



# 4D-Nucleofector® Manual

Your guide to setup and usage of  
the 4D-Nucleofector® System

*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.

# Content

<b>1</b>	<b>Nucleofector® Technology</b>	<b>4</b>	<b>2.13</b>	<b>Additional settings and options</b>	<b>31</b>
How it works	4	2.13.1	Settings menu on the right	31	
Transfection of any cell	4	2.13.1.1	System settings	31	
Transfection of any substrate	4	2.13.1.2	Cleaning the Nucleofection® Vessel Tray (X, Y, or 96-well Unit)	32	
Moving transfection into the fourth dimension	5	2.13.2	Left menu	32	
Experience the advanced Nucleofector® Platform:	5	2.13.2.1	Experiments	32	
		2.13.2.2	Results	32	
		2.13.2.3	Custom Cell Type Codes	32	
<b>2</b>	<b>Operating instructions</b>	<b>6</b>	2.13.2	Lonza programs	33
<b>2.1</b>	<b>Restrictions</b>	<b>6</b>	2.13.4	Firmware update	33
<b>2.2</b>	<b>Maintenance</b>	<b>6</b>	2.14	4D-Nucleofector® PC Editor Software	34
<b>2.3</b>	<b>Safety instructions – please read carefully</b>	<b>6</b>	2.14.1	Installation of the PC Editor	34
2.3.1	Consignes de sécurité - à lire attentivement	7	2.14.2	Transfer of experiments from the PC Editor	35
<b>2.4</b>	<b>Waste disposal</b>	<b>8</b>	2.14.3	Viewing and printing result files	35
<b>2.5</b>	<b>4D-Nucleofector® System components</b>	<b>9</b>	<b>3</b>	<b>Troubleshooting</b>	<b>36</b>
<b>2.6</b>	<b>Set-up instructions</b>	<b>10</b>	<b>3.1</b>	<b>Suboptimal transfection results</b>	<b>36</b>
<b>2.7</b>	<b>General use instructions</b>	<b>10</b>	<b>3.2</b>	<b>Rescue of samples (X and Y Unit)</b>	<b>37</b>
2.7.1	Turning on the 4D-Nucleofector® System	10	<b>4</b>	<b>Error codes of the</b>	
2.7.2	Operating software	10		<b>4D-Nucleofector® System</b>	<b>38</b>
2.7.3	Turning off the 4D-Nucleofector® System	11	<b>4.1</b>	<b>Color coding of wells</b>	<b>38</b>
<b>2.8</b>	<b>General instructions for running a</b>	<b>11</b>	<b>4.2</b>	<b>Result rating (LV Unit)</b>	<b>38</b>
	<b>Nucleofection® Experiment</b>		<b>4.3</b>	<b>Arc discharge correction</b>	<b>38</b>
2.8.1	Overview	11	<b>4.4</b>	<b>Error codes</b>	<b>38</b>
2.8.2	Unit and vessel selection	11	<b>5</b>	<b>Purchaser notification</b>	<b>41</b>
2.8.3	Experiment parameters – overview	12		<b>Purchaser notification</b>	<b>41</b>
2.8.4	Using a predefined experiment	13		<b>Representations and warranties</b>	<b>41</b>
<b>2.9</b>	<b>4D-Nucleofector® X Unit</b>	<b>14</b>	<b>6</b>	<b>Technical specifications</b>	<b>42</b>
2.9.1	Defining a new experiment	14			
2.9.2	Loading samples	16			
2.9.3	Running the experiment	16			
<b>2.10</b>	<b>4D-Nucleofector® Y Unit</b>	<b>17</b>			
2.10.1	Defining a new experiment	17			
2.10.2	Loading samples	18			
2.10.3	Running the experiment	18			
<b>2.11</b>	<b>4D-Nucleofector® 96-well Unit</b>	<b>19</b>			
2.11.1	Defining a new experiment	19			
2.11.2	Loading samples	21			
2.11.3	Running the experiment	21			
<b>2.12</b>	<b>4D-Nucleofector® LV Unit</b>	<b>22</b>			
2.12.1	Using the 1 mL Nucleocuvette® Cartridge (Fixed volume)	23			
2.12.1.1	Defining a new experiment	23			
2.12.1.2	Loading Samples	24			
2.12.1.3	Running the experiment	24			
2.12.2	Using the LV Nucleocuvette® Cartridge (Scalable volume)	25			
2.12.2.1	Defining a new experiment	26			
2.12.2.2	Mounting an LV Nucleocuvette® Cartridge	26			
2.12.2.3	Loading Samples	28			
2.12.2.4	Running the experiment	30			

# 1 Nucleofector® Technology

Since its introduction in 2001, Nucleofector® Technology has transformed transfection. Nucleofection® Technology is 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 (Visit the Lonza Knowledge Center to learn more: <https://knowledge.lonza.com>). Nucleofector® Technology provides an answer to almost every transfection challenge.

## How it works

Nucleofector® Technology is based on two proprietary 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 325 protocols optimized by Lonza's R&D Team for cell lines and primary cells and more than 750 entries in the online Cell Transfection Database, Nucleofector® Technology is the transfection method of choice for difficult-to-transfect cell types. Moreover, the electroporation-based

Nucleofection® Method allows 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. Nucleofection® Technology can be 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.

## Optimized protocols

This manual describes how to operate the 4D-Nucleofector® System with its different functional units, e.g. how to program an experiment for execution of the Nucleofection® Process. For details on setting up a Nucleofection® Sample, including how to prepare cells for Nucleofection® experiments and how to handle them after the process, please refer to our cell type-specific Optimized Protocols in our cell database.

 [www.lonza.com/celldatabase](https://www.lonza.com/celldatabase)





## The 4D-Nucleofector® System

### Moving transfection into the fourth 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® Conditions for cells in adherence
- Closed, scalable transfection of larger cell numbers in the range of  $1 \times 10^7$  to  $1 \times 10^9$  cells per reaction

[lonza.com/transfection](https://lonza.com/transfection)

### Scientific Support Teams

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# 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, nor 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 only 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 terms with any such entities.

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## 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 2.1). 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 2.1: 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 the 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.
- Never unplug the high voltage cable while system is running. It will destroy the device.
- Only use the power cord that comes with the 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.
- Nucleofector® Solutions used with the Nucleofector® I/II/2b System are not compatible with the 4D-Nucleofector® System. Only use dedicated 4D-Nucleofector® or 96-well Nucleofector® Solutions.
- 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 cannot 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 leak-proof containers and avoid unprotected handling of such parts.
- Lonza disclaims all warranties and shall in no event be liable for any kind of damages caused by or arising out of any operation or use in violation with the above safety and handling instructions.

### 2.3.1 Consignes de sécurité - à lire attentivement

**Ce symbole signifie qu'il existe un risque de choc électrique. Un choc électrique peut provoquer des blessures graves, voire mortelles.**



L'appareil 4D-Nucleofector® a été certifié par les normes de sécurité internationales et peut être utilisé en toute sécurité lorsqu'il est utilisé conformément à ce manuel.

