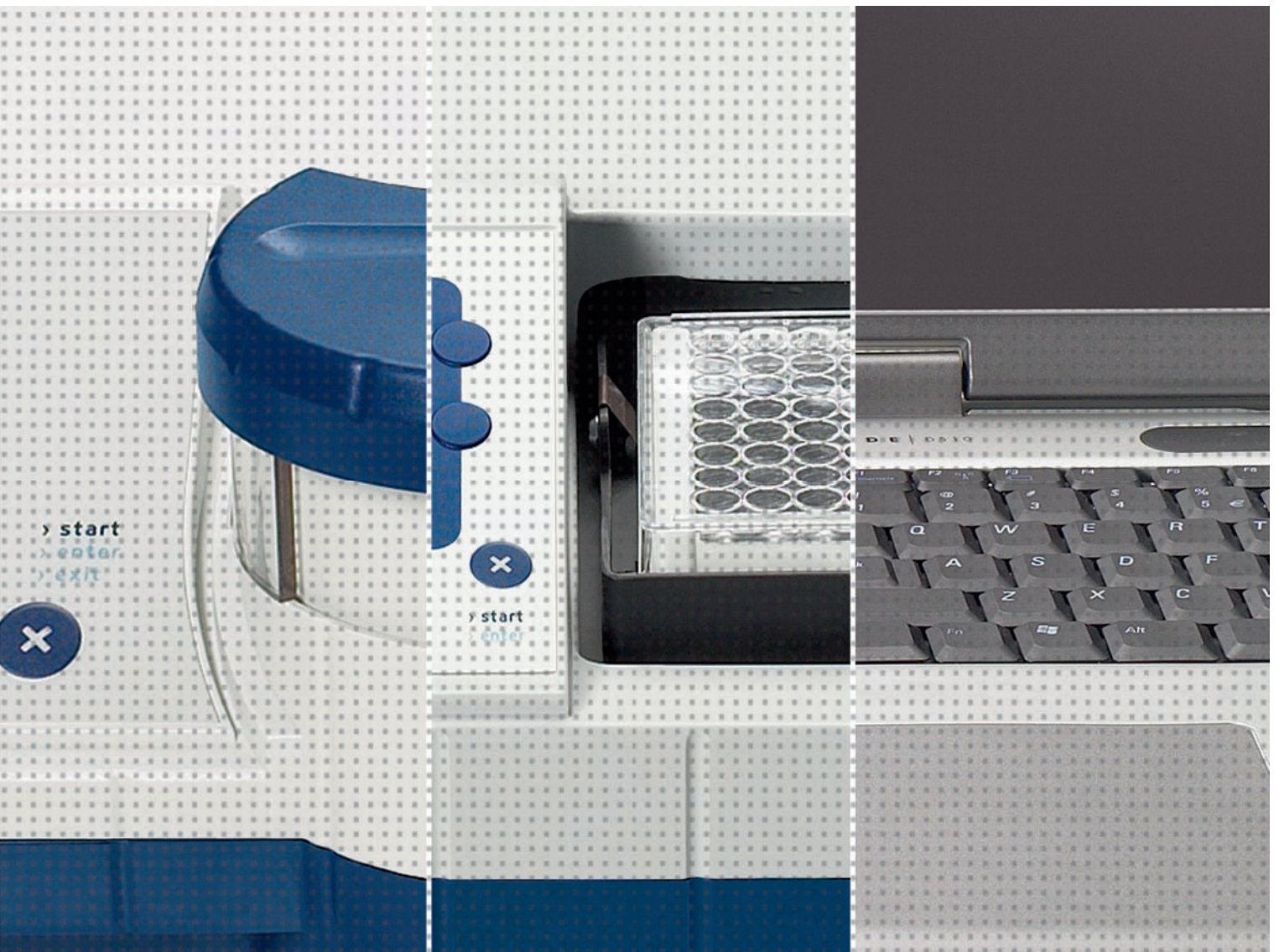


## Amaxa™ Nucleofector™ 96-well Shuttle™ System Software Manual



Nucleofector™ 96-well Shuttle™ Software Installation CD

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# 1.0 Introduction

The Nucleofector™ 96-well Shuttle™ Software is the graphic user interface required for running the Nucleofector™ 96-well Shuttle™ System. It is used to setup, control and save the results of a 96-well Nucleofection™ Experiment. This manual is valid for 96-well Shuttle™ Software versions 1.5b and 1.51b. In former versions, some functionalities might slightly differ. Manuals for all versions are available for download at [www.lonza.com/nucleofection-resources](http://www.lonza.com/nucleofection-resources).

## 2.0 General Specifications and Instructions

### 2.1 Soft-/Hardware Specifications

The Nucleofector™ 96-well Shuttle™ Software is pre-installed on a laptop computer provided by Lonza. For specifications and warranty information, refer to [www.lonza.com/96w-software](http://www.lonza.com/96w-software).

In case a replacement of the laptop is necessary, the following hardware and system specifications are required:

Processor/CPU	minimum 1.7 GHz, e.g., Pentium 3 or higher
Memory	minimum 1024 MB RAM
Data storage	minimum 10 GB hard disk
Monitor	15 inch XGA
System	Windows® XP or 2000
Hardware	USB connection, CD/DVD writer

**Installation CD:** The installation CD can be used to re-install the software on the laptop computer of the Nucleofector™ 96-well Shuttle™ System. Furthermore, the owner is allowed to install and to use the software on the owner's other computers, but such installation of the software may cause damage to the computer or loss of data stored on the computer or the internal network server. The user assumes the entire risk of using the software and for any computer or internal network server and/or data damage arising out of or in connection with the use of the software.

**Software updates:** Lonza reserves its rights to change the described software without announcement or to generate updates of the software. Software updates will be provided on a CD or as download from our website ([www.lonza.com/96w-software](http://www.lonza.com/96w-software)). Please register at [www.lonza.com/96w-software](http://www.lonza.com/96w-software) to receive announcements for Software updates.

