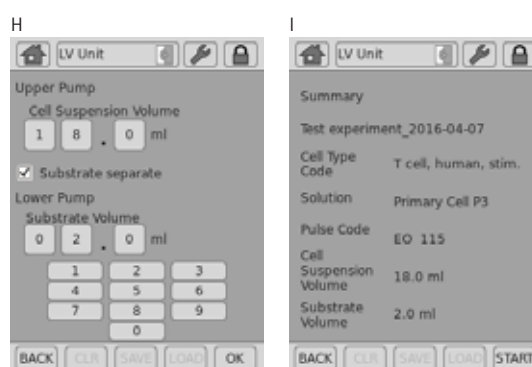
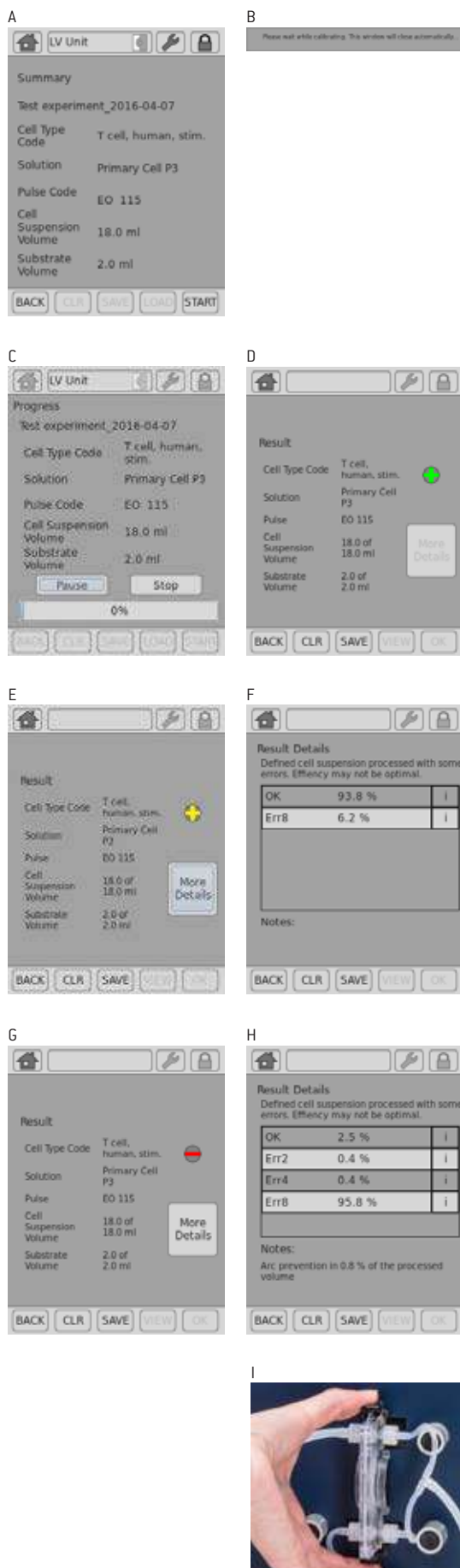


Figure 2.25: Setup Checklist for LV Nucleocuvette™ Cartridge



1. Cartridge inserted in slot
2. Short venting tube with filter attached to the male Luer connector on the front plate
3. Tubes inserted in pinch valves (press black round surface of the valves while pulling tubes fully into the valves)
4. T connector inserted into T connector holder. Two larger diameter tubes completely inserted into liquid sensors
5. Upper tube (for cell suspension!) inserted into the upper pump. Flap closed.
6. Optional - when feeding substrate separately - lower tube inserted into lower pump. Flap closed.
7. Holder for cell suspension reservoir placed on a magnetic stirring platform (stirring speed to approx. 300 rpm)
8. All reservoir-tube connections established to
  - a. Input reservoir for cell suspension
  - b. Input reservoir for substrate (optional)
  - c. Output reservoir

Figure 2.26: Experiment definition (LV Unit, LV Nucleocuvette™ Cartridge)