- Cet appareil est conçu pour délivrer des impulsions électriques variables à haute tension dans le but d'introduire des substrats dans des cellules eucaryotes.

**Ces impulsions électriques peuvent être mortelles!**

**Par conséquent, utilisez cet appareil avec attention et prenez les précautions suivantes:**

- Utilisez l'appareil uniquement après avoir lu et compris le manuel 4D-Nucleofector®. Le manuel devrait être accessible à tous les utilisateurs. Assurez-vous que chaque utilisateur potentiel le lit et le comprend.
- Ne pas ouvrir l'appareil. L'appareil ne contient pas de pièces réparables par l'utilisateur. Les composants du circuit ne doivent en aucun cas être perturbés, car ils peuvent provoquer un choc électrique même lorsque le système n'est pas en fonctionnement.
- Ne modifiez pas l'appareil de quelque manière que ce soit.

- Ne gênez pas le mouvement du tiroir maintenant les cuvettes de Nucleofector® ou les plaques de cultures.
- Ne branchez aucun connecteur non certifié par Lonza sur les prises d'électrodes haute-tension externes situées à l'arrière du système 4D-Nucleofector®. Ils sont conçus pour connecter des systèmes certifiés complémentaires au système 4D-Nucleofector®, par exemple l'appareil X Unit ou 96-well Unit.
- Utilisez l'appareil uniquement lorsqu'il est installé sur une table ou paillasse sûre, plate et stable.
- Placez l'appareil de manière à pouvoir retirer facilement le cordon d'alimentation à tout moment.
- N'exposez pas l'appareil à un environnement humide.
- L'appareil ne doit pas être exposé à la lumière directe du soleil ni être placé dans un environnement chaud
- L'appareil n'est pas approuvé pour une utilisation dans des zones présentant un danger d'incendie ou d'explosion, ni pour une utilisation avec des milieux inflammables ou explosifs.
- Prendre des précautions contre les chocs et les vibrations lors des déplacements et transports de l'appareil 4D-Nucleofector®.
- Utilisez l'appareil avec les solutions et cuvettes Nucleofector® certifiées par Lonza uniquement (cuvette Nucleocuvette® de 100 µL ou barrette Nucleocuvette® de 20 µL pour Unité X; cartouche Nucleocuvette® de 1 mL ou LV pour Unité LV; plaque d'électrodes à immersion 24-puits pour Unité Y). L'utilisation de consommable de toute autre source que Lonza exclura toute réclamation appelant la garantie et la responsabilité de Lonza.
- Ne modifiez pas la configuration de la cartouche Nucleocuvette® LV préassemblée.
- Les solutions Nucleofector® pour Nucleofector®I/II/2b ne sont pas compatibles avec le système 4D-Nucleofector®.
- Ne débranchez jamais le câble haute tension lorsque le système est en marche.
- Déballez les Nucleocuvette® ou les plaques d'électrodes à immersion de 24-puits juste avant l'expérience. Assurez-vous que les zones de contact extérieures soient sèches.
- La sécurité peut être compromise si du liquide a été répandu à proximité du système 4D-Nucleofector®. Assurez-vous qu'aucun fluide n'est en contact avec ou n'a pénétré dans le système.
- Si du liquide a pénétré l'appareil, la sécurité peut être compromise. Pour confirmer que l'utilisation de l'appareil est sécurisée, contactez le Service du Support Scientifique de Lonza pour les actions ou les précautions à prendre.
- Ne pas insérer ou placer d'objets étrangers dans les tiroirs électriques du système 4D-Nucleofector®.
- La sécurité peut être compromise si un objet étranger est entré dans le système 4D-Nucleofector®. Pour vous

assurer que le système est sûr à utiliser, contactez le Service du Support Scientifique de Lonza pour obtenir des conseils sur les actions et précautions à prendre.

- Si le système 4D-Nucleofector® a été endommagé, assurez-vous qu'il ne puisse être utilisé par aucun membre du personnel et contactez le Service du Support Scientifique de Lonza pour assistance.
- Toute réparation ne doit être effectuée que par un personnel autorisé par Lonza.
- La manipulation des pièces de l'appareil pouvant présenter un risque de contamination des échantillons doit toujours être effectuée avec des gants de protection. Toute élimination de ces pièces doit être conforme aux procédures fédérales, régionales ou nationales relatives à la manipulation et à l'élimination des déchets cliniques. Utilisez des conteneurs étanches et évitez toute manipulation non protégée de telles pièces.

Lonza Cologne décline toute garantie et ne pourra en aucun cas être tenue responsable de tout type de dommages causés par ou résultant d'une opération ou d'une utilisation contraire aux consignes de sécurité et de manipulation susmentionnées.

## 2.4 Waste disposal

Dispose of 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, the number of units in a system might vary, as one Nucleofector® Core Unit can operate up to three functional units. The units may also belong to different 4D Nucleofector® System variants. These variants can be distinguished by their part codes and serial numbers (Table 2.1).

This manual only describes the use of variant C. For older variants A or B please refer to the manual of the first generation 4D-Nucleofector® System.

Table 2.1: Overview of model variants

Model	Variant A	Variant B	Variant C
Years	2010 – 2013	2013 – 2020	2021 - 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-1002	Core Unit: AAF-1003B X Unit: AAF-1003X Y Unit: AAF-1003Y LV Unit: AAF-1002LW 96-well: AAF-1003S
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*** Shuttle: 9**S****	Core Unit: 9**B2*** X Unit: 9**X2*** Y Unit: 9**Y2*** LV Unit: 9**LV2** 96-well: 9**S2***
Unit arrangement	Horizontal or vertical (via demountable side panels)	Vertical only (fixed side panels)	
Core Unit – display frame	Silver	Blue	Blue

A typical 4D-Nucleofector® System includes (Figure 2.2 and Figure 2.3 A and B):

- 1. 4D-Nucleofector® Core Unit
- 2. At least one functional unit from the following list:
  - 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
  - 96-well Unit: for medium throughput transfection of up to 96 samples in 20 µL format
  - LV Unit: for large volume transfection of up to 10<sup>9</sup> cells

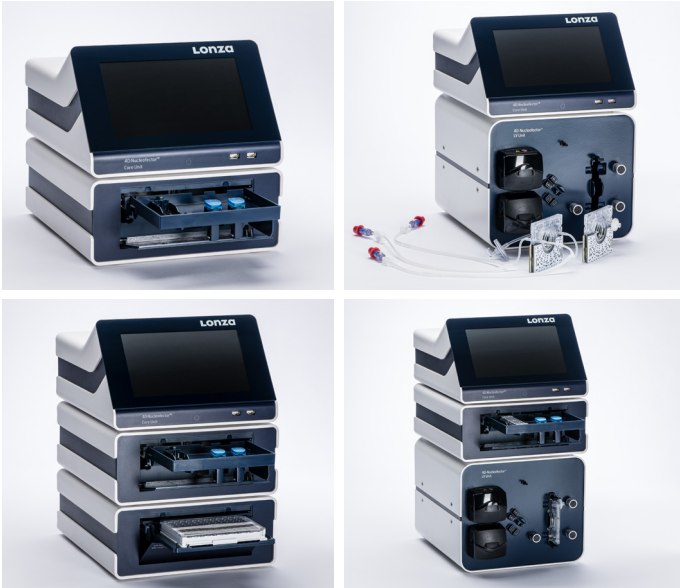


Figure 2.2: 4D-Nucleofector® System in different configurations. Up to three functional Nucleofector® Units can be operated with on Nucleofector® Core Unit.