#### License statement

Lonza Cologne GmbH is holder of various patents, patent applications, copyrights and technical and scientific experience with respect to the Nucleofector™ Technology. Use of Lonza's Nucleofector™ 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 Nucleofector™ Technology and related software for research and development purposes, the terms of which are disclosed in detail in the license agreements accompanying the shipped Nucleofector™ Device or 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 Nucleofector™ 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](mailto:ip.cologne@lonza.com).

## 2.2 Handling Instructions

Only use the software once you have read and understood the Nucleofector™ 96-well Shuttle™ Software Manual. The manual must be accessible for all users. Make sure that each potential user reads and understands it.

- Do NOT alter the software in any manner.
- Only use the software in connection with the Nucleofector™ 96-well Shuttle™ System.
- **Installation of additional software on the laptop:** All the software necessary for system operation has been pre-installed. It is not recommended to install additional software—including virus scanners or network tools—on the system unless it is absolutely necessary. Even then, be sure it can be uninstalled should it become necessary. Additional software installation is not under the control of Lonza and may void your system warranty. Additional software may cause conflicts with existing software and system operation while it is running or may implement configuration changes during installation that could cause erratic system performance or failure. If additional software is installed on the system, it is done so at the customer's own risk.
- **Installation of Lonza software on another computer:** Installation of the software on another computer than the one provided by Lonza occurs at the users own risk. Installation of software may cause damage to your computer or loss of data stored on the computer. User assumes the entire risk of using the software and for any computer and/or data damage arising out of or in connection with the use of the software.

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 of the above safety and handling instructions.

# 3.0 Operating Instructions

## 3.1 Short Instructions

Step	Description	For details see
1	Connect Nucleofector™ II Device, 96-well Shuttle™ Device and Laptop.	96-well Shuttle™ Manual
2	Switch on all 3 components.	
3	Start the 96-well Shuttle™ Software on the laptop and login.	Chapter 3.2
4	Adapt your user settings (optional).	Chapter 3.4.2
5	Check USB connection between laptop and Nucleofector™ II Device or re-connect.	Chapter 3.4.4
6	Define your experiment by opening an existing Parameter file or creating a new one.	Chapter 3.5
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.	96-well Shuttle™ Manual
9	Upload programs and start Nucleofection™ Process from a) the laptop or b) the 96-well Shuttle™ Device.	Chapter 3.8 96-well Shuttle™ Manual
10	Monitor Nucleofection™ Process during program execution a) either via the Nucleofector™ 96-well Shuttle™ Software b) or the display of the 96-well Shuttle™ Device.	Chapter 3.9 96-well Shuttle™ Manual
11	After run completion, remove 96-well Nucleocuvette™ Plate from the 96-well Shuttle™ Device and transfer samples to culture plates.	
12	View results on the laptop to check if errors occurred.	Chapter 3.9
13	Close files and exit 96-well Shuttle™ Software and switch off all components.	Chapter 3.4.1

## 3.2 Start and Log-In

Switch on the laptop (ignore safety message and wireless message) and start the Nucleofector™ 96-well Shuttle™ Software via the link on the desktop or in the Windows® start menu or via "96wellShuttleSW.exe" under C:\amata\96-well Shuttle™ Software. Upon starting the software, a login window appears asking for user name and password.

Your user name and password for the first login is:

**User name:** Administrator

**Password:** amata

Once you are logged in, you can change your administrator login or create additional users using the "My Profile" command (located in the "Profiles" menu, see 3.4.2).



### 3.3 Compatibility Check and Automatic Software Update

There are two different login levels:

- a) **Administrator:** The Administrator can create new users (see 3.4.2), unlock accounts after entry of a wrong password (see below) and is allowed to import program updates (see 3.4.3).
- b) **User:** normal user

Enter your user name and password.

#### Note

- 1) The login is case-sensitive.
- 2) The account will be locked if a wrong password is entered three times. Please contact the Administrator to unlock your account. In case the Administrator forgot his/her password please contact Lonza's Scientific Support.

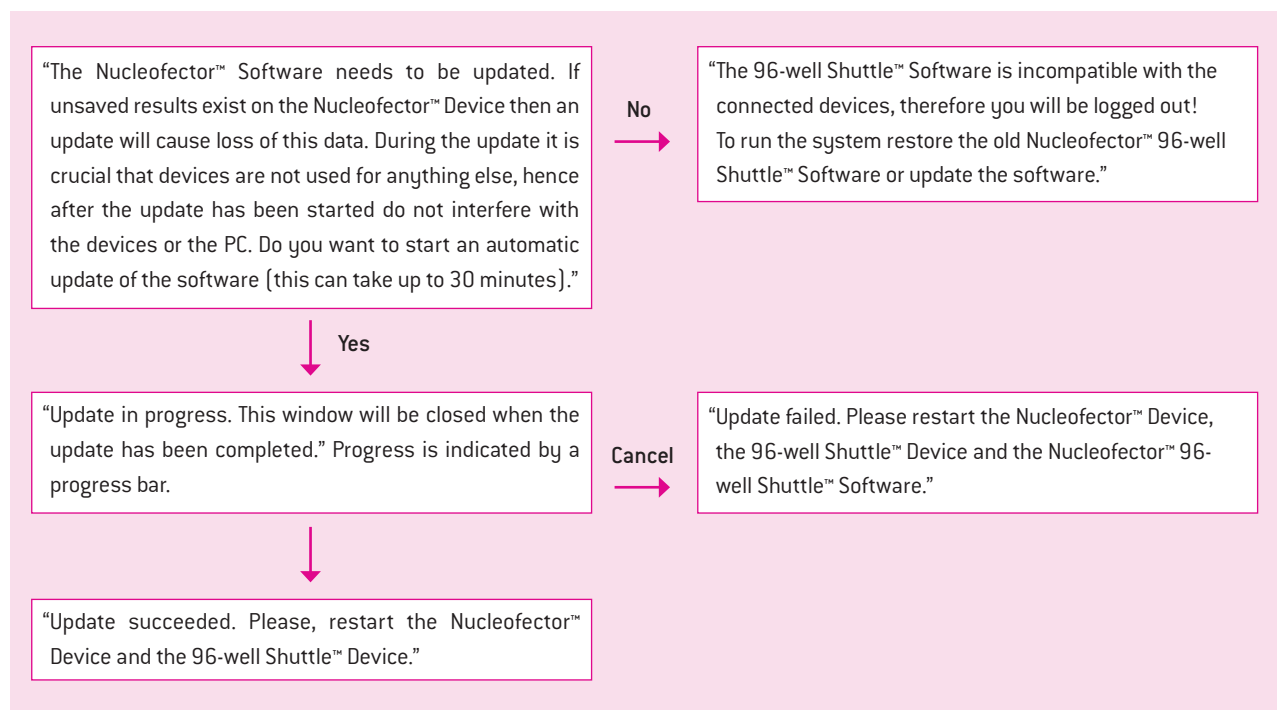
As soon as the Nucleofector™ 96-well Shuttle™ Software has been started and the USB connection is active (see 3.4.4), the Nucleofector™ II Device switches into "96-well mode" (as indicated in its display) and the buttons of the Nucleofector™ II Device are disabled.

