Lonza

HT Nucleofector® Software Manual

Content

1 Introduction

The HT Nucleofector® Software is the graphic user interface
required to operate the HT Nucleofector® System. It is used
to set up, control and save the results of an experiment.
This manual describes the HT Nucleofector® Software and
is valid for HT Nucleofector® Software version 2.0 and above.

The HT Nucleofector® System — designed for high-throughput approaches

The HT Nucleofector® System was developed for efficient, reproducible, and flexible high-throughput transfection. With up to 95% transfection efficiency and the ability to deliver various substrates into virtually any cell type, the HT Nucleofector® System meets your screening requirements.

Experience the benefits of the HT Nucleofector® Device:

Automation-compatible – Full integration into liquid handling platforms
Fast – Process 384 samples in one minute
Screening-compatible – Low cell numbers required per sample

For fine-tuning of the optimization process, please contact our scientific support teams.

Europe and International
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2 General specifications/installation

2.1 Soft-/hardware specifications

The HT Nucleofector® Software is pre-installed on a laptop computer provided by Lonza. In case a replacement of the computer is necessary, the following hardware and system specifications are required:

System	Specifications
Processor/CPU	minimum 2.5 GHz, e.g., Intel Core i5
Memory	minimum 4 GB RAM
Data storage	minimum 512 GB free hard disk space
Monitor/resolution	FullHD (at least 1920 x 1080 pixels)
System	Windows® 10
Hardware	USB 3.0

Installation USB Memory Stick

The USB memory stick provided with the HT Nucleofector® System can be used to re-install the software on the computer operating the HT Nucleofector® System. The software may be installed on other computers owned by the user. Installation and use of the software is at the user's own risk, and Lonza assumes no responsibility for any computer or internal network server and/or data damage arising from 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 announced by e-mail and provided on a CD or as download from our website.

2.2 Handling instructions

Read this HT Nucleofector® Software Manual before using the software. This manual should be accessible to all users. Make sure that each potential user has read it.
Do **not** alter the software in any manner.
Only use the software in connection with the HT Nucleofector® System.

Installation of Lonza software on a personal computer

Installation of the software on a personal computer occurs at the user's own risk. Lonza disclaims all warranties and shall in no event be liable for any damage caused by or arising out of any operation or use in violation of the safety and handling instructions set forth herein.

3 Initializing the system

3.1 Start and login

Switch on the laptop computer and start the HT Nucleofector® Software via the link on the desktop or in the Windows® start menu. Upon starting the software, a login window appears asking for a user name and password.
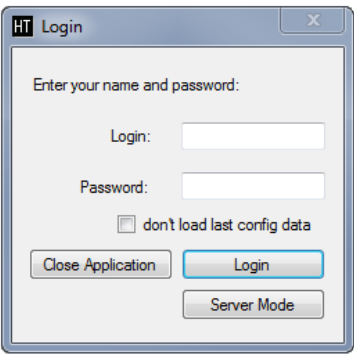
The user name and password for the first login is:

User name: Admin

Password: <empty>

Once you are logged in you can change your administrator login user name and password and create additional by selecting "**Profiles** > **Add New"** (see 4.3.5).

There are two different login levels:

Administrator: The administrator can create new users, unlock accounts after repeated entry of a wrong password and import program updates (see 4.3.4).

User: Normal users with no administration rights.

The login screen also allows the start of the automation server for operating the HT Nucleofector® within an automated environment.
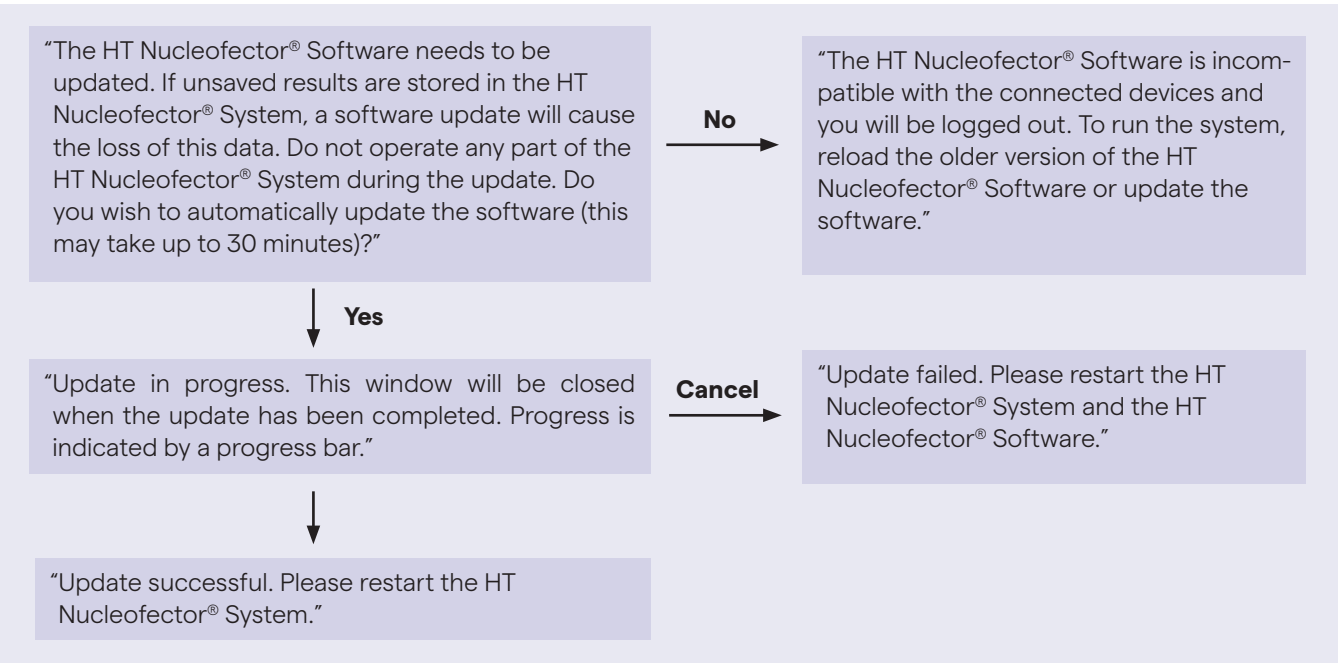
Figure 3.2: Initial HT Nucleofector® Software Screen
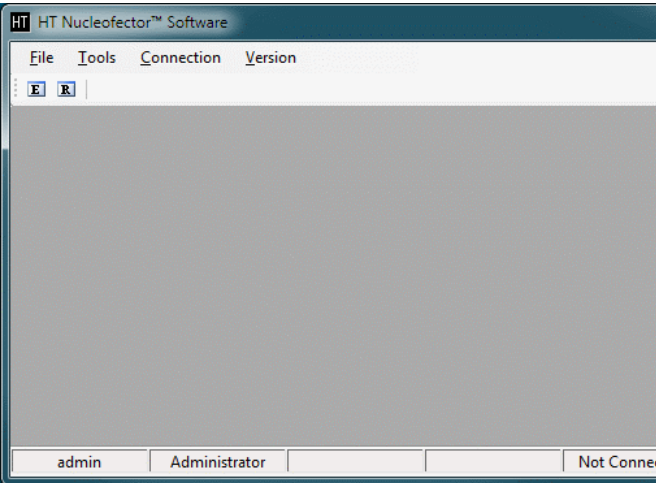
The HT Nucleofector® Software starts with an empty screen, showing the three basic navigation bars (main menu bar, icon bar and the status bar at the bottom of the screen). Initially, the main menu bar contains the four menus "**File,**" "**Tools,**" "**Connection,**" and "**Version.**"