- 1. Touch screen (graphical user interface of the system)
- 2. Power Switch LED
- 3. USB ports (for USB sticks)



Figure 2.3: (A) 4D-Nucleofector® Core Unit, (B) 4D-Nucleofector® System comprising of Core, X, and 96-well Unit in rear view.

- 4. Power cord socket with main power switch and fuses
- 5. Power cord
- 6. USB port(s)
- 7. Lonza interface outlet port to connect functional units
- 8. Lonza interface inlet port to connect functional units
- 9. Lonza interface inlet port of last functional unit
- 10. Lonza interface cable connected to outlet and inlet port
- 11. Lonza interface outlet port of functional unit
- 12. Lonza outlet port of last functional unit with termination plug



## 2.6 Set-up instructions

1. Unpack the components of the 4D-Nucleofector® System and check for damaged or missing components.
2. Stack the units with the Core Unit (containing the touch screen) on top (Figure 2.2)
3. Connect the units (Figure 2.3) by using the interface cables:
  - Connect the interface outlet port of the Core Unit (7) with the interface inlet port of the first functional unit (X Y, or 96-well Unit; 8). 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 (11) and the inlet port (9) of adjacent units as described above.
  - Important: Plug in the interface terminator cap into the outlet of the last unit (12).
4. Attach the power cord (5) to the power cord socket (4) at the rear side of the Core Unit and plug it into an appropriate power outlet. Use only the power cord that comes with the device.
5. Check all connections before turning on the system for the first time.

**Important note:** If the device is not used for longer periods, it will require occasional recharging to avoid battery discharge and ensure functionality. To avoid a deep discharge of the battery and loss of data, switch on the system at least once a month.

The 4D-Nucleofector® System comes with default programs and includes USB ports at the front and rear for software updates and import and export of experiments and results.

## 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 (<https://knowledge.lonza.com>).

**NOTE:** All screenshots shown in this section refer to software version 5.1.

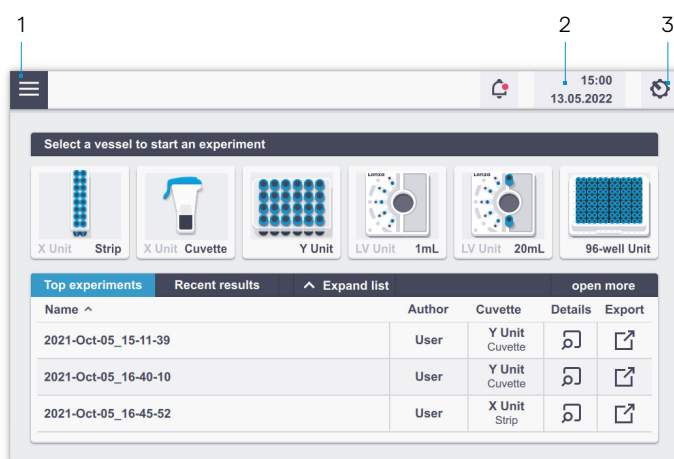
### 2.7.1 Turning on the 4D-Nucleofector® System

Turn on the system using the main power switch at the rear of the Core Unit. The blue LED at the front of the Core Unit will be lit. Then press the power button at the front of the Core Unit. The system will boot — this process may take a few moments. Once the start-up procedure is complete, the 4D-Nucleofector® Graphical User Interface (touch screen) will display the software's main screen. The main screen (home screen, Figure 2.4) displays all vessel types that can be used with the respective functional units that

are attached to the Core Unit. It is possible to connect up to three functional units with one 4D-Nucleofector® Core Unit. In addition, at the bottom the recent results and the top experiments used are shown.

### 2.7.2 Operating software

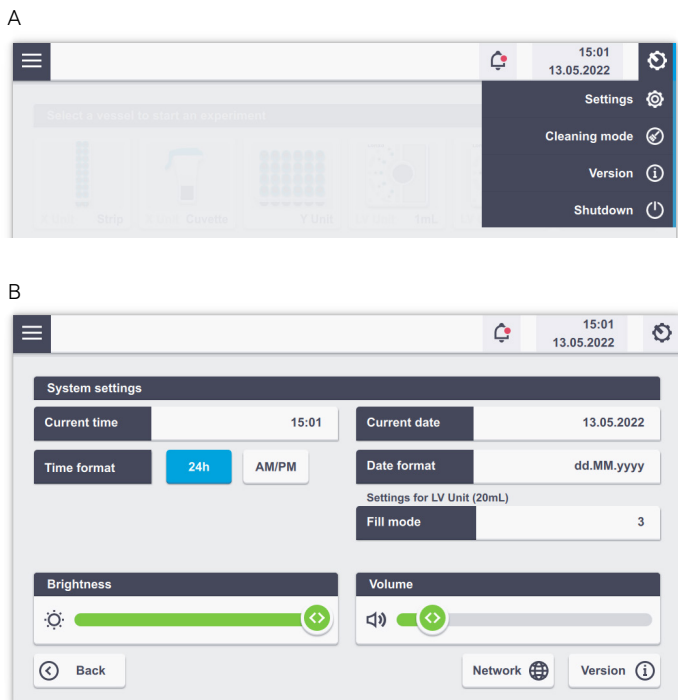
The 4D-Nucleofector® System is operated via an 8-inch touch screen display and is controlled by intuitive software. Figure 2.4 and Table 2.2 will familiarize you with the functions of the touch fields and the basic features of the operating software.



**Figure 2.4:** Main screen (home screen) elements and general icons.

**Table 2.2:** Main menu and general commands or icons

Menu item	Description
<b>Accordion menu (1)</b>	Return to the main screen (home screen) <b>Experiment section</b> – Search, import and export of experiments <b>Results section</b> – Search import and export of results <b>Cell type section</b> – Search for, create new and edit existing cell type specific protocols
<b>Time and date (2)</b>	Shows time and date (changes can be made in the Settings menu)
<b>Gear wheel icon (3) menus</b>	Select "Settings" to set the following instrument parameters: <ul style="list-style-type: none"> <li>• Date and date format (select date and different formats to display)</li> <li>• Time and time format (setting time and switching between 24 h and am/pm display)</li> <li>• Display brightness (slide to adjust the brightness)</li> <li>• Volume or mute sound (slide to adjust volume)</li> <li>• Setting the fill mode for LV Unit use (for details, see chapter 2.12.1.1) If a functional unit is active, additional commands are available:               <ul style="list-style-type: none"> <li>• Open/close drawer</li> <li>• Cleaning mode (opens the drawer to clean the retainer)</li> </ul> </li> <li>• Version</li> <li>• Shut down</li> </ul>



**Figure 2.5:** System settings screen (A) dropdown menu after tapping on the gear icon. (B) Settings menu after tapping on "Settings".