Upon starting, the software automatically checks the compatibility of the software versions of all connected Nucleofector™ 96-well Shuttle™ System components, i.e., the Nucleofector™ II Device and the 96-well Shuttle™ Device. This process only works if the system components are properly connected and switched on.

#### Note

For an overview of compatible device and PC software versions, please refer to our website [www.lonza.com/96w-software](http://www.lonza.com/96w-software).

If an update of the Nucleofector™ 96-well Shuttle™ Software version was installed which is not compatible with the software of the connected devices (Nucleofector™ II Device or 96-well Shuttle™ Device), you will be guided through an update process by message windows (see flow chart below). You can either allow the update by pressing the YES button or refuse or cancel the process by pressing the NO or CANCEL button.



## 3.4 Main Menu

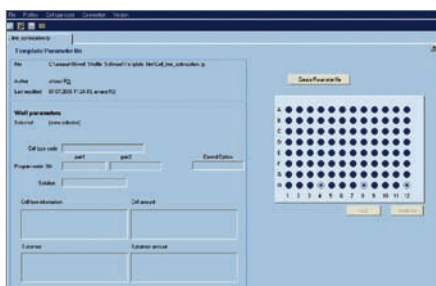
This chapter gives an overview of the menu for the Nucleofector™ 96-well Shuttle™ Software. A more detailed description regarding how to use software files can be found in the subsequent chapters (see 3.5–3.10).

### 3.4.1 Menu “File”

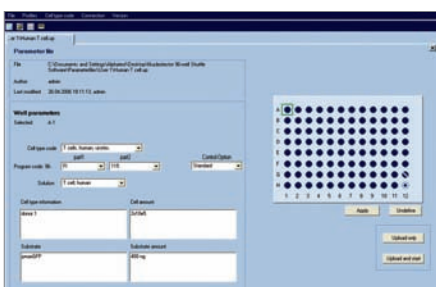
#### Menu “File” > Open

Via this menu point, three file types can be opened:

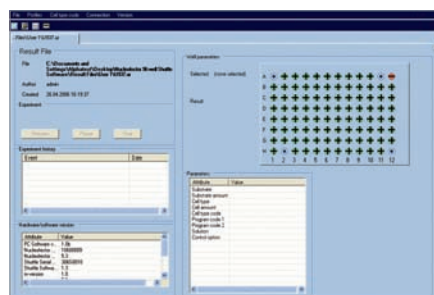
- a) **Template parameter files (\*.tp):** A template parameter file is a parameter file pre-defined by Lonza, e.g., “Cell Line 96-well Optimization Strategy”. Template parameter files are stored in the folder “Nucleofector™ 96-well Shuttle™ Software/amaxa Template Files”. Such a file cannot be edited by a user and it is not suitable to directly start an experiment. For usage and editing it has to be converted into a parameter file saved under a new user-specific name (see 3.5.2).



- b) **Parameter files (\*.up):** These files can be individually created and saved by the user for his specific experimental setups.



- c) **Result files (\*.ur):** A result file is generated out of a parameter file as soon as the user starts a 96-well Nucleofection™ Experiment. The result file inherits all information of the parameter file and additionally logs the results of the Nucleofection™ Process.



#### Menu “File” > New

This menu point is used to create a new parameter file (see 3.5). New template parameter files can be only created by Lonza.

#### Menu “File” > Save

#### Menu “File” > Save as

#### Shortcut <Ctrl> + S

Changes to an existing parameter file can be saved under the same name by choosing “Save” or under a new name by choosing “Save as”.

#### Menu “File” > Report

This menu point opens a tabulated report of a parameter or result file, which can be further exported to the Microsoft® Excel® compatible csv-format.

#### Menu “File” > Close file

Via this menu point any file can be closed. Alternatively, files can be closed via the X button in the upper right edge of the file window.

#### Menu “File” > Logout

Via this menu point, a user can logout either to lock the software without closing it or to enable another user to log-in (see 3.2). Upon logout, the USB connection to the Nucleofector™ II Device is inactivated. If the same or another user logs in again, the connection has to be re-activated (see 3.4.4).

#### Menu “File” > Quit

This menu point quits the Nucleofector™ 96-well Shuttle™ Software. Alternatively, the software can be closed via the X button in the upper right edge of the software window.