An additional "**Experiment**" or "**Result**" submenu is inserted when selecting the corresponding option in the "**File**" menu (see 4.3.1).

The icon bar below the main menu bar contains the "E" and "R" buttons enabling quick access to either the experiments list or the results list which are retrieved from the SQL database.

An active connection between the HT Nucleofector® Software and the HT Nucleofector® System is indicated by a "Connected" entry in the bottom status bar.

3.2 Compatibility check and automatic software update

During connection, the software automatically checks the comp-atibility of the laptop/PC software version and the firmware versions of all HT Nucleofector® System components to ensure proper operation. If an update of the HT Nucleofector® Software version was installed which is not compatible with the software of the connected HT Nucleofector® System, you will be guided through an update process by message windows (see flow chart below). You can either allow the update by clicking **"Yes"** or refuse or cancel the process by clicking "**No**" or "**Cancel.**"

Chart 1: Compatibility check and software update for the HT Nucleofector® System

"The HT Nucleofector® Software needs to be updated. If unsaved results are stored in the HT Nucleofector® System, a software update will cause the loss of this data. Do not operate any part of the HT Nucleofector® System during the update. Do you wish to automatically update the software (this may take up to 30 minutes)?"

No →

"The HT Nucleofector® Software is incompatible with the connected devices and you will be logged out. To run the system, reload the older version of the HT Nucleofector® Software or update the software."

↓ **Yes**

"Update in progress. This window will be closed when the update has been completed. Progress is indicated by a progress bar."

Cancel →

"Update failed. Please restart the HT Nucleofector® System and the HT Nucleofector® Software."

↓

"Update successful. Please restart the HT Nucleofector® System."

4 Operating instructions

4.1 Short instructions

The table below gives a brief overview of the steps required for a typical Nucleofection® Workflow.

Step	Description	For more information see
1	Switch on the HT Nucleofector® System.	Hardware manual
2	Start the HT Nucleofector® Software on the computer and log in.	Software manual
3	Check your user settings (optional).	Software manual
4	Check that all connections are active / reconnected	Software manual
5	Open an existing experiment or create a new experiment.	Software manual
6	Prepare and transfer samples into the 384-well Nucleocuvette® Plate.	Optimized protocol
7	Insert the 384-well Nucleocuvette® Plate into the HT Nucleofector® Plate Handler.	Hardware manual
8	Start Nucleofection® Process from the computer.	Software manual
9	Monitor Nucleofection® Process during program execution (optional).	Software manual
10	Remove 384-well Nucleocuvette® Plate from the plate handler and transfer samples to appropriate culture plates.	Optimized protocol
11	Check result file on the computer for potential errors.	Software manual
12	Close files, exit HT Nucleofector® Software and switch off all components.	Software manual

4.2 Experiments, templates and results

All experiments, results, optimized protocols and settings are stored in a SQL database, located in the installation directory of the HT Nucleofector® Software.

Experiment templates

Experiment templates are experimental plate configurations that are pre-defined by Lonza, e.g. primary cell optimization. The templates are stored in the HT Nucleofector® Database on the computer on which the software is installed. Experiment templates cannot be altered by a user and cannot be used to directly start an experiment. They can be used as a basis for new experiments by editing the template and saving it as an experiment under a new user-specific name (see 4.3 and 4.4).

Experiments

Experiments are plate configurations that can be created from experiment templates (see above) or from scratch and saved by the user for their specific experimental setups. Parameters defined in experiments are used to perform the Nucleofection® Experiment. An experiment can contain between one and 255 plates.

Results

A result is generated as soon as the user starts an HT Nucleofector® Experiment. The result tab records all information and results from the Nucleofection® Experiment.