### 2.11.2.3 Running the Experiment

28. Check defined experiment including volumes again and press “START” to initiate the Nucleofection process (figure 2.26, A).
29. Before starting the system performs a calibration step (figure 2.26, B).
30. The progress of the experiment is indicated by a progress bar (figure 2.26, C).
31. The overall result is indicated by a color code: green for “OK” (figure 2.26, D), yellow in case of few errors (figure 2.26, E) and red in case of multiple errors (figure 2.26, G). If errors occurred you can check for more details by pressing “More details”. The result details (figure 2.26, F, H) will display the type of error and its percent occurrence in the processed volume. For additional information on the error press “;”.
32. Once the process is completed disconnect the outlet reservoir and transfer transfected cell suspension into cell culture vessels.
33. Disassemble the tubing and take out the LV Nucleocuvette™ Cartridge by pressing the black levers (figure 2.26, I) and discard it.

## 2.12 Additional Settings and Options

This chapter explains how to change settings like date and time and how to organize and manage your stored experiments, results and custom programs.

- Activate the “Settings” menu by either pressing the “Core Unit” icon or the “Wrench” icon submenu “Settings” (figure 2.27, A).
- A screen appears displaying a list of setting options (figure 2.27, B):
  - **Experiments** – display user-defined experiments (see chapter 2.12.1 for more information)
  - **Custom programs** – create and manage custom Nucleofection programs (see chapter 2.12.3 for more information)
  - **Results** – reopen or load result files onto a USB stick plugged into the USB port in front of the Core Unit (see chapter 2.12.2 for more information)
  - **Lonza programs** – version information about the cell type list and option to upload a new cell type program list (see chapter 2.12.6)
  - **Display and audio** – adjust brightness of the touch screen display, time to activate standby and deactivate the touch tone
  - **Date and time** – set and format date and time
  - **Language and keyboard** – change language settings for display functions and keyboard
  - **... more** – to switch between pages of the settings list
  - **Firmware update** – update the system software (see chapter 2.12.7 for details)
  - **Device cleaning** – opens the tray of the functional modules to remove it for cleaning (see chapter 2.12.5 for details)
  - **System restart** – restart system
  - **Shut down** – activates the transportation lock (for X and Y Unit) and shuts down the system (software version 4.0 or later, in older versions it may not be available or only activate the transport mode; also see chapter 2.7.4)
  - **Synchronize** – supports synchronization of the actual firmware with the PC Editor (see chapter 2.12.4 and chapter 2.13 for more information)
  - **Version** – displays firmware version and serial number of all modules

To open one of the setting menus touch the appropriate field. Depending on the menu, several parameter settings will appear. Most of the menus are intuitive. The menus requiring additional information are described below.

### 2.12.1 Experiments

This menu displays a list of experiments and allows you to view additional information by pressing the “i” field. Erase existing experiments by pressing “CLR”. To load a predefined experiment from a USB stick, insert the stick into the USB port on the front of the Core Unit, press “LOAD” and follow the instructions displayed on the screen (figure 2.27, C).

Figure 2.27: Additional settings and options for organizing stored experiments

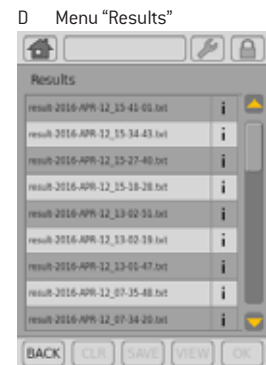
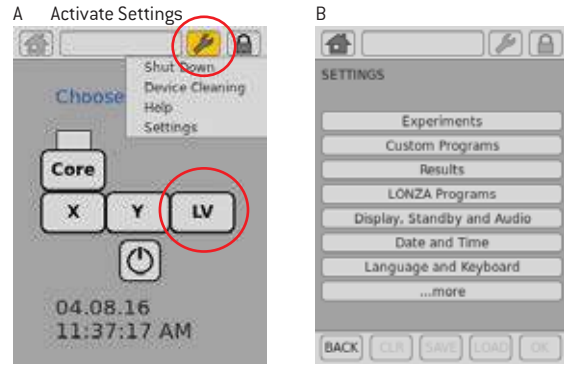