### 3.4.2 Menu “Profiles”

#### Menu “Profiles” > My profile > Password

Via this menu point, the user-specific password can be changed.

#### Menu “Profiles” > My profile > Settings

Via this menu point, a user can define his personal system settings.

**Path to save user** Default setting is

**Parameter files:** C:/amaxes/96-well Shuttle Software/  
Parameter files

**Path to save user** Default setting is

**Result files:** C:/amaxes/96-well Shuttle Software/Result  
files

**Autosaving of files:** The user can either define the required autosave intervals from 1 to 30 min or inactivate autosaving by deleting the mark. The default setting is active autosaving every 30 min.

**Automatic or manual opening of the 96-well Shuttle™ Device retainer:** By marking the box on or off, the user has the option to define if the retainer of the 96-well Shuttle™ Device shall open automatically at the end of 96-well Nucleofection™ Experiment or not.

#### Menu “Profiles” > Profile management

This menu point is only accessible for the Administrator to create new users, delete a user or unlock a user in case the wrong password was used three times.

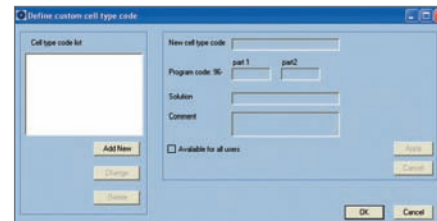
### 3.4.3 Menu “Cell type code”

#### Menu “Cell type code” > Edit

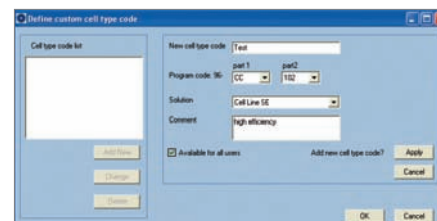
The software offers the possibility to add custom cell-type codes to the cell-type code list for choosing a Nucleofection™ Program, e.g., after determination of a specific program code as optimal for a certain cell-type. Existing custom cell type codes can be only edited or deleted by the user who created them or by the Administrator. Lonza cell-type codes cannot be edited or deleted at all. To add, edit or delete a custom cell-type code all files must be closed.

#### Addition of a new cell-type code:

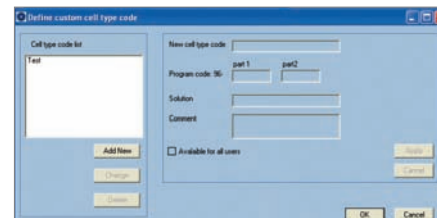
Press “Add new” to add a new cell-type code.



- Enter name for new cell type code.
- Choose the appropriate 96-well program code (part 1 and part 2).
- Choose the respective 96-well solution code to define the solution which works optimal for the new cell type.
- Optional: Define if new cell-type code shall be available for other users by checking the box. In the cell-type code drop-down list of a parameter file, custom cell-type codes will be highlighted in red (if available for all users) or blue (if just available for the user who added it).
- Optional: Add a comment to this code.



- Press “Apply”.
- By pressing “Cancel” all changes will be deleted.



### Editing of an existing cell-type code:

This function is only valid for the users' own cell type codes, but not for Lonza codes or codes of another user. Only the Administrator is allowed to edit all custom cell-type codes.

- Mark the respective cell-type code in the list and press "Change".
- After the changes were done press "Apply".
- By pressing "Cancel" all changes will be ignored.

### Deletion of an existing cell-type code:

This function is only valid for the users own cell type codes, but not for Lonza codes or codes of another user. Only the Administrator is allowed to delete all custom cell-type codes.

- Mark cell-type code in the list and press "Delete".
- Press "Apply" to confirm deletion.
- By pressing "Cancel" the deletion will be ignored.

To close window and accept the changes press "OK". By pressing "Cancel" or closing the window without confirmation by OK, all additions or changes will be ignored.

### Note

If a new Lonza program list is imported (see below) custom cell-type codes won't be overwritten.

### Menu "Cell type code" > Import

This menu point can be used to import a new 96-well Shuttle™ Program List provided by Lonza (program definition files = \*.pd). Importing of a new program list will overwrite the complete list of Lonza program and cell type codes, except for the custom codes (see above).

### Menu "Cell type code" > Nucleofector™ Device

The program list of the Nucleofector™ II Device which is used for its standalone functionality is not related to the 96-well program list stored in the software. However, as an alternative to a chipcard update (see Nucleofector™ II Manual) this menu point offers the Administrator the possibility to upload a new program list provided by Lonza onto the Nucleofector™ II Device (Nucleofector™ Program Definition Files = \*.npd).

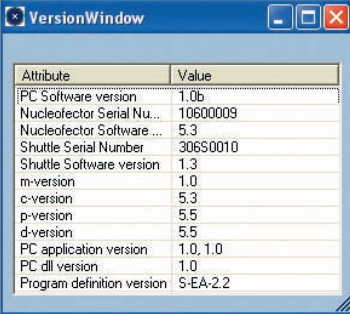
## 3.4.4 Menu "Connection" and Menu "Version"

### Menu "Connection" > Reconnect

The status of USB connection is indicated in the lower bar of the software window ("Connected" or "Not connected"). In case USB connection was not built up while starting the software or in case it is lost or after a re-login press "Reconnect". The connecting process takes a few seconds. In case reconnection fails, try again or re-start your laptop.

### Menu "Version" > Details

This menu point provides information about the system components: the serial numbers of connected devices (Nucleofector™ II Device and 96-well Shuttle™ Device), the software version numbers and the version of the 96-well program list.