4.3 Main menu

This section gives an overview of the HT Nucleofector® Software menu and functions. A more detailed description can be found in the subsequent chapters (see 4.4 – 4.11).
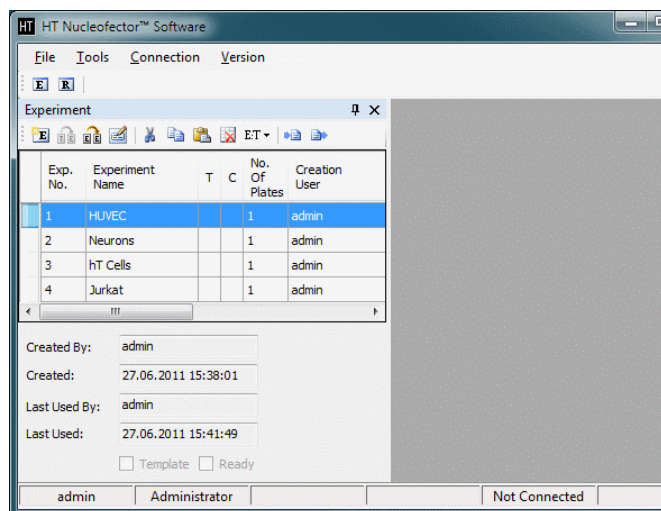
4.3.1 "File" menu

Figure 4.1: "File" menu
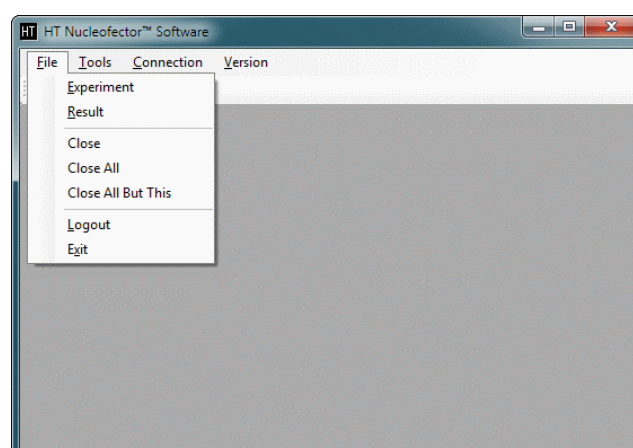
Select **"File > Experiment"** to access the database section containing the experiment data sets and experiment templates. The "Experiment" menu appears to the right of the "File" menu in the main menu bar and the database list of experiments appears in a separate window on the left hand side of the screen (see Figure 4.2). The experiment list can also be accessed by clicking the "E" icon on the left hand side of the menu bar below the main menu bar.

Figure 4.2: Experiment list view

Select **"File > Result"** to access the list of results obtained from previous experiments. The "Result" menu appears to the right of the "File" menu in the main menu bar and the database list of results appears on the left hand side of the screen (see Figure 4.3).

Figure 4.3: Result list view
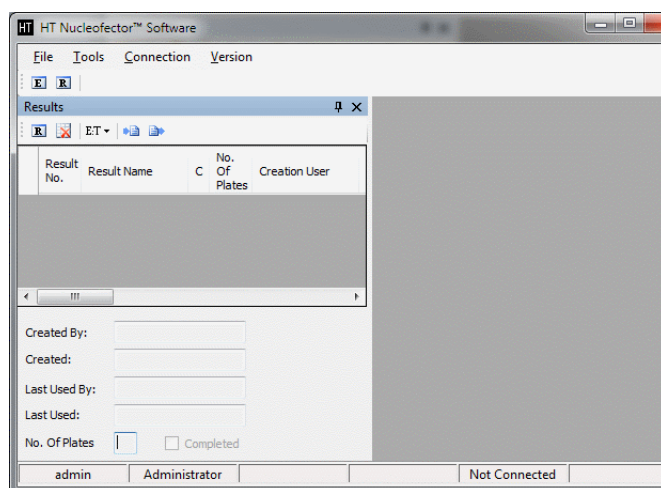
The list of results can also be accessed by clicking the "**R**" icon on the left hand side of the menu bar below the main menu bar. Entries in the experiment and result columns can be sorted by left-clicking the relevant column heading. The lower section of the experiment list displays creator, creation date, user ID, date of last use and plate number.

Open one or more experiments or results by double clicking on the respective entries in the experiment respectively result list.

Select **"File > Close"** to close individual experiment or result tabs.
Select **"File > Close All"** to close all experiment or result tabs simultaneously.
Select **"File > Close All But This"** to close all except the currently active tab.
Select **"File > Logout"** to log out and lock the software without closing it.

It is still possible for other users to log in using their account (see 3.1). Upon logout, the USB connection to the HT Nucleofector® System is deactivated. The connection will be re-activated when the next user logs in.

Select **"File > Exit"** to exit the HT Nucleofector® Software. Alternatively, the software can be closed via the "X" button in the upper right corner of the software window.

4.3.2 "Experiment" menu
Select **"Experiment > Save Experiment"** to save changes to an existing experiment.
Select **"Experiment > Close Experiment"** to close the selected experiment.
Select **"Experiment > Close All But This"** to close all except the currently active file.
Select **"Experiment > Report"** to generate a tabulated report of an experiment that can be exported in a Microsoft® Excel®-compatible CSV format.

4.3.3 "Result" menu
Select **"Result > Save Result"** to save changes to an existing result.
Select **"Result > Report"** to generate a tabulated result report that can be exported in a Microsoft® Excel® compatible CSV format.