### 2.7.3 Turning off the 4D-Nucleofector® System

For a safe shut down of the system and protection against damage of interior fragile parts, press the power button at the front of the Core Unit and tap on "Shutdown" on the screen. Alternatively, tap on the "Settings" icon (Figure 2.5, A) and then select "Shut down". Both ways, the 4D-Nucleofector® System will shut down and the transportation lock will be applied to delicate parts of the system.

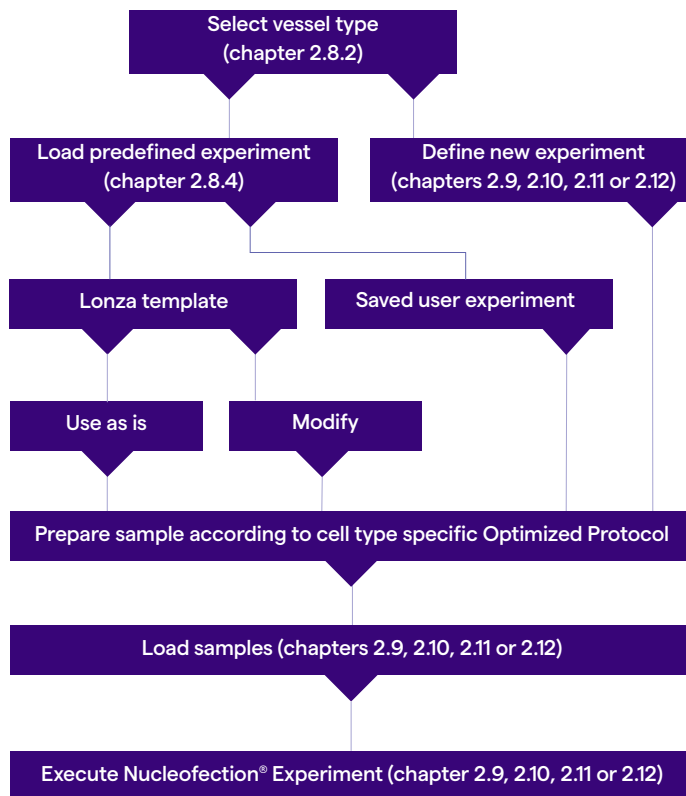
Turn off the main power switch at the rear of the device before relocating or transporting the system.

**Important note:** A hard shutdown of the system by pressing the rear power switch of the Core Unit without a previous software shutdown should be avoided as this may lead to loss of data.

## 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 – 2.12.

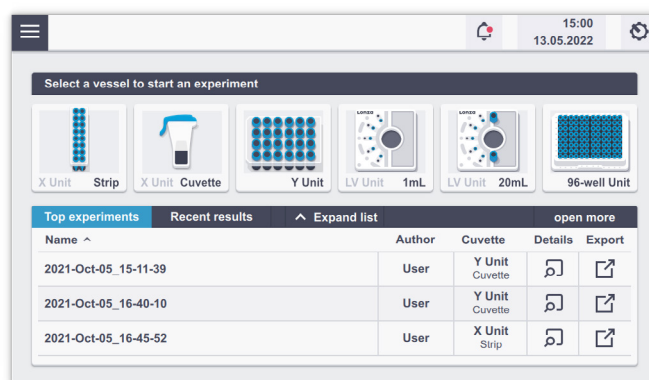
### 2.8.1 Overview



**Figure 2.6:** Schematic workflow overview of a typical Nucleofection® Experiment.

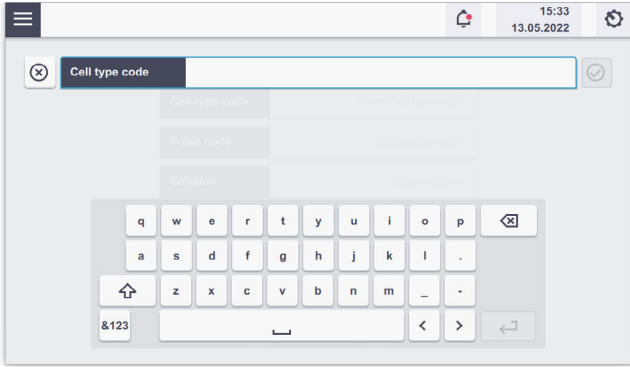
### 2.8.2 Unit and vessel selection

The main screen (Figure 2.7) shows all available vessels for attached functional units (X, Y, 96-well, and LV Unit)



**Figure 2.7:** Example of main screen elements, depending on attached modules, up to three functional modules can be attached to one Core Unit.

By selecting the vessel type a new screen opens where either a new experiment can be started or one of the predefined experiments can be loaded. For new experiments, a name generated with date and time stamp will be suggested. After either changing the name of the experiment or accepting the suggested name, you can proceed to the Experimental setup screen by tapping on the green checkmark (Figure 2.8)



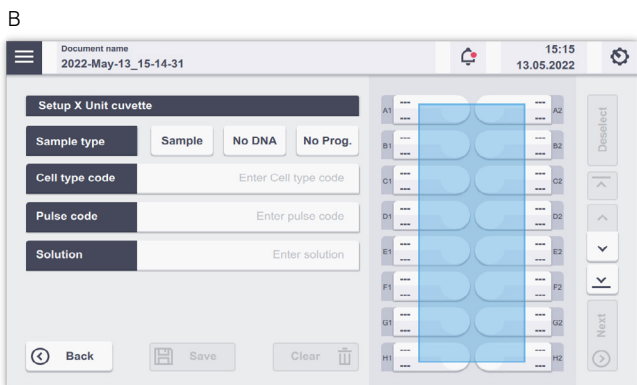
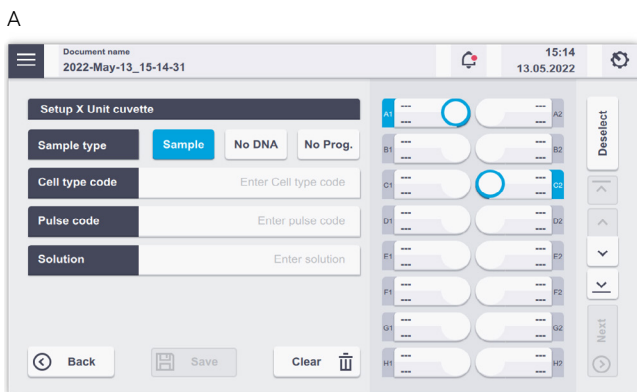
**Figure 2.8:** Start experiment by typing in the experiment name or click the “load” button to load a predefined experiment (see chapter 8.4.2).