Figure 2.27: Continued

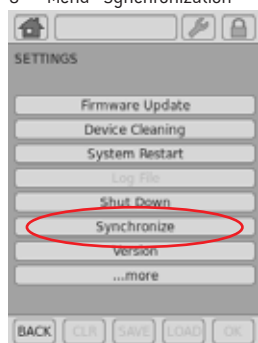
E Menu "Custom Programs"



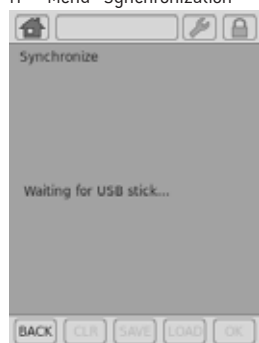
F Menu "Custom Programs"



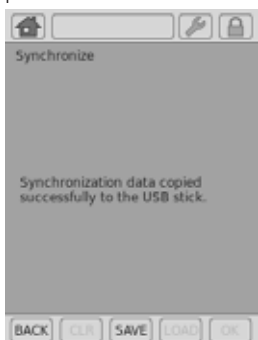
G Menu "Synchronization"



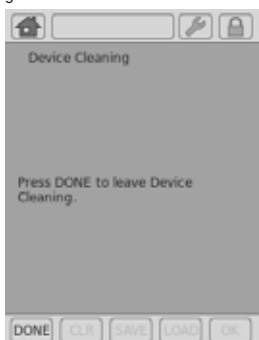
H Menu "Synchronization"



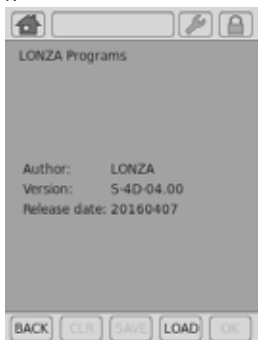
I



J



K



L



## 2.12.2 Results

This menu will display a list of result files and allows you to manage them. Result files are listed in chronological order with newest results at the top. To open existing result files just select the file you'd like to open and press "VIEW". To erase result files that are no longer required, select the file from the list and press "CLR". To store result files on a USB stick, insert the USB device into the USB port on the front of the Core Unit and press "SAVE". Pressing "i" displays further information related to the result file (figure 2.27, D).

## 2.12.3 Custom Programs

The software offers the possibility to add custom program codes for choosing a defined combination of a Nucleofector™ Program and Solution, e.g., after an optimization. Custom program codes are included in alphabetical order into the "Cell Type Program" list and can be differentiated from Lonza defined code by color:

**Blue:** Lonza defined cell type program codes

**Black:** Customer defined codes. You can create new custom programs by pressing the "NEW" field. Edit existing programs by pressing "EDIT" or save your programs onto a USB stick by pressing "SAVE" (figure 2.27, E).

To create a new custom program or to edit an existing program:

- Press "NEW" or select an existing program and press "EDIT".
- A screen will open comprising a keyboard, "PULSE CODE" setting, a "SOLUTION" field and a "VOLUME" selection (figure 2.27, F).
- Define a name for your custom program by typing, e.g., the cell name.
- Press "SOLUTION" to display a list of available solutions.
- Select the solution most suited for your cell from the list and confirm your selection with "OK".
- Define the Nucleofection program by typing in the "PULSE CODE" — you can determine the best program by using one of our optimization kits or simply use the code from an existing optimized protocol.
- Select functional units for which the code should apply (either X and LV Unit or Y Unit).
- Confirm the setting for your custom program by pressing "OK".
- The custom program can now be selected for an experiment by pressing the "CELL TYPE LIST" field.

Custom program codes won't be overwritten in case an updated program definition file is installed (see chapter 2.12.6).

## 2.12.4 Synchronize

This menu is used to synchronize the firmware of your 4D-Nucleofector™ System with the PC Editor software (see chapter 2.13). It is necessary to perform synchronization with each firmware update.

To start synchronization:

- Insert a USB stick into the USB port on the front of the Core Unit.
- Press **“Synchronize”** (figure 2.17, G).
- The 4D-Nucleofector™ System is now creating a synchronization file and saves it to the USB stick (figure 2.27, H).
- Remove the USB stick and connect it to the PC your PC Editor software is installed.
- Open the PC Editor software as described in chapter 2.13
- Select the menu Settings and press **“Synchronize”**.
- A window will open enabling you to select the USB stick.
- Select the USB stick as storage device and press **“OK”**.
- Confirm that you’d like to update the PC Editor by pressing **“YES”** or cancel by pressing **“NO”**.