4.3.4 "Tools" menu
Select **"Tools > Profiles"** to open a dialog window that enables users or the administrator to edit general program settings and save individual configurations as profiles.

Figure 4.4: Profiles administration
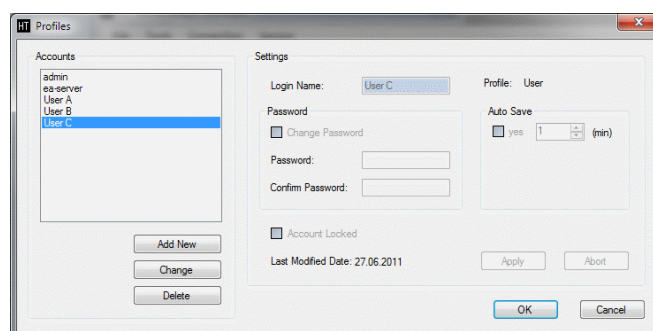
Accounts

Used by the administrator to create new users, delete users or unlock a user account when a wrong password has been entered three times.

Settings

Enables users or the administrator to define their personal system settings, such as login name or the time interval for the "Auto Save" function.

Password

Enables users to change their individual password.
The administrator can lock/unlock accounts if required.

Select **"Tools > Cell Type Code"** to open a new submenu consisting of functions relating to Lonza program and cell type codes.

Figure 4.5: Editing cell type codes
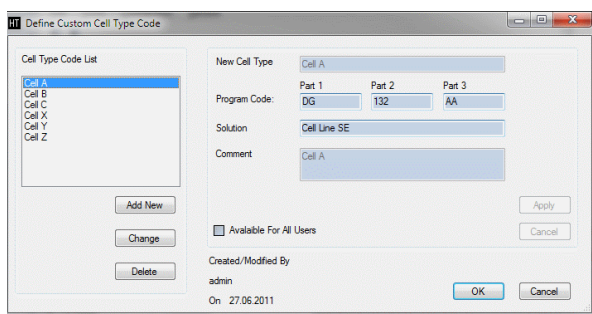
Editing cell type codes

If a specific program code is found to be optimal for a certain cell type, custom cell type codes can be added to the cell type code list to choose a specific Nucleofection® Program. Existing custom cell type codes can only be edited or deleted by the user who created them or by the administrator. To add, edit or delete a custom cell type code, all files must be closed. Lonza cell type codes cannot be edited or deleted.

- **Adding a new cell type code**
- Select **"Tools > Cell Type Code > Edit."**
- Click on "**Add New**" to add a new cell type code.
- Enter a name for new cell type code.
- Choose the appropriate 384-well program code (part 1, part 2 and part 3). Part 3 should be left empty or defined as AA for all experiments using 20 µL of reaction volume.
- Choose the respective 384-well solution code to define the solution which works optimally for the new cell type.
- Optional: If the new cell type code should be available to other users, check the relevant box. Custom cell type codes available to all users are **highlighted in blue** in the cell type code drop-down list of an experiment tab. Custom cell type codes **highlighted in grey** are only available to the user who added them.

- Optional: Add a comment to the new cell type code.
- Click "**Apply**."
- Close the window and create the new cell type code by clicking "**OK**."
- Click "**Cancel**" to discard all changes.

Editing an existing cell type code

Users may only edit custom cell type codes that they created themselves. Alteration of cell type codes created by other users or Lonza is not possible. Only the administrator is allowed to edit all custom cell type codes.

- Select **"Tools > Cell Type Code > Edit."**
- Highlight the respective cell type code in the list and click "**Change**."
- After changes have been made, confirm changes by clicking "**Apply**."
- Close the window and apply the changes by clicking "**OK**."
- Click "**Cancel**" to discard all changes.

Deleting an existing cell type code

Users may only delete custom cell type codes that they created themselves. Deletion of cell type codes created by other users or Lonza is not possible. Only the administrator is allowed to delete any custom cell type code.

- Select **"Tools** > **Cell type code > Edit."**
- Highlight the respective cell type code in the list and click "**Delete**."
- Click "**Apply**" to confirm deletion.
- Click "**Cancel**" to cancel the deletion.
- Close the window and accept the changes by clicking "**OK**." Clicking "**Cancel**" or closing the window without clicking "**OK**" will mean that all additions or changes will be ignored.

NOTE: Importing a new Lonza program list (see below) will not overwrite custom cell type codes.

Importing cell type codes

Select "**Cell Type Code > Import**" to import a new HT Nucleofector® program list provided by Lonza (program definition files = *.pd). Importing a new program list will overwrite the complete list of Lonza program and cell type codes, except for the custom codes (see note above).

Database backup

Select **"Tools > Backup Results > Create Result Backup"** to shift all results from the working database to a read-only database. Previously backed-up results are listed in the drop-down list next to the experiment and result buttons and can also be opened from there (read-only).

Select "**Tools > Backup Results > Open Result Backup**" allows opening a previously generated backup of the result section. The name of the backup is automatically generated. To distinguish several backups each backup name shows the date and time when it was generated in the following format HT__YYMMDDhhmmss.

4.3.5   "Connection" men

The current status of the USB connection ("**Connected**" or "**Not Connected**") is indicated in the status bar at the bottom of the screen. If the USB connection has not been established while starting the software, or if the connection is lost (e.g. after a re-login), select "**Connection > Reconnect.**"

The connection process takes a few seconds. If reconnection fails, retry or restart your laptop.

Select "**Connection > Manual**" to change the position of the carousel and to align it.