### 2.8.3 Experiment parameters – overview

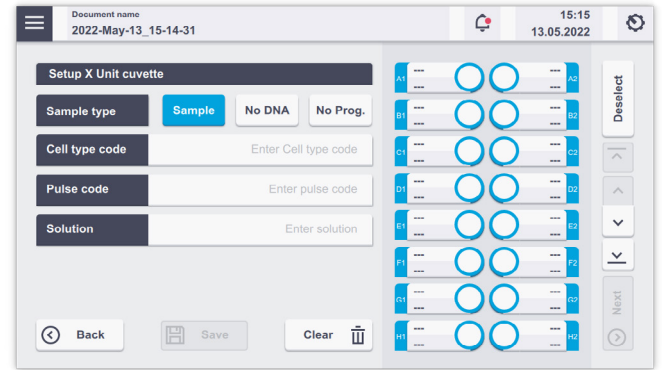
An experiment comprises a selected set of vessels or wells that can only be processed with defined Nucleofection® Parameters.

To select a single well or a set of vessels, simply touch the wells displayed or drag along a set of vessels with your fingertip.

After selection, there are several options for parameter definition. Table 2.3 describes the parameters that can be defined.



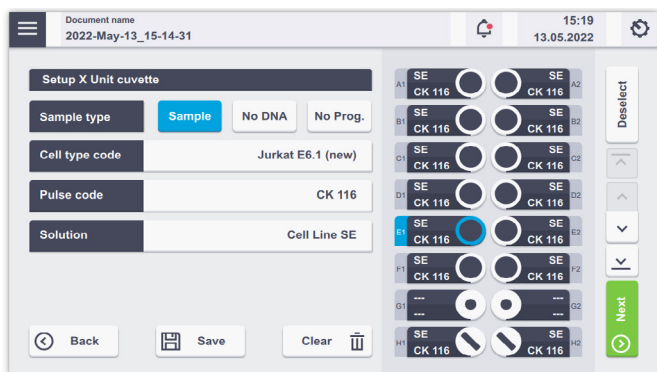
C



**Figure 2.9:** (A) Selecting single vessels by touching the screen at the respective well position. (B/C) Multiple vessel selections by dragging across the screen.

**Table 2.3:** Entering Nucleofection® Conditions

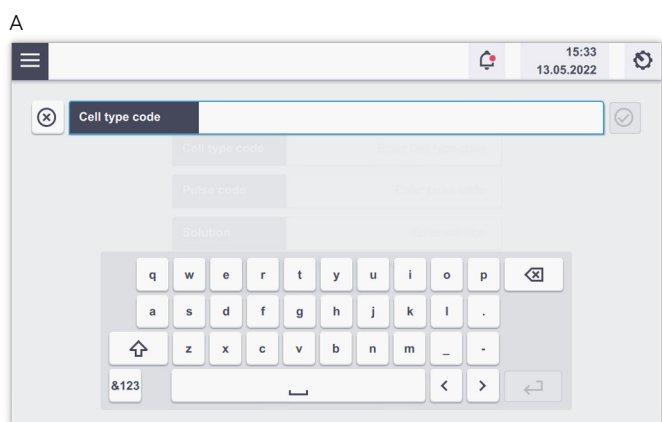
Parameter	Description
<b>Sample type</b> (X,Y and 96-well Unit)	<ol style="list-style-type: none"> <li><b>Sample:</b> This selection defines a normal sample containing cells and substrate. The selected Nucleofection® Program will be applied to this position.</li> <li><b>No DNA:</b> Negative control. Nucleofection® Program applied to vessel with cells but without substrate</li> <li><b>No Prog.:</b> Negative control. No Nucleofection® Program applied to a vessel containing cells and substrate. 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.</li> </ol>
<b>Cell type code</b>	Via the “Cell type code” a predefined combination of a “Pulse code” and a “Solution” can be selected. All Lonza optimized conditions for a cell type are available in the “Cell type code” drop down menu. Customized cell type codes (highlighted in black) can be added in addition to the Lonza-defined codes (highlighted in blue), for example, after cell line optimization (for details, see 2.13.2.3).
<b>Solution</b>	If the recommended Nucleofector® Program is not selected automatically via the “Cell type code” option, the “Solution” can be entered manually.
<b>Pulse code</b>	In case the recommended Nucleofector® Program is not selected automatically via the “Cell type code” option, a “Pulse code” (e.g. FI-115) can be entered manually. Note: For manual “Pulse code” selection, the “Solution” has to be selected first (see below).
<b>Volume (LV Unit with LV Nucleocuvette® Cartridge)</b>	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.
<b>Fill mode (LV Unit with LV Nucleocuvette® Cartridge)</b>	When using the scalable cuvette version, the LV Nucleocuvette® Cartridge, the fill mode (selected via “Settings”) is indicated. It can be changed for an individual experiment on the experiment screen.



**Figure 2.10:** Setting up Nucleofection® Conditions.

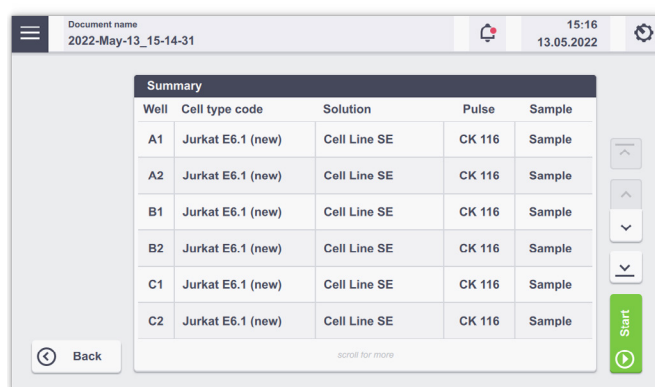
### 2.8.4 Using a predefined experiment

Besides setting up a new experiment (as described in the unit-specific chapters), users can also execute or modify a predefined experiment. A predefined experiment can either be a ready-to-use template pre-installed by Lonza (i.e. optimization schemes for X, Y, and 96-well Unit only) or a previous experiment that was saved by the user.



**Figure 2.11:** (A) Search for an experiment via name (B) Selection of a predefined experiment

1. After vessel type selection (see 2.8.2) a screen appears prompting you to enter an experiment name (Figure 2.11, A).
2. Selecting "Load" will open a list of experiments for the selected vessel type (Figure 2.11, B). The list comprises experiments pre-defined by Lonza (template files; blue) and all experiments saved by the user (black). Tapping on "Details" will provide information about name, vessel type and number of defined vessels. Choosing an experiment and tapping the "Load" button will open the experiment.
3. You can load predefined experiments by tapping on the accordion menu (Figure 2.4 (1)) and select "Experiments". Tapping on "Open" will show you the Experimental set up screen.
4. If 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, 2.11 or 2.12) once loaded. You can save the changed experiments under a new name by pressing "Save". Alternatively, you are prompted to change the name once you proceed to the summary screen (see next step) and then save it. By tapping the "New" button, you can name the altered experiment and proceed.
5. To accept all settings, press "Next".
6. The summary screen appears (Figure 2.12) listing the settings for each well-defined and the drawer will open.
7. Load the samples (for unit-specific details, see chapters 2.9.1, 2.10, 2.11 or 2.12) and press "Start".