## 2.12.5 Cleaning the Nucleofection Vessel Tray (X or Y Unit)

To allow convenient cleaning, the Nucleofection vessel tray can be removed from the X and the Y Unit. To dismount the tray please follow the instructions below:

- Open the **“Settings”** menu
- Press **“Device Cleaning”**.
- The drawer will open, giving access to the Nucleofection vessel tray.
  - Remove the tray from its support by pressing the two plastic noses on the left and right side of the tray.
  - Wash the tray with a cloth damped in water and rinse it with tap water until all leftovers are washed off.
  - Dry the tray carefully.
  - Remount the dried tray to the support.
  - Press **“DONE”**.
- The drawer will close and the tray will be moved to its home position.

## 2.12.6 Lonza Programs

The **“Cell Type Program”** list can be exchanged independently from a firmware update (see chapter 2.12.7). For uploading a new **“Cell Type Program”** list follow instructions below:

- Open the **“Settings”** menu.
- Press **“LONZA Programs”** (figure 2.27, K).
- Insert a USB stick comprising the file you want to upload (\*.pd)
- Press **“Load”** and browse for file location.
- Double-click on USB folder and select new file.
- Press **“OK”** for upload.

## 2.12.7 Firmware Update

Due to technical improvement of the operation software, the 4D-Nucleofector™ System may require an update of its firmware. To update the firmware proceed as follows:

- Start the 4D-Nucleofector™ System.
- The **“Choose a Device”** main screen will appear.
- Click on the Core Unit icon.
- Select **“Firmware Update”** within the **“Settings”** menu.
- A dialog box will appear asking to plug in the USB stick.
- Plug in the USB stick containing the new firmware (as provided by Lonza) and press **UPD”** (figure 2.27, L).
- Follow the instructions on the screen to update the firmware.

**NOTE:** More comprehensive firmware updates may not be possible via USB upload and require return of the system to Lonza.





## Transfer of Experiments from the PC Editor

- Open PC Editor by double clicking on the file 4D-Nucleofector\_Editor.exe.
- Define and save your experiment with the PC Editor
- Plug USB stick into a USB port of your PC.
- Open the “Settings” menu by pressing the “Wrench” icon and select the menu “Experiments”.
- Select your new experiment by clicking the appropriate field (figure 2.29, A).
- This will activate the “SAVE” keys of the PC Editor
- Press “SAVE” .
- A Windows® Explorer® screen will open allowing you to save the experiment to the appropriate location (figure 2.29, B).
- Select the USB stick and press “SAVE”.
- Unplug the USB stick from your PC as soon as the experiment file has been saved.

**NOTE:** Do not unplug the stick while saving!

- Turn on the 4D-Nucleofector™ System.
- Plug USB stick with new experiment into the USB port at the front of the Core Unit (figure 2.29, C).
- Press the Core icon to open the “Settings” menu (figure 2.29, D).
- Press “EXPERIMENTS” to open the experiment menu.
- Press “LOAD”.
- A screen appears displaying “Load experiment from” (figure 2.29, E)
- Select the USB stick by double clicking on “USB”.
- Select your experiment and press “OK” to load it.
- Press “OK” to leave the experiment menu.
- Your new experiment is now saved onto the 4D-Nucleofector™ System and can be used directly.