Attribute	Value
PC Software version	1.06
Nucleofector Serial Nu...	10600009
Nucleofector Software ...	5.3
Shuttle Serial Number	306S0010
Shuttle Software version	1.3
m-version	1.0
c-version	5.3
p-version	5.5
d-version	5.5
PC application version	1.0, 1.0
PC dll version	1.0
Program definition version	S-EA-2.2

## 3.5 Working with Parameter Files

A parameter file defines a 96-well Nucleofection™ Experiment, i.e., which 96-well program shall be applied to which well. For convenient assignment of parameters to a well the 96-well Nucleocuvette™ Plate is represented graphically and each well can be selected individually (see 3.6).

### 3.5.1 Opening an existing parameter file

#### Menu "File" > Open > Parameter file

An existing parameter file can be directly used to start an experiment or it can be edited as described under 3.6. Up to three parameter files can be opened in parallel.

### 3.5.2 Use of template parameter files

#### Menu “File” > Open > Template parameter file

Pre-defined Lonza template parameter files are stored under C:/amaxes/96-well Shuttle Software/Template files. Such a file cannot be used directly, it has to be converted to a parameter file by pressing “Create parameter file”. The software will ask you to save the file under a new name and the new parameter file opens inheriting the assigned well parameters from the Template parameter file. This new parameter file can be directly used to start an experiment or it can be edited as described under 3.6.

### 3.5.3 Creating a new parameter file

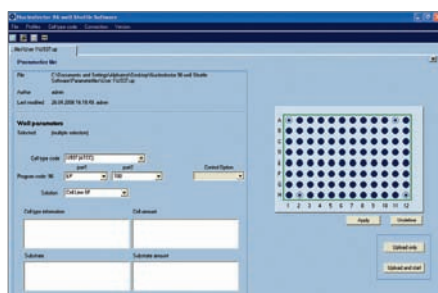
#### Menu “File” > New > Parameter file

The user can generate a new parameter file either by changing an existing one or creating it by opening a new parameter file. The system will ask the user to save it under a new file name. It can be edited as described under 3.6.

## 3.6 Well Selection and Assignment of Well Parameters

### 3.6.1 Well selection

Each well can be selected or activated individually via a left mouse click. Holding the left mouse button and dragging selects multiple wells. Selected wells are indicated by a box. Upon selection of a well the parameter boxes are activated (as indicated by a white color; for color codes of drop-down and text boxes refer to Appendix 5.1).



### 3.6.2 Well parameters

Each well can be defined via the following parameters:

- 96-well Nucleofector™ Program:** This is a mandatory well parameter which can be chosen via two options (drop-downs).  
**By “cell-type code”:** Each cell-type code is assigned to the optimal program for the certain cell type as defined in the Amaxes™ Optimized Protocol (e.g., “T cells, human, unstim.” is affiliated to the optimal 96-well Nucleofection™ Program for unstimulated human T cells). The “cell-type code” list can be extended by the user with customized cell-type codes (e.g., after a cell line optimization) (see 3.4.3).  
**By “program code” (e.g., 96-CA-116):** Part 1 (letters) and part 2 (numbers) can be selected separately. In this case the “cell-type code” box stays empty. Furthermore, if the program code is changed after a program was selected via “cell-type code” the cell-type code entry disappears.
- Control options (drop-down):**  
**“standard”:** This is the default setting, reflecting a normal sample.  
**“no program control”:** This option offers the possibility to add the information “no program control” to a certain well in order to track control wells. A “no program control” is indicated by a dark blue dot in a light blue well (see Appendix 5.2). The fields “cell-type code” and “program code” part 1 and 2 will stay empty and no program will be applied to this well.  
**“no DNA control”:** This option offers the possibility to add the information “no DNA control” to a certain well indicated by a light blue backslash in the dark blue well (see Appendix 5.2). In this case, the selected program will be applied. This option helps to track those wells as controls without substrate.
- 96-well Nucleofector™ Solution (optional):** This drop-down list is connected to the “cell-type code” drop-down: e.g., if cell-type code “T cells, human” is chosen, solution drop-down automatically shows “TC, human” indicating the optimal 96-well Nucleofector™ Solution for human T cells. However, it is a one-directional connection: By changing the solution the “cell-type code” disappears because one solution might be connected to different cell types.
- Further parameters (optional):** can be entered as free text to further describe the experimental parameters
  - Cell-type information: Offers the possibility to add any kind of required additional information to the cell type (e.g., passage no.)
  - Cell amount
  - Substrate
  - Substrate amount

### 3.6.3 Applying of well parameters

For defining the parameters for one or more wells, select/ activate them by using the left mouse button. A selected well is represented by a surrounding box.

After parameters (see 3.6.2) are defined for one or more wells, press the APPLY button to confirm the definition. Upon pressing APPLY, the well color will change from light to dark blue (see Appendix 5.2).

#### Copying of assigned well parameters to another well

The software allows for copying parameters from one well to another within one parameter file or between different parameter files. If selecting different numbers and/or arrangements of wells for copying and pasting please refer to Appendix 5.4 for details how such cases are addressed by the software.

1. Activate/select the well(s) to be copied using the left mouse button.
2. Click the right mouse button to open the “well editing menu” (copy, paste, undefine) and choose “copy” via left mouse click.
3. Activate/select the well(s) into which you wish to paste the copied parameters using the left mouse button.
4. Click the right mouse button to open the “well editing menu” (copy, paste, undefine) and choose “paste” via left mouse click.

As long as no new well parameters are copied, the copy memory is not deleted, i.e., well parameters can be pasted multiple times into different wells.

#### Note

As an alternative to the menu-based copy and paste commands, you can use the shortcuts <Ctrl>+C (copy) and <Ctrl>+P (paste). An autofill function is implemented: e.g., if 4 wells are copied but only one is selected for pasting, the other 3 will be filled automatically. If more than 4 wells are selected for pasting the other wells will be filled accordingly.

### 3.6.4 Removal of assigned well parameters

To remove the definition of a well, select respective well(s) and press the UNDEFINE button.