**Figure 2.12:** Summary screen after setup.

2.9 4D-Nucleofector® X Unit

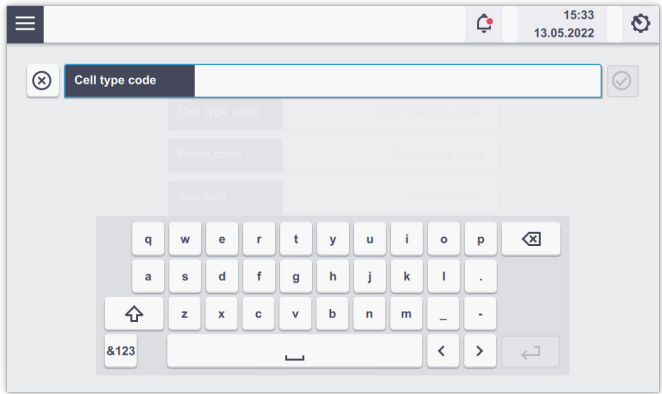


**Figure 2.13:** 4D-Nucleofector® Core and X Unit (A) and Nucleocuvette® Vessels and Strips for the use with the X Unit (B).

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

- 16-well, 20 µL Nucleocuvette® Strips for cell numbers ranging from 2 x 10<sup>4</sup> to 1 x 10<sup>6</sup> cells
- Single 100 µL Nucleocuvette® Vessels for cell numbers ranging from 2 x 10<sup>5</sup> to 2 x 10<sup>7</sup> cells

By choosing the respective vessel type, you will be directed to the Experiment setup screen.

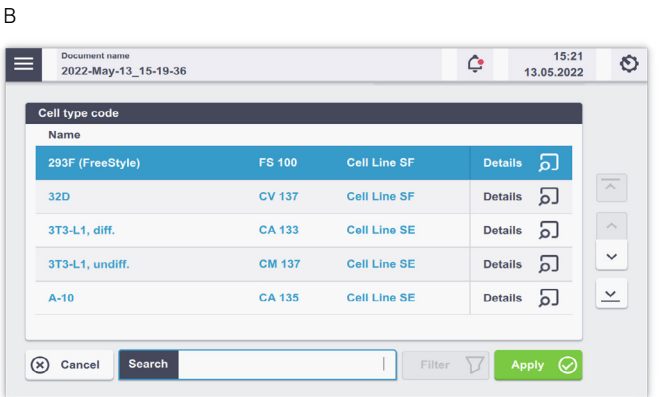
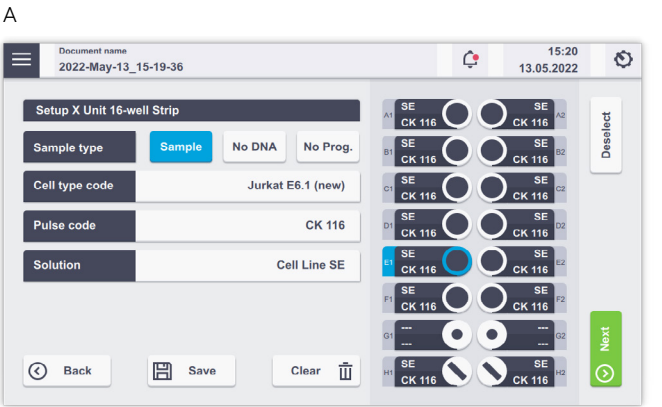


**Figure 2.14:** Experiment naming (X Unit; example: 16-well Nucleocuvette® Strips)

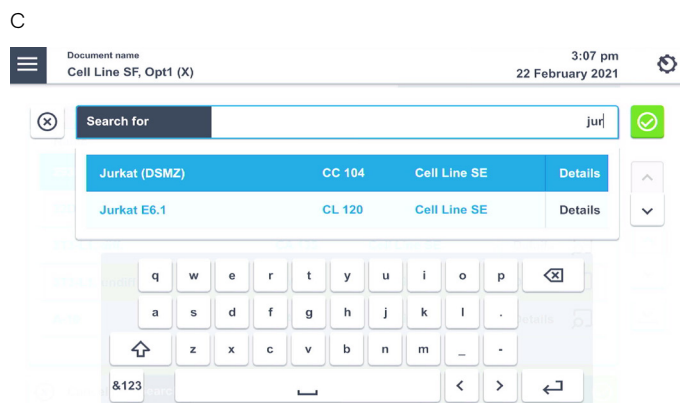
2.9.1 Defining a new experiment

After selecting a vessel type (see chapter 2.8.2), you can either select a predefined experiment (see chapter 2.8.4) or create a new experiment.

If you want to create a new experiment, you may tap in the field “Experiment name” and enter a specific experiment name or accept the suggested name generated with date and time stamp (see chapter 2.8.4). Tap on the checkmark button to get to the Experiment Setup screen (Figure 2.15).







**Figure 2.15:** (A) Experiment setup screen for 16-well strip in a 4D-Nucleofector® X Unit. Tapping on “Cell type code” will open a searchable screen for picking predefined Cell type codes. (B) Scroll the screen, or use the arrows to navigate. (C) Use the search field and start typing if you know the name of the Cell type code.

Depending on the vessel type you selected, a screen with either “Setup X Unit cuvette” or “Setup X Unit 16-well strip” (Figure 2.15, A) will appear.

### 1. Select well(s) to define Nucleofection® Conditions.

By tapping on a well position one sample/well is selected marked by a blue circle around the well position. Multiple samples/wells can be selected by dragging across the wells.

Tapping on additional wells will add more wells to the pre-selected wells.

Remove a well from your selection by tapping on the selected well again. Dragging across all selected wells will deselect them. Dragging across selected and deselected wells again will toggle your selection. Alternatively, use “Deselect” to remove all selections.

### 2. Choose your sample type

Choose “Sample” for substrate-containing samples that will be pulsed

Select “No DNA” for samples that do not contain any substrate (pulse control)

Select “No Prog” for samples with substrate not being subjected to an electrical pulse (no-pulse control)

### 3. Enter Cell type code (optional)

Press the field “Cell type code” to choose predefined Nucleofection® Conditions from a Cell type list (Figure 2.15, B). A list with all available Cell type codes (defined by Lonza or custom) will appear. Select the code that shall be used by scrolling through the menu or start typing in the search field (Figure 2.15, C).

To confirm your selection press “Apply”. If you pick a preset Cell type code the next two steps do not apply.