Figure 2.29: Transferring experiments from the PC Editor

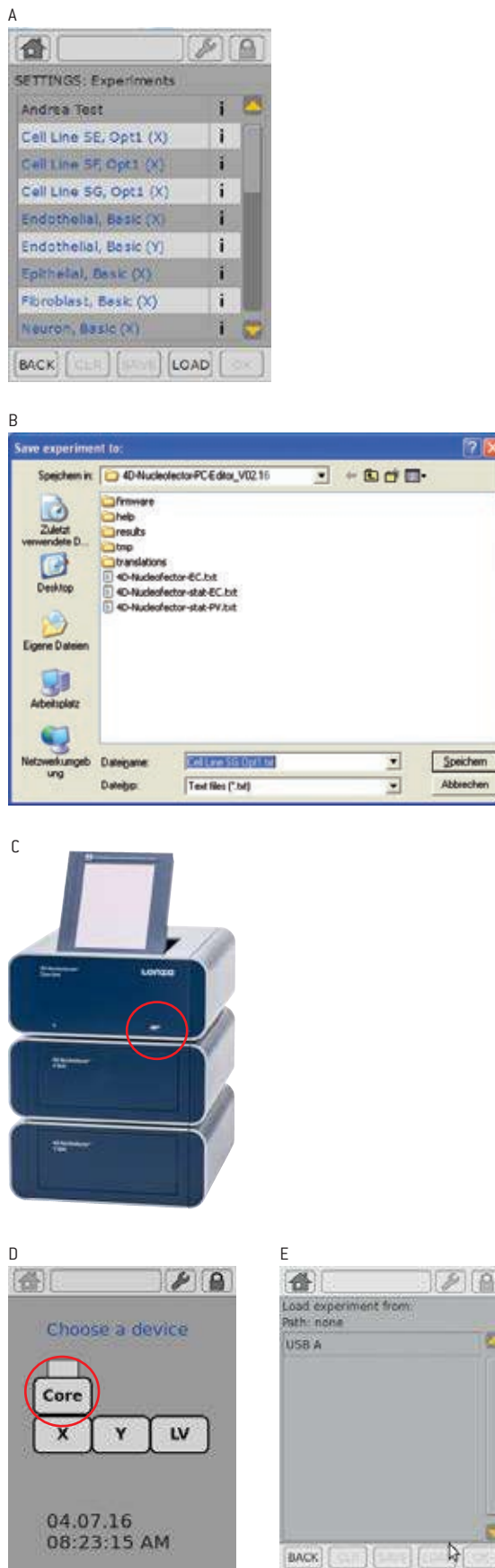




Figure 2.30: Shuttle connectivity on the 4D-Nucleofector™ System



## 2.14 96-well Shuttle™ Mode

To operate the 96-well Shuttle™ Device a 4D-Nucleofector™ System comprising Core and X Unit is required (for details about operating the “Nucleofector™ 96-well Shuttle™ System” please refer to the respective 96-well Shuttle™ Manual). Three connections have to be established:

- High voltage cable between 96-well Shuttle™ Device and X Unit (figure 2.30, 1)
- USB data cable between laptop and Core Unit (figure 2.30, 2)
- Serial data cable between 96-well Shuttle™ Device and Core Unit (figure 2.30, 3)

As soon as the Nucleofector™ 96-well Shuttle™ Software controlling the system is started, the 4D-Nucleofector™ Device switches into “96-well mode” (shown on the display) and the graphical user interface of the 4D-Nucleofector™ Device is locked. It is then controlled by the PC-based Nucleofector™ 96-well Shuttle™ Software. To exit the “96-well mode” press “Abort”.

# 3 Troubleshooting

## 3.1 Suboptimal Transfection Results

The following troubleshooting guidelines may be helpful if experiments using the 4D-Nucleofector™ System do not provide the expected results. The comments are intended to help optimize experimental conditions. If you require further help, please contact our Scientific Support Team.

Issue	Possible Error	Solution
Low survival rate	Cells were kept in Nucleofector™ Solution too long	Transfer cells immediately into pre-warmed medium as recommended in the optimized protocol.
	Cells were damaged by harvesting procedure or through handling	Avoid harsh conditions during cell harvesting, especially centrifugation at higher speed or overexposure to trypsin. Pipette cells smoothly as they are quite stressed already. Use a plastic pipette as recommended in the optimized protocols.
	Cells culture conditions were suboptimal	Cells should be viable and in culture for several passages. Avoid excessive cell densities or cell confluencies since this may decrease cell viability post Nucleofection. For further details please refer to the dedicated optimized protocol.
	Multiple use of cuvettes	We strongly recommend using the Nucleofection vessels only once, because the high voltage pulses that are applied drastically affect their physical integrity.
	Poor DNA quality	DNA used for Nucleofection should be of high purity. We strongly recommend endotoxin-free preparation of the DNA. Do not use procedures involving phenol/chloroform treatment.
Low efficiency	DNA amount is too low	We recommend a certain DNA amount per sample (depending on cell type and Nucleofection vessel; for details please refer to respective optimized protocol). If both transfer efficiency and cell mortality are low, the DNA amount could be increased. Increasing DNA amount may lead to higher transfection efficiency, but at the same time result in higher cell mortality.
	Cell number in Nucleofection sample too high or too low	Please use the cell numbers recommended in the dedicated optimized protocol.
	Poor DNA quality	DNA used for Nucleofection should be of high purity. We strongly recommend endotoxin-free preparation of the DNA. Do not use procedures involving phenol/chloroform treatment.