### 3.6.5 Change of parameters for a group of wells

The software allows for parameters from a group of selected wells to be changed at the same time:

1. Select the desired wells: if parameters differ between members of the group this will be indicated by either an empty grey box (in the case of drop-down boxes) or a grey box with red text stating “values differ” (in the case of free text boxes).
2. Change parameter(s) of interest. These values will be changed for all selected wells.
3. Confirm changes by pressing APPLY (all previous definitions of each well will be kept except the newly entered parameter(s)).

#### Note

In the case that you wish to make sure that all parameters for a selected group of wells are identical, it is often useful to first press UNDEFINE (which resets all parameters) and then to re-define each parameter.

## 3.7 Report of Parameter File

#### File > Report

For overview purposes, in addition to the graphical representation of the 96 wells, a table with assigned well parameters is available as printable report in a new window. The table can be sorted individually by clicking on the header of a column. The report can be printed and also exported as an Excel-compatible csv-file.

The screenshot shows a window titled "Parameter file report" with a file path: C:\Documents and Settings\Alphast\Desktop\Nucleostar 36-well-Grids\Software\Parameters\user 1\1037214p. The table below represents the data shown in the screenshot.

File	Lab	Plates	Program	Calibration	Substrate	Well Type	Well Amount	Substrate Am.	Control notes
B	1	EP	100	UNDEF (ATCC2)	CellLine SF				No Program
B	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
C	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
D	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
E	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
F	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
G	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
H	1	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
A	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
B	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
C	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
D	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
E	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
F	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
G	2	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
H	2	EP	100	UNDEF (ATCC2)	CellLine SF				No Program
A	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
B	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
C	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
D	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
E	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
F	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard
G	3	EP	100	UNDEF (ATCC2)	CellLine SF				Standard

## 3.8 Starting a 96-well Nucleofection™ Experiment

A 96-well Nucleofection™ Experiment is started from the active parameter file (up to 3 parameter files can be open in parallel, the active one is the one visible on the screen). For starting an experiment there are two options:

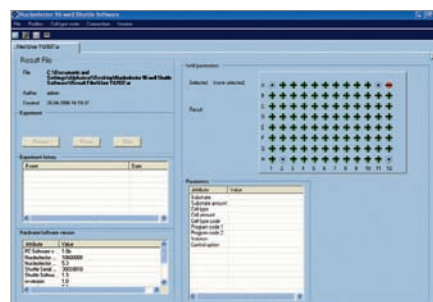
### Start directly via software

- Press “Upload and Start” to start the experiment directly (prerequisite: 96-well Nucleocuvette™ Plate is already inserted in the retainer of the 96-well Shuttle™ Device).
- The software will ask you to enter a name for the result file.
- The parameter file is sent to the Nucleofector™ II Device. The progress of this transfer is shown by a running bar.
- The retainer of the 96-well Shuttle™ Device closes automatically.
- After a short lag phase, the Nucleofection™ Process starts, i.e., the defined programs are applied well-by-well.

### Start from 96-well Shuttle™ Device

- Press “Upload” to upload the experiment.
- The software will ask you to enter a name for the result file.
- The parameter file is sent to the Nucleofector™ II Device. The progress of this transfer is shown by a running bar.
- Successful upload will be indicated in the software by “The experiment was successfully uploaded to the device”.
- Insert the loaded 96-well Nucleocuvette™ Plate into the retainer of the 96-well Shuttle™ Device.
- Press the START button on the 96-well Shuttle™ Device.
- After a short lag phase, the Nucleofection™ Process starts, i.e., the defined programs are applied well-by-well.

plus error explanation including handling recommendations) please refer to chapter 4. The experiment history logs processing information as start, pause, stop and completion.



### Note

In case of a software crash or loss of the USB connection during execution only the monitoring process is interrupted while the execution is not affected. Upon re-start of the software or re-connection monitoring continues and results will be downloaded as usual.

### 3.9.1 Interruption of the 96-well Nucleofection™ Process

The experiment can be interrupted from the result file or via the 96-well Shuttle™ Device menu (please refer to 96-well Shuttle™ Manual) at any time by pressing the PAUSE button. The retainer will open automatically (if “retainer opens automatically at end” is selected in the user settings, see 3.4.2).

Options during the paused status:

- a) **Abortion of the experiment:** The experiment can be completely aborted by pressing the STOP button. The results of the wells processed so far will be downloaded into the result file. The download is indicated by a progress bar and finished with the message “Download completed”.
- b) **Resumption of the experiment:** The experiment can be resumed by pressing the RESUME button. The 96-well Shuttle™ Device continues with the next well in line and the process can be monitored on the screen of the Nucleofector™ 96-well Shuttle™ Software.

## 3.9 Working with Result Files (\*.ur)

The result file opens automatically once “Upload” or “Upload and start” has been pressed and a result file name has been entered (see 3.8). During the Nucleofection™ Process, the progress of the experiment is monitored and shown graphically (green = OK, red = error occurred). After completion of the Nucleofection™ Process and final download of the results, the result of each well is saved automatically in the result file (each partial or completed run will be saved automatically). Result files can't be closed before the download is completed. In case of an error, the respective error code can be viewed by clicking on the respective well or printing the report (see 3.10). For extended error information (error code



### 3.9.2 Completion of the 96-well Nucleofection™ Experiment

At the end of a Nucleofection™ Experiment, the retainer opens automatically (if “retainer opens automatically at end” is selected in the user settings, see 3.4.2). The results will be downloaded into the result file. The download is indicated by a progress bar and finished with the message “Download completed”.

### 3.9.3 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.

When an experiment is completed, the Nucleofector™ II Device stays in 96-well Nucleofection™ Mode. To reset the status of the Nucleofector™ II Device to stand-alone functionality the device has to be re-started.

## 3.10 Report and Export of a Result File

### File > Report

The results can be also viewed as a printable and exportable report.