Align In Position 1 – moves the carousel to position 1 and aligns it for liquid handler teaching purposes
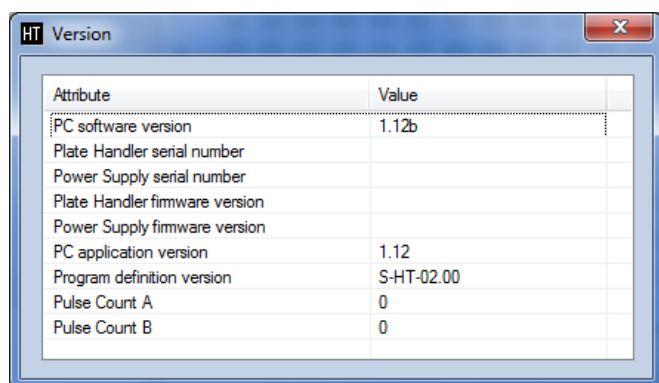
Align In Position 2 – moves the carousel to position 2 and aligns it for liquid handler teaching purpose

Release carousel:

If this function is activated the carousel motor does not pull the carousel in a defined position. Using this function repeatedly toggles the carousel between "aligned" and "released". Use the "aligned" state to teach the robot gripper position for 384-well Nucleocuvette® Plates.

4.3.6   "Version" menu

Figure 4.6: "Version" window

Select "**Version > Details**" to display information about system components; i.e. the serial numbers of connected devices, the software version numbers and the version number of the HT Nucleofector® Program list.

4.4   Working with experiments

4.4.1 Opening an experiment template

Pre-defined Lonza experiment templates are stored in the HT Nucleofector® Database in the same section as the experiment data sets. They are marked with the template flag.

Selecting "**File > Experiment**" opens a list of experiment templates and custom experiments on the left hand side of the screen. By double-clicking on one of the templates the corresponding experiment tab is opened in a separate window. This tab shows the wells of a 384-well plate and various parameter and plate data entry fields.

Figure 4.7: Experiment functions
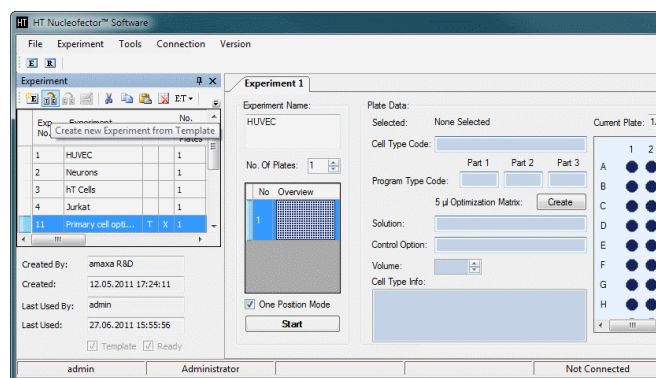
An experiment template cannot be used directly to perform a Nucleofection® Experiment. Before use, the template must be converted into a custom experiment by selecting the "**Create new Experiment from Template**" button in the top row of the experiment list (see Figure 4.7). The software generates and opens a new experiment that inherits the assigned well parameters from the experiment template. This new experiment can be used directly to start an experiment or it can be edited as described in chapter 4.5.

4.4.2 Opening an existing experiment

An existing experiment stored in the HT Nucleofector® Database can be opened as described in section 4.3.1 and directly used to start an experiment or it can be edited as described in chapter 4.5.
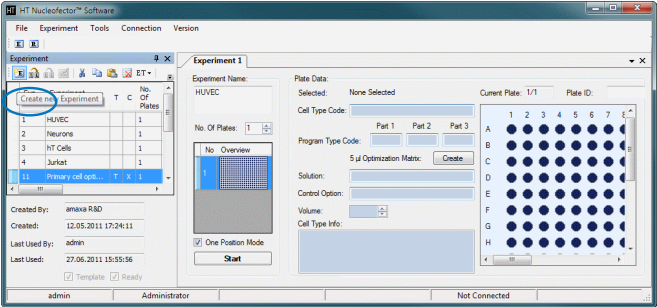
Changes to an existing experiment can be saved by selecting **"File > Experiment > Save experiment"** or via the context menu that is opened by right-clicking on the experiment register tab (see Figure 4.8).

Figure 4.8: Experiment / result register tab context menu
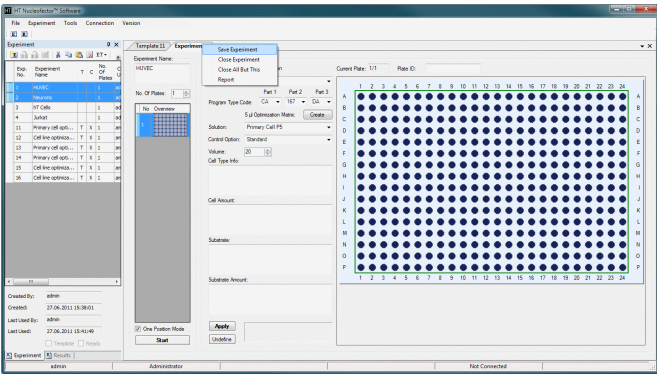
4.4.3 Creating a new experiment

Figure 4.9: Creating a new experiment

The user can generate a new experiment either by creating it from an experiment template (Figure 4.7) or by using the buttons shown in Figure 4.9 (blue circle). Use the button "Create a new experiment" to create a new empty experiment. Use the button "Clone new experiment" to duplicate the selected experiment. The text inside the field "Experiment Name" is also shown in the experiment list to enable various experiments to be found and distinguished quickly. Experiments can be modified as described in chapter 4.5.

By default, new experiments are executed from plate position "1" only. Deselecting the check option "One Position Mode" above the "START" button activates loading of the second position while position "1" is being processed. Once the plate in position "1" has been processed, the carousel revolves and the plate in position "2" can be inserted and processed.