## 3.2 Rescue of Samples (X and Y Unit)

This chapter describes how to rescue your samples from the X or Y Unit in case of system malfunction or break down of electricity. Depending on the model variant (see table 2.1 on page 8) the process differs slightly (figure 3.1).

### Model variant A (Older):

- Unplug system from main power
- Remove side panel from the X or Y Unit (figure 3.1, A)
- Turn wheel into direction of the red arrow as far as it will go (figure 3.1, B)

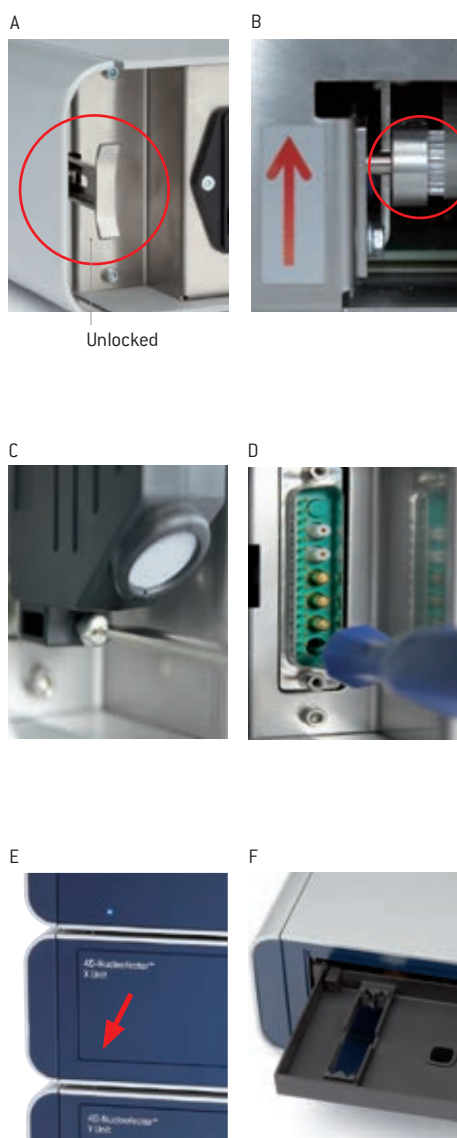
### Model variant B (Newer):

- Unplug system from main power
- Remove the terminator cap or cable connection to next unit (figure 3.1, C)
- Insert screw driver supplied with the 4D-Nucleofector™ System in the lower hole of the plug receptacle (figure 3.1, D)
- Turn the screw manually counter-clockwise until the screw blocks (may require > 60 turns). Turn back clockwise one full turn.

### Further steps for both model variants (Both):


- Open the front flap manually (figure 3.1, E)
- Pull the tray cautiously towards you (figure 3.1, F)
- Remove the Nucleocuvette™ Vessels and rescue your cells
- Contact your Lonza representative to solve potential technical issue

Figure 3.1: Rescue of samples



# 4 Error Codes of the 4D-Nucleofector™ System

The color of a well indicates the completion status of each well (figure 4.1). In case of an error, the respective error code can be displayed by clicking on the well in the result file. This section describes Nucleofector™ Error Codes and provides suggestions for solving the problem.