The report contains the following information:

- Time needed for the complete Nucleofection™ Process
- Assigned Well parameters
- Result for each well: OK or errors with extended error information
- Time stamp and UserID (for regulated environments)

The report can be printed and also exported as an Excel-compatible csv-file.

Row	Cols	Program	CellType	Solution	Cell Type	Cell Area	Subst	Subst Area	Control	Error	Time
E	10	EP	100	VRSP paTCC2	CellLine SF				Standard OK		133
F	10	EP	100	VRSP paTCC2	CellLine SF				Standard OK		135
G	10	EP	100	VRSP paTCC2	CellLine SF				Standard OK		136
H	10	EP	100	VRSP paTCC2	CellLine SF				Standard OK		138
B	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		140
C	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		141
D	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		143
E	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		145
F	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		146
G	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		148
H	11	EP	100	VRSP paTCC2	CellLine SF				Standard OK		150
A	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK	Ex 6- Acc Discharge c	162
B	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		164
C	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		166
D	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		167
E	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		169
F	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		171
G	12	EP	100	VRSP paTCC2	CellLine SF				Standard OK		173

Well	Program	CellType	Solution	Cell Type	Cell Area	Subst	Subst Area	Control	Error	Time
A1	EP	100	VRSP paTCC2	CellLine SF				Standard OK		133
B1	EP	100	VRSP paTCC2	CellLine SF				Standard OK		135
C1	EP	100	VRSP paTCC2	CellLine SF				Standard OK		136
D1	EP	100	VRSP paTCC2	CellLine SF				Standard OK		138
A2	EP	100	VRSP paTCC2	CellLine SF				Standard OK		140
B2	EP	100	VRSP paTCC2	CellLine SF				Standard OK		141
C2	EP	100	VRSP paTCC2	CellLine SF				Standard OK		143
D2	EP	100	VRSP paTCC2	CellLine SF				Standard OK		145
A3	EP	100	VRSP paTCC2	CellLine SF				Standard OK		146
B3	EP	100	VRSP paTCC2	CellLine SF				Standard OK		148
C3	EP	100	VRSP paTCC2	CellLine SF				Standard OK		150
D3	EP	100	VRSP paTCC2	CellLine SF				Standard OK		162
A4	EP	100	VRSP paTCC2	CellLine SF				Standard OK		164
B4	EP	100	VRSP paTCC2	CellLine SF				Standard OK		166
C4	EP	100	VRSP paTCC2	CellLine SF				Standard OK		167
D4	EP	100	VRSP paTCC2	CellLine SF				Standard OK		169
A5	EP	100	VRSP paTCC2	CellLine SF				Standard OK		171
B5	EP	100	VRSP paTCC2	CellLine SF				Standard OK		173



# 4.0 Troubleshooting

## 4.1 Troubleshooting

The following troubleshooting guide may be helpful if the Nucleofector™ 96-well Shuttle™ Software does not work as expected. Should you have any questions regarding the use of the software, please do not hesitate to contact Lonza's Scientific Support Teams:

### Europe

Phone +49 221 99199 400

Fax +49 221 99199 499

scientific.support.eu@lonza.com

www.lonza.com/nucleofection-support

### North America

Phone 800 521 0390 (toll free)

Fax +1 301 845 8338

scientific.support@lonza.com

www.lonza.com/nucleofection-support

In case of troubles with any hardware part of your laptop, please contact the original suppliers (e.g. Dell™) worldwide service teams. A list of international contact addresses is provided with the laptop.

If you have questions relating to a Microsoft® program (Windows® or Office®), please contact Microsoft® Technical Service.

Problem	Procedure
Your log-in request was ignored due to wrong user name and/or password.	Try it again and check for case-sensitivity. The account will be locked if a wrong password word is entered three times. Please contact the Administrator to unlock your account. In case the Administrator forgot his/her password please contact Lonza's Scientific Support.
The Nucleofector™ II Device does not switch to 96-well mode after the software was started.	Check USB status. If inactive ("not connected") perform a re-connect.
"Not connected" is indicated in the lower bar of the software window.	Check physical USB connection and perform a re-connect. If this does not solve the problem, re-start the software or re-start the whole system.
The csv-file opened in Excel does not result in column-separated data.	Mark the whole column containing the data, go to the menu point "data" and choose "text in columns" to open the conversion wizard. In the first dialog choose the upper option "separated" and press continue. In the second dialog choose "semicolon" as separation marker and press finish.

## 4.2 Error Codes

There are three different sources for the generation of errors:

1. Program execution errors (see below)
2. Software or Communication errors: Such errors will be communicated by the software via a message window.
3. Mechanical errors of the 96-well Shuttle™ [www.lonza.com/nucleofection-support](http://www.lonza.com/nucleofection-support) (for details, please refer to 96-well Shuttle™ Manual)

### 4.2.1 Program execution errors

Each program execution error will be logged by its error code (e.g., Err1A) in the result file of a 96-well Nucleofection™ Experiment. In parallel the display of the 96-well Shuttle™ Device will count up the number of errors ( $\sum E = n+1$ ).

Some errors (Err2, Err3 and Err7) – if they occur on more than 16 successive wells – will lead to an interruption of the process. All well results generated before interruption will be downloaded. In that case, the display of the 96-well Shuttle™ Device will show its “Stopped” screen.