4.4.4 Export and import experiments

This chapter explains how to export and import experiment files by:

● Selecting a single experiment
● Choosing multiple experiments by pressing CTRL while selecting the experiments
● Selecting all experiments by choosing one experiment from the list and pressing CTRL+A in parallel

The files selected can than be exported by choosing the icon "Export Experiments to XML Data" displayed in the tool bar. To import an exported file (extension.xp) press the icon "Import XML Data to Experiments" displayed in the tool bar. This will import all exported experiment files into the actual database. In addition this function will generate a new experiment number for each imported file.

4.5 Well selection and defining and applying well parameters

The user can define how a 384-well Nucleofection® Experiment is per-formed (i.e., which transfection conditions shall be applied to which well) in the experiment view. For convenient assignment of parameters to each well the 384-well Nucleocuvette® Plate is represented graphically (see section 4.4.1) and each well can be selected individually.

4.5.1 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 surrounded by a green box. Selecting a well displays the relevant information in the parameter and plate data entry fields; for color codes of drop-down lists and text fields refer to Appendix 6.1.

4.5.2 Defining well parameters

The following parameters can be defined individually for each well:

1. **384-well Nucleofector® Program (drop-down list):**
   This mandatory parameter can be assigned either via the cell type code or the program code.
   By cell type code: Entering a cell type code assigns the optimal 384-well Nucleofector® program for a specific cell type as defined in the Lonza optimized protocol. For example,"T cells, human, unstim." assigns the optimal 384-well Nucleofection® Program for unstimulated human T cells. Customized cell type codes can be added to the cell type code list, e.g. after a cell line optimization, by selecting **"Tools" > Cell Type Code > Edit (see section 4.3.5)."**
   By program code (e.g. CA-116-AA): When directly entering a program code, part 1 (letters), part 2 (numbers) and part 3 (letters) can be selected separately. In this case the "Cell Type Code" field remains empty. Direct entry of a "Program Code" deletes and cancels any entry in cell type code field.
2. **Control options (drop-down list):**
   "Standard": This is the default setting used for normal samples. "No Program Control": Select this option for control wells to which no program code will be applied. A "No Program Control" well is indicated by a dark blue dot on a light blue background (see Appendix 6.2). The fields "Cell Type Code" and "Program Code" part 1 and 2 will remain empty."No Substrate Control": Select this option for control wells to which no DNA will be added. A "No Substrate Control" well is indicated by a light blue backslash in a dark blue well (see Appendix 6.2).
3. **HT Nucleofector® Solution (drop-down list):**
   This drop-down list is linked to the "Cell Type Code" drop-down list. For example, if cell type code "T cells, human, stim." is chosen, the HT Nucleofector® Solution drop-down list automatically displays "Primary Cell P3" indicating the optimal HT Nucleofector® Solution for human T cells. However by selecting an HT Nucleofector® Solution from the drop-down list, the entry in the "Cell Type Code" field disappears because a solution may be connected to numerous cell types.
4. **Further information (optional, free text fields):**
   Information can be entered in the following fields as free text to further describe the experiment

   - "Cell Type Info:" Add any kind of required additional information to the cell type (e.g. passage no.)
   - "Cell Amount"
   - "Substrate"
   - "Substrate Amount"
   - "Plate ID"

4.5.3 Applying well parameters

To apply the selected parameters to one or more wells, activate them using the left mouse button (see section 4.5.1). Selected wells are surrounded by a green box. After parameters are defined for one or more wells (see section 4.5.2), clicking the "Apply" button will save the chosen parameters for each selected well and the well background color will change from light to dark blue (see Appendix 6.2).

4.5.4 Copying parameters to other wells

Parameters can be copied from one well to another, either within an experiment or between different experiments.

5. Activate / select the well(s) whose parameters are to be copied using the left mouse button.
6. Click the right mouse button to open the context menu and select "Copy".
7. Activate/select the well(s) into which you wish to paste the copied parameters using the left mouse button.
8. Click the right mouse button to open the context menu and select "Paste".

Well parameters are stored on the clipboard, i.e. well parameters can be pasted multiple times into different wells.

NOTE: The shortcuts Ctrl + C (copy) and Ctrl + V (paste) can be used as an alternative to the menu-based copy and paste commands.

Further information on the parameter copy / paste functionality can be found in Appendix 6.4.

4.5.5 Deleting assigned well parameters

To remove the parameters defined for a well, select the respective well(s), click the right mouse button to open the context menu and select "Undefine". Alternatively, select the respective well(s) and click on the "Undefine" button under the "Substrate Amount" info field.

4.5.6 Changing parameters for a group of wells

Parameters for a group of selected wells can be changed at the same time:

1. Select the desired wells. If wells in the selected group have different parameters, this will be indicated either by an empty grey box (in the case of drop-down lists) or a grey box with red text stating "Value differs" (in the case of free text fields).
2. Change parameter(s) of interest. These values will be changed for all selected wells.
3. Confirm changes by pressing the "Apply" button (all previous definitions of each well will be kept except the newly entered parameter(s).

NOTE: To ensure that all parameters for a selected group of wells are identical, it is useful to first clear all defined parameters using "Undefine" (which resets all parameters) and then to re-define each parameter.