 If the problem cannot be resolved, please call Lonza's Scientific Support Team

## Europe

Scientific Support: +32 87 321 611  
scientific.support.eu@lonza.com

















## North America

Scientific Support: +1 800 521 0390 (toll free)  
scientific.support@lonza.com

## 4.1 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 4D-Nucleofector™ System is equipped with a hardware safety feature that immediately detects arc formation and protects the cells from damage. After the arc interruption the 4D-Nucleofector™ System resumes program execution. Normally, the program can be completed successfully ("arc discharge correction 1 or 2") and only minor differences in transfection efficiency are observed. Such events are not considered as an error and just indicated as a notes. When repeated arc discharges occur within one program, it may be impossible for the 4D-Nucleofector™ System to complete execution of the program. In this case ("Err8 – Arc discharge"), significant impact on transfection efficiency may be observed. Discard the cuvette (which is possibly damaged) and its content, reset the device by pressing any button and repeat the experiment with a new cuvette. It is not necessary to switch off the 4D-Nucleofector™ System.

Figure 4.1: Color code of wells

	Parameter File	Result File, OK	Result File, Warning	Result File, Error	Skipped Well
Undefined					
Sample					
No Program					
No DNA					

Error Code	What Happened?	Possible Error	Procedure
Err1A	No pulse generated	Internal communication failure, internal error or device possibly defective.	Clear the error message by pressing any button and try to apply program a second time. If Err1A re-occurs, switch off the device, check the interface cable, wait for 2 seconds and switch on again. If error persists, contact our Scientific Support.
Err2	No pulse generated	No or inappropriate cuvette loaded. Inappropriate Nucleofector™ Solution or volume.	Check cuvette. Check type and volume of Nucleofector™ Solution. Cuvette can be reloaded.
Err3	No pulse generated	Improper interconnection or system defective.	Switch off the device. Check proper connection of the interface cable as well as the terminator cap on the last interface outlet. Switch on the device and repeat the experiment with the same sample.
Err 4 WEAK	A weak pulse occurred. Efficiency may be sub-optimal.	Re-used cuvette or inappropriate Nucleofector™ Solution.	Clear the error message by pressing any button. Use the sample if maximum efficiency is not essential. Otherwise, use a new sample.
Err7	No pulse generated	No lid detected on the cuvette.	Clear the error message by pressing any button. Check proper placement of the lid and rerun experiment with the same cuvette.
Err8	Device possibly generated an arc discharge leading to incomplete program execution. A substantial reduction in efficiency and viability must be assumed.	Inappropriate cuvette, Nucleofector™ Solution or volume in the cuvette.	Clear the error message by pressing any button. Check the volume of Nucleofector™ Solution. Try again with a new cuvette.
Err11	No pulse generated.	The device case is open.	Switch the device off, check the correct mounting of all side panels and the interlock between all modules. Try again with the same samples. Can only occur with model variants A (see 2.5).
Err12	Pulse possibly omitted.	Internal device temperature too high for pulsing.	Switch off the device, wait for 2 minutes and run experiment with a new cuvette.
Err13	No pulse generated.	The device front flap is open.	Check proper closing of front flap after the tray is closed and rerun the experiment.
Err 21	Used well: Multiple use of well. Nucleofection may be suboptimal.	Dipping electrode for that well position was used twice.	Utilize sample if maximum performance is not essential. Avoid re-use of dipping electrodes that had been used before.
Err 22	Used well: A weak pulse occurred. Efficiency may be sub-optimal.	Re-used dipping electrode or inappropriate Nucleofector™ Solution.	Clear the error message by pressing any button. Use the sample if maximum efficiency is not essential. Otherwise, use a new sample.
Err 23 & Err24	Used well: Device possibly generated an arc discharge. Program could be resumed and successfully completed. A limited impairment on performance has to be presumed.	Inappropriate solution or well volume, occasional arcing.	Utilize sample if maximum performance is not essential. Check solution and volume.
Err 25	Used well: Device possibly generated an arc discharge leading to incomplete program execution. A substantial reduction in efficiency and viability must be assumed.	Inappropriate dipping electrode, Nucleofector™ Solution or volume in the dipping electrode. Device possibly defective.	Clear the error message by pressing any button. Check the volume of Nucleofector™ Solution. Try again with a new dipping electrode.
Err 26	Used well: Multiple use of well Nucleofection suboptimal	Dipping electrode for that well position was used multiple times.	Utilize sample if maximum performance is not essential. Avoid re-use of dipping electrodes that had been used before.
Res 27	Used well: Well was skipped	User confirmed to skip the well as it had been used before	–
Err 30	Cartridge not detected	Cartridge not properly inserted/arrested	Ensure proper interlocking of cartridge. Remove and insert cartridge again and check snapping of lower and upper release lever.
Err 31	No liquid was detected at liquid sensor 1 (upper sensor)	Cell suspension reservoir not filled or not attached. Pump not closed. Liquid in reservoir too viscous.	Check correct filling and attachment of reservoirs. Close pump.
Err 32	No liquid was detected at liquid sensor 2 (lower sensor)	Substrate reservoir not filled or not attached. Pump not closed. Liquid in reservoir too viscous.	Check correct filling and attachment of reservoirs. Close pump.
Err 33	Target volume not reached for reservoir 1 (upper pump)	Cell suspension reservoir empty before the entered volume has been completely processed. Bubbles in tubing.	Check if reservoir is empty. Check if filling should have matched entered values. Reduce speed of magnetic stirrer. Do not re-use consumables.
Err 34	Target volume not reached for reservoir 2 (lower pump)	Substrate reservoir empty before the entered volume has been completely processed. Bubbles in tubing.	Check if reservoir is empty. Check if filling should have matched entered values. Do not re-use consumables.