**NOTE:** Instead of defining solution and Pulse code via the Cell type code, both parameters can also be specified independently of each other, e.g. if no predefined Cell type code is available. For adding new Cell type codes, please refer to chapter 2.13.2.3.

### 4. Enter Pulse code

Modify the pulse code if necessary by pressing the pulse code field. A keyboard will appear, enabling you to change settings. Press the “Apply” field to confirm the selection. To confirm your choice, click “Apply”.

- The Solution code can be modified by selecting the “Solution” field and clicking on the desired solution. Then press “Apply” to confirm the selection
- Optional: At this stage, you can save your defined experiment or only the pulse-solution code combination (as a custom Cell type code) by pressing the “Save” button. Select whether the Cell type code or the experiment shall be saved. A keyboard will appear, allowing you to define a name (max. length: 26 characters) for either the Cell type code or the experiment. Confirm and save the experiment parameters by pressing “Next” (saves the experiment and opens the drawer) or “Save” (saves the experiment for later use).

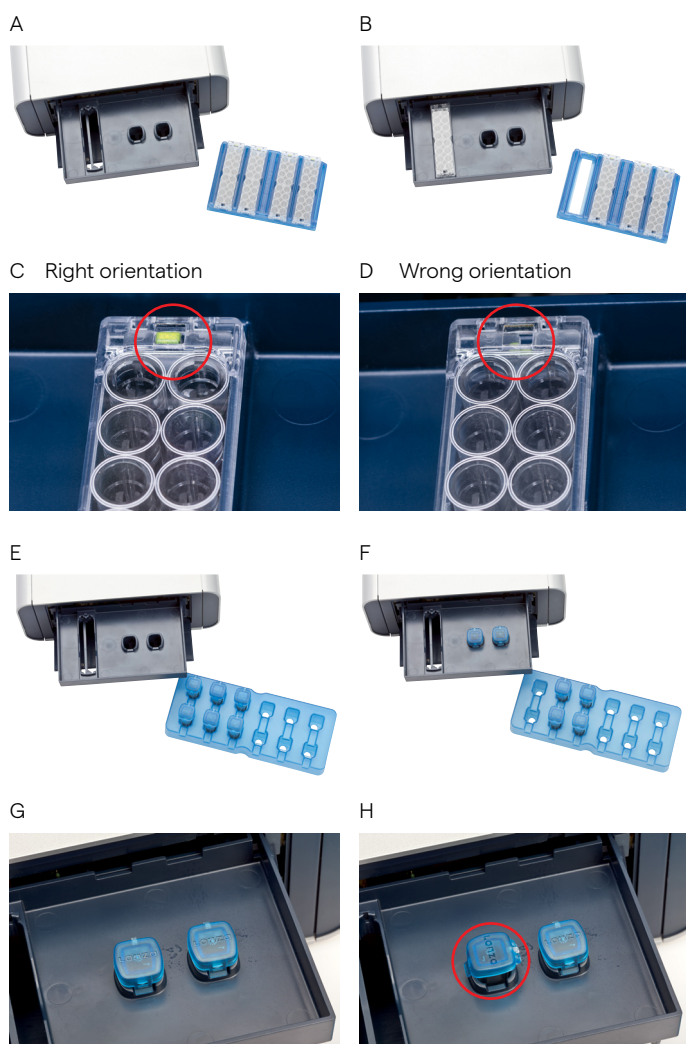
Well	Cell type code	Solution	Pulse	Sample
A1	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample
A2	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample
B1	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample
B2	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample
C1	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample
C2	Jurkat E6.1 (new)	Cell Line SE	CK 116	Sample

A summary of the defined settings will be displayed (Figure 2.16). Before loading samples and pressing “Start” (see chapter 2.9.3), please double-check for accuracy.

### 2.9.2 Loading samples

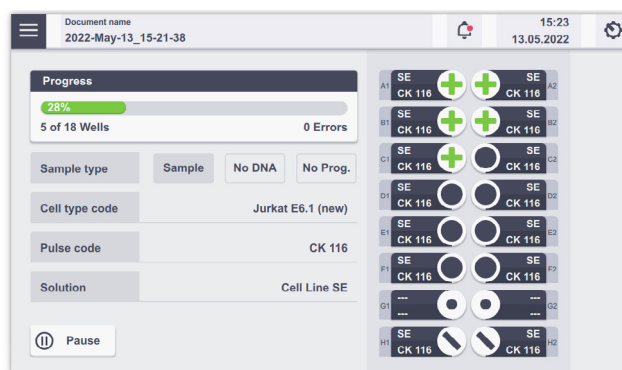
1. Load the 16-well Nucleocuvette® Strip (Figure 2.17, A, B) or one or two 100  $\mu$ L Nucleocuvette® Vessels into the sample tray (Figure 2.17, E, F)
2. Make sure that the 16-well Nucleocuvette® Strip is positioned correctly to avoid damaging the X Unit:

For the 16-well Nucleocuvette® Strip, the yellow pin at the rear end of the strip must be visible (Figure 2.17, C). If the strip is mounted in the wrong orientation, its rear end will stay above the strip holder and the yellow pin is barely visible (Figure 2.17, D). For the 100  $\mu$ L 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 2.17, G, H).



**Figure 2.17:** Loading vessels into 4D Nucleofector® X Unit

### 2.9.3 Running the experiment

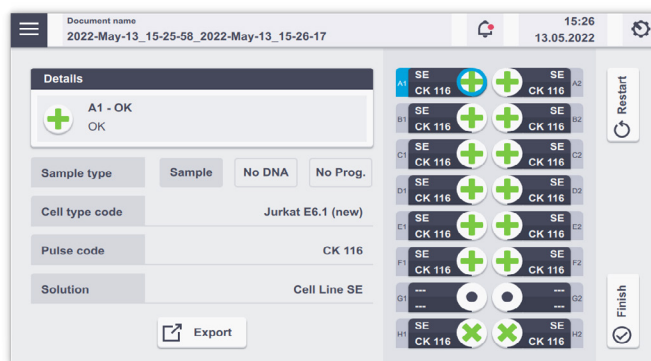


**Figure 2.18:** Experiment progress (X Unit)

1. After loading the samples, press “Start” to run the experiment (Figure 2.16).

The progress of the experiment is indicated by changing the color of the cuvette or well positions (Figure 2.18; 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 insert cuvette for...” will be displayed. Load next samples and press “OK” to continue or “No” to interrupt the experiment.
2. When the experiment is complete, a result file summarizing the Nucleofection® Process will be displayed (Figure 2.19).
  3. The result file will be saved automatically by the system (either as Result + Date + Time or in case you saved the experiment under an individual name as “Experiment name + Date + Time) and can be reopened as described in chapter 2.13.2.2.
  4. Press “Restart” to start the experiment or “Finish” to return to the home screen.



**Figure 2.19** Result screen (A). Tap on wells to receive details about the experiment. For explanation of symbols, refer to chapter 4.1.