4.5.7 Adding plates to an experiment

Each experiment can consist of one or more (up to 255) plates. You can increase or decrease the number of plates in an experiment using the up/down arrow buttons shown in Figure 4.10. Plates can be cloned by right-clicking in the plate preview and selecting "Clone" (see Figure 4.11). The selected plate is copied and the copy is inserted after the selected plate.
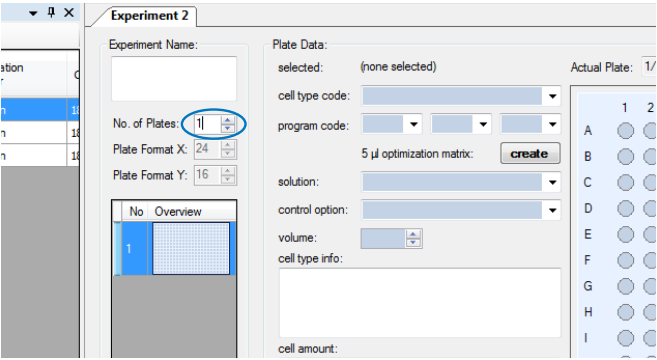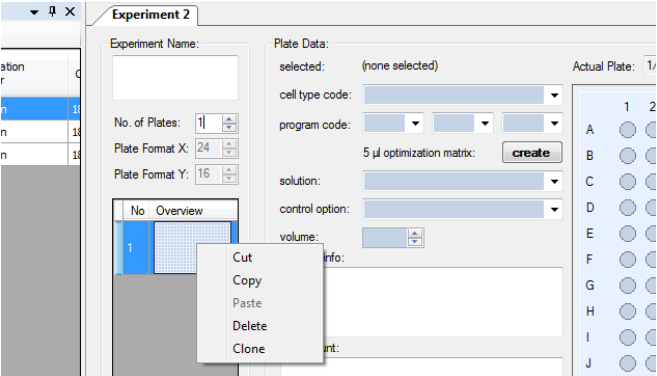
Figure 4.10: Increasing / decreasing the number of plates

Figure 4.11: Plate preview and context menu

A complete plate configuration can be copied by right-clicking in the plate preview and selecting "Copy" (equivalent to using Ctrl+A and Ctrl+C inside the large plate view). Apply the complete plate configuration onto another plate by right-clicking in the plate preview of the target plate and selecting "Paste".

4.6 Generating a report from an experiment

"Experiment > Report"

In addition to the graphical representation of the 384-wells, a report table with assigned well parameters is available. Table entries can be sorted by clicking on the header of the respective column. The report can be printed or exported as an Excel®-compatible CSV format file. Generate an experiment report for an open experiment by selecting "**Experiment** > **Report**" or right-clicking in the experiment register tab and selecting "Report" (see Figure 4.8).

Figure 4.12: Experiment report

4.7 Starting an HT Nucleofector® Experiment

To start an HT Nucleofector® Experiment:

1. Define an experiment as described before
2. Ensure that a 384-well Nucleocuvette® Plate is inserted in the retainer of the HT Nucleofector® Plate carousel.
3. Press "START" to start the experiment.
4. When prompted, confirm that the plate is loaded to start processing.

The experiment data and all corresponding parameters are sent to the HT Nucleofector® Device. Progress of this transfer is shown by a progress bar.

4.8 Working with results

The result tab for the experiment opens automatically once the "Start" button has been pressed and the Nucleofection® Process has started (see section 4.7). During the Nucleofection® Process, the progress of the experiment is monitored and shown graphically (green = OK, yellow=potential error, 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 section of the database (each partial or completed run will be saved automatically). Result tabs cannot 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 section 4.11). For extended error information (error code plus error explanation and handling recommendations) see chapter 5. The experiment history logs processing information such as experiment start, pause, error, stop and completion.

NOTE: In case of a software crash or loss of the USB connection during the experiment, only the monitoring process is interrupted: the experiment is not affected. Upon restart of the software or reconnection of the USB connection, monitoring continues and results will be downloaded as usual.

4.9 Interrupting the 384-well Nucleofection® Experiment

An experiment can be interrupted at any time by pressing the "Pause" button. Options available while the experiment is paused:

a. **Abort the experiment:** Abort the experiement by pressing the "Stop" button. Results of the wells processed before the abort will be downloaded into the result file. Download progress is indicated by a progress bar and ends with the message "Download Completed".

b. **Resume the experiment:** Resume a paused experiment by pressing the "Resume" button. The HT Nucleofector® System continues the experiment. The process can be monitored on-screen using the HT Nucleofector® Software.

4.10 Completion of the 384-well Nucleofection® Experiment
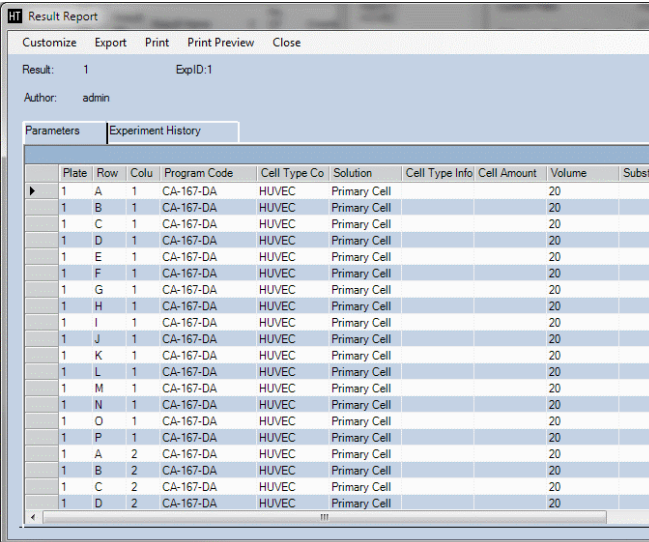
After processing a 384-well Nucleocuvette® Plate, the plate carousel will rotate automatically to return the plate. The results will be downloaded and stored within the database.