# 5 Purchaser Notification

## Purchaser Notification

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# 6 Technical Specifications

Technische Daten, Specifications, Spécifications	
Spannung / power / électrique	100–240 VAC
Frequenz / frequency / fréquence	50–60 Hz
Stromverbrauch / consumption / consommation	0 VA (power off) / 20 VA (idle) / 140 VA (busy - during transfection)
Sicherung / fuse / fusible	T 2.5A, L250V
Betriebsumgebung / operating temperature / environnement	+15°C to +40°C, (59°F - 104°F) non-condensing
Transfektionen pro Minute / transfections per minute / transfections par minute	X Unit: 16-32 reactions (using 16-well Nucleocuvette™ Strips) 20-26 reactions (using 100 µL Nucleocuvette™ Vessels) Y Unit: 8-10 reactions (using Dipping Electrodes) LV Unit: 2-3 mL (using LV Nucleocuvette™ Cartridges)
Schnittstellen / interfaces / interface	RS422 USB Lonza interface for module connection Shuttle HV Out (X Unit only)
Spannungsbegrenzung / voltage limitation / limitation de tension	1500 V
Strombegrenzung / current limitation / limitation de courant	100 A
Klassifikation/ classification/ classification	IP 20 - protection against foreign materials Splash-proof until 15° inclination Group 1, Class B
Abmessungen / dimensions / dimensions (w x h x d)	24.5×10.5×28 cm / 9.7×4.1×11.0 in (Core, X, or Y) 24.5×20.7×28 cm / 9.7×8.5×11.0 in (LV)
Gewicht / weight / poids	Core Unit: 4.4 kg (9.7 lb) X Unit: 4.7 kg (10.4 lb) Y Unit: 5.0 kg (11.0 lb) LV Unit: 5.7 kg (12.5 lb)
Hersteller / supplier / manufacture	Lonza Cologne GmbH Nattermannallee 1 D-50829 Köln, Germany
Herstellungsdatum / date of manufacture /date de production	The manufacturing year is encoded by the second and third digit of the serial number, e.g., serial number x16xxxxx was manufactured in 2016.



Core Unit

X or Y Unit

LV Unit





## Contact Information

### North America

Customer Service: +1 800 638 8174 (toll free)  
order.us@lonza.com  
Scientific Support: +1 800 521 0390 (toll free)  
scientific.support@lonza.com

### Europe

Customer Service: +32 87 321 611  
order.europe@lonza.com  
Scientific Support: +32 87 321 611  
scientific.support.eu@lonza.com

### International

Contact your local Lonza distributor  
Customer Service: +1 301 898 7025  
Fax: +1 301 845 8291  
scientific.support@lonza.com

### International Offices

Australia	+ 61 3 9550 0883
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+ 91 22 4342 4000
Japan	+ 81 3 6264 0660
Luxemburg	+ 32 87 321 611
Singapore	+ 65 6521 4379
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

#### Lonza Cologne GmbH – 50829 Cologne, Germany

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