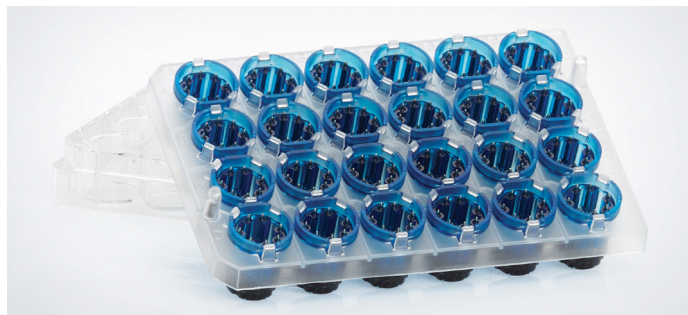
## 2.10 4D-Nucleofector® Y Unit

The Y Unit allows for transfection of cells in adherence. A specially designed dipping electrode pulses cells plated in a 24-well dish from the top.

A



B

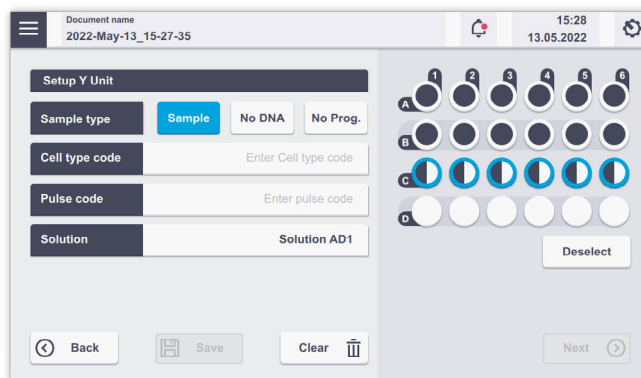


**Figure 2.20:** 4D-Nucleofector® Core and Y Unit and Dipping Electrode to use with the Y Unit.

### 2.10.1 Defining a new experiment

After selecting a vessel type (see chapter 2.8.2), you can either select a predefined experiment (see chapter 2.8.4) or create a new experiment.

If you want to create a new Experiment, you may tap in the field "Experiment name" and enter a specific experiment name or accept the suggested name generated with date and time stamp. Tap on the checkmark button to get to the Experiment setup screen (Figure 2.21).



**Figure 2.21:** Experiment setup screen for 24-well plate in 4D-Nucleofector® Y Unit

A screen appears with the title "Setup Y Unit" (Figure 2.21).

#### 1. Select well(s) to define Nucleofection® Conditions

By tapping on a well position one sample/well is selected marked by a blue circle around the well position. Multiple samples/wells can be selected by dragging across the wells.

Tapping on additional wells will add more wells to the pre-selected wells.

Remove a well from your selection by tapping on the selected well again. Dragging across all selected wells will deselect them. Dragging across selected and deselected wells again will toggle your selection. Alternatively, use "Deselect" to remove all selections.

#### 2. Choose your sample type

Choose "Sample" for substrate-containing samples that will be pulsed.

Select "No DNA" for samples that do not contain any substrate (pulse control).

Select "No Prog" for samples with substrate not being subjected to an electrical pulse (no-pulse control).

#### 3. Enter Cell type code (optional)

Press the field "Cell type code" to choose predefined Nucleofection® Conditions from a cell type list (Figure 2.15, B). A list with all available Cell type codes (defined by Lonza or custom) will appear. Select the code that shall be used by scrolling through the menu or start typing in the search field (Figure 2.15, C).

To confirm your selection, press "Apply". If you pick a preset Cell type code the next two steps do not apply.

**NOTE:** Instead of defining solution and Pulse code via the Cell type code, both parameters can also be specified independently of each other, e.g. if no predefined Cell type code is available. For adding new Cell type codes, please refer to chapter 2.13.2.3.

#### 4. Enter Pulse code

Modify the pulse code if necessary by pressing the “Pulse code” field. A keyboard will appear, enabling you to change settings. Press the “Apply” field to confirm the selection.

5. The solution code can be modified by selecting the “Solution” field and clicking on the desired solution. Then press “Apply” to confirm the selection.

Optional: At this stage, you can save your defined experiment or only the pulse-solution code combination (as a custom Cell type code) by pressing the “Save” button. Select whether the Cell type code or the experiment will be saved. A keyboard will appear allowing you to define a name (max. length: 26 characters) for either the Cell type code or the experiment.

Confirm and save the experiment parameters by pressing “Next” (saves the experiment and opens the drawer) or “Save” (saves the experiment for later use). A summary of the defined settings will be displayed. Before loading samples and pressing “Start” (see chapter 2.10.2), please double-check for accuracy.

### 2.10.2 Loading samples



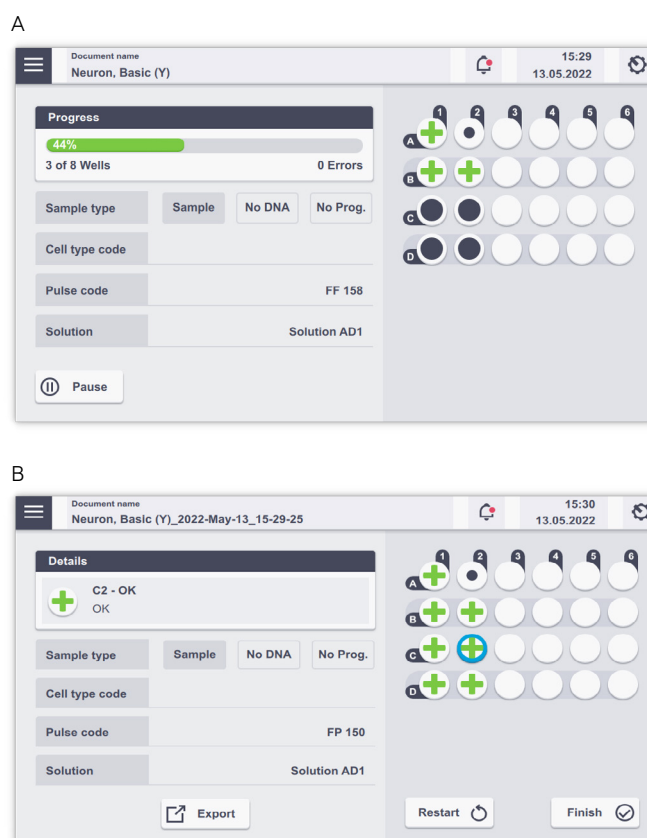
**Figure 2.22:** Loading of samples (Y Unit). Watch the orientation of the plate. (Well A1 is placed in the upper left position).

1. 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.
2. Place the 24-well plate with inserted dipping electrode array into the retainer of the 4D-Nucleofector® Y Unit. Well “A1” must be in the upper left position (Figure 2.22).

3. It is not recommended to re-use dipping electrodes as this leads to suboptimal transfection efficiencies.

### 2.10.3 Running the experiment

1. After loading the samples, press “Start” to run the experiment.
2. The progress of the experiment is