Error Code (logged in result file)	What happened?	Possible error	What to do?
<b>Err1A –</b> Internal communication failure	No pulse generated.	Internal Nucleofector™ II Device error or device possibly defective.	If you want to retry the failed wells, generate a new Parameter file containing parameters for the previously failed wells only. If the experiment was interrupted due to continuous Err1A, switch off the Nucleofector™ Device, wait for 2 seconds, switch on again and re-start program execution.
<b>Err1B –</b> Internal communication failure	Pulse generated with unclear outcome.	Internal Nucleofector™ II Device error or device possibly defective.	If you want to retry the failed wells with new samples, generate a new Parameter file containing parameters for the previously failed wells only. If the experiment was interrupted due to continuous Err1B, switch off the Nucleofector™ Device, wait for 2 seconds, switch on again and re-start program execution with new samples.
<b>Err2 –</b> Inappropriate or no solution, contacting error, HV cables disconnected	No pulse generated.	Inappropriate or no solution or inappropriate well volume or inefficient contacting of well(s) or high voltage cables not connected.	If you want to retry the failed wells, verify the volume in the corresponding well, check type of solution, check connection of high voltage cables and generate a new Parameter file containing parameters for the previously failed wells only. If the experiment was interrupted due to continuous Err2, check connection of high voltage cables or switch off the Nucleofector™ Device, wait for 2 seconds, switch on again and re-start program execution.

Error Code (logged in result file)	What happened?	Possible error	What to do?
<b>Err3 –</b> Device possibly defective	Very weak pulse generated.	Nucleofector™ II Device overheated or possibly defective or inefficient contacting of well(s).	Switch off the Nucleofector™ II Device, wait for 1 minute, switch it on again and re-start program execution with new samples.
<b>Err5 –</b> Arc discharge correction 1	Device possibly generated an arc discharge. Program could be resumed and successfully completed. A limited impairment on efficiency and vitality has to be presumed.	Inappropriate solution or inappropriate well volume, occasional arcing.	Utilize samples if maximum efficiency is not essential. If you want to retry failed wells with new samples, check whether combination of 96-well program and solution fits the protocol recommendations and check sample volume.
<b>Err6 –</b> Arc discharge correction 2	Device possibly generated an arc discharge. Program could be resumed and successfully completed. A limited impairment on efficiency and vitality has to be presumed.	Inappropriate solution or inappropriate well volume, occasional arcing.	Utilize samples if maximum efficiency is not essential. If you want to retry failed wells with new samples, check whether combination of 96-well program and Solution fits the protocol recommendations and check sample volume.
<b>Err7 –</b> Supply voltage too low	No pulse generated.	Supply voltage insufficient or Nucleofector™ II Device defective.	If you want to retry the failed wells, generate a new Parameter file containing parameters for the previously failed wells only. If the experiment was interrupted due to continuous Err7, check supply voltage and re-start program execution.
<b>Err8 –</b> Arc discharge	Device possibly generated an arc discharge leading to incomplete program execution. A substantial impairment on efficiency and viability has to be presumed.	Inappropriate solution or volume, occasional arcing.	If you want to retry failed wells with new samples, check whether combination of 96-well program and solution fits the protocol recommendations and check sample volume.
<b>Err9 –</b> Inappropriate solution, volume	Device possibly generated an over-current. A substantial impairment on efficiency and viability has to be presumed.	Inappropriate solution or volume or Nucleofector™ II Device possibly defective.	If you want to retry failed wells with new samples, check whether combination of 96-well program and solution fits the protocol recommendations and check sample volume.
<b>Err10 –</b> Inappropriate solution, volume	Device possibly generated an over-current. A substantial impairment on efficiency and viability has to be presumed.	Inappropriate solution or volume or Nucleofector™ II Device possibly defective.	If you want to retry failed wells with new samples, check whether combination of 96-well program and solution fits the protocol recommendations and check sample volume.

Error Code (logged in result file)	What happened?	Possible error	What to do?
<b>Err11</b> – DSP error	Pulse possibly omitted.	Internal Nucleofector™ II Device error or device possibly defective.	If you want to retry failed wells with new samples, switch off the Nucleofector™ Device, wait for 2 seconds, switch on again and re-start program execution with new samples.
<b>WEAK</b> – Inappropriate solution	A weak pulse occurred. Only sub-optimal efficiency might be achieved.	Inappropriate solution or combination with program, well volume too low.	Utilize samples if maximum efficiency is not essential. If you want to retry failed wells with new samples, check whether combination of 96-well program and solution fits the protocol recommendations and check sample volume.












## 5.0 Appendix

### 5.1 Color Code of Boxes (Drop-Downs, Text Boxes)

	Background	Text	Values
Inactive box – no well selected	Pale blue	No text	No values
Active box – one well selected	White	Black text	Shown
Active box – several wells selected with same parameters	White	Black text	shown
Active box – several wells selected with different parameters for some boxes*	Boxes with same parameters: white	Boxes with same parameters: black	Boxes with same parameters: shown
	Boxes with different parameters: light grey	Boxes with different parameters: red	Boxes with different parameters: “values differ”

\* It is possible to change text in these boxes. After pressing APPLY the newly entered information is true for all wells that were selected. Respective boxes will get white with black text.

### 5.2 Color Code of Wells

	Parameter file	Result file OK	Result file Error
Undefined			
Defined			
No program control			
No DNA control			

## 5.3 List of Shortcuts

<Ctrl> + C	Copy
<Ctrl> + V	Paste
<Ctrl> + S	Save
<Ctrl> + Z	Undo
<Ctrl> + Y	Redo
<Ctrl> + D	Undefine
Shift + arrow keys	Selection of several wells

## 5.4 Copy-Paste Functionality

### 1. Copy

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

### 2. Paste

a) To same number of selected wells:

Selection of wells in which copied parameters shall be pasted:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Result of pasting:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

b) To a smaller number of selected wells:

Selection of wells in which copied parameters shall be pasted:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Result of pasting:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

c) To a higher number of selected wells:

Selection of wells in which copied parameters shall be pasted:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Result of pasting:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

## Contact Information

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 scientific.support.eu@lonza.com  
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Italy	+39 0363 45710
Japan	+81 3 5566 0612
Poland	+48 22 833 87 45
Singapore	+65 64914214
Spain	+34 902 531 366
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