4.11 Generating a report and exporting result data

Each user-accessible result in the database can be viewed as a printable and exportable report. The report contains the following information:

a. Time taken for the complete Nucleofection® Process Parameters and free text information assigned to each well

b. Result for each well: "OK" or errors with extended error information

c. Time stamp and UserID (for regulated environments). The report can be printed or exported as an Excel® compatible CSV file.

Figure 4.13: Result report

Generate a result report by selecting "**Result** > **Report**" or right-clicking in the result register tab and selecting "**Report.**"

5 Troubleshooting and error codes

5.1 Troubleshooting

The following troubleshooting guide may be helpful if the HT Nucleofector® Software does not function as expected. If you have any questions regarding the use of the software, please do not hesitate to contact Lonza's scientific support teams:

For fine-tuning after the optimization process, please contact our scientific support teams

Europe and International
Phone: + 49 221 99199 400
E-mail: scientific.support.eu@lonza.com

Americas
Phone: 800 521 0390 (toll free)
E-mail: scientific.support@lonza.com

Problem	Solution
Your login request was ignored due to wrong user name and/or password.	Try to log in again and check for case sensitivity. The account will be locked if a wrong password is entered three times. Please contact the administrator to unlock your account. If the administrator forgets the password, please contact Lonza's scientific support.
"Not connected" is indicated in the status bar at the bottom of the software window.	Check physical USB connection and try to reconnect. If this does not solve the problem, restart the software or restart the PC and the HT Nucleofector®.
The CSV format file opened in Excel® does not contain column-separated data.	In Excel®, mark the whole column containing the data, go to the "Data" menu and choose the option "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".

5.2 Error codes

There are three different types of error that will generate an error code:

- Program execution errors (see table in section 5.2.1)
- Software or communication errors: such errors will be communicated by the software via a message window
- Mechanical errors of the HT Nucleofector® System (for details, please refer to the HT Nucleofector® System manual)

5.2.1 Program execution errors

Each program execution error will be logged by its error code (e.g. "Err 1A") in the result file of a 384-well Nucleofection® Experiment.

Error code (logged in result file)	What occured?	Possible cause of error	Solving the problem
Err 1A	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 Err 1A re-occurs, switch off the device, check all cables, wait for 2 seconds and switch on again. If error persists, contact our scientific support.
Err 1B	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 Err 1B re-occurs, switch off the device, check all cables, wait for 2 seconds and switch on again. If error persists, contact our scientific support.
Err 2	No pulse generated	Inappropriate or no solution; inappropriate well volume; 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 all cables and generate a new experiment containing parameters for the failed wells only. If the experiment was interrupted due to continuous "Err 2" errors, check connection of all cables or switch off the HT Nucleofector® System, wait for 2 seconds, switch on again and restart program execution.
Err 3	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 Err 3 re-occurs, switch off the device, check all cables, wait for 2 seconds and switch on again. If error persists, contact our scientific support.
Err 4 WEAK	Re-used cuvette or inappropriate Nucleofector® solution.	A weak pulse occurred. Efficiency may be sub-optimal.	Clear the error message by pressing any button. Use the sample if maximum efficiency is not essential. Otherwise, use a new sample.
Err 5 & Err 6	The HT Nucleofector® System detected and corrected an arc discharge. Program could be resumed and successfully completed. A limited impairment of transfection efficiency and cell viability is probable.	Inappropriate solution or inappropriate well volume, occasional arcing.	Use samples if maximum efficiency is not essential. Before reprocessing failed wells with new samples, check that the combination of 384-well program and 384-well Nucleofector® Solution is suitable for the protocol recommendations and check sample volume.
Err 8	The HT Nucleofector® System detected a reoccurring arc discharge leading to incomplete program execution. A limited impairment on efficiency and vitality is probable.	Inappropriate solution or volume, occasional arcing.	Before reprocessing failed wells with new samples, check that the combination of 384-well program and HT Nucleofector® Solution is suitable for the protocol recommendations and check sample volume.
Err 21	Multiple use of well detected	Well already used, expect lower efficiency	Use a new plate
Err 22	Multiple errors occurred	Combination of error 21 and 4	Use a new plate
Err 23	Multiple errors occurred	Combination of error 21 and 5	Use a new plate
Err 24	Multiple errors occurred	Combination of error 21 and 6	Use a new plate
Err 25	Multiple errors occurred	Combination of error 21 and 8	Use a new plate
Err 26	Multiple use of well detected	Well used too often, no pulse generated	Use a new plate

5

6 Appendix

6.1 Color code of boxes (drop-downs, text fields)

	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 different parameters: light grey	Boxes with same parameters: black Boxes with different parameters: red, "values differ"	Boxes with same parameters: show Boxes with different parameters: "values differ"

6.2 Well representation color codes

	Experiment tab	Result tab OK	Result tab, error	Skipped well	Unskipped well	Unskipped well, error
Undefined	⬤	⬤				
Defined	⬤	⊕	⊖	⬤	⊕	⊖
No program control	⬤	⬤				
No substrate control	⬤	⊗	⬤	⬤	⊗	⬤

6.3 List of shortcuts

Command	Function
<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

6.4 Copy-paste functionality

1. Copy – select wells

Copy – complete plate

2. Paste

a) To same number of selected wells

b) To a smaller number of selected wells

c) To a larger number of selected wells

d) To a new plate

Selection of the same number of wells into which copied parameters shall be pasted:

Selection of a smaller number of wells into which copied parameters shall be pasted:

Selection of a larger number of wells in which copied parameters shall be pasted:

Selection of a entire plate of wells in which copied parameters shall be pasted:

Result of pasting:

Result of pasting:

Result of pasting:

Result of pasting into a new plate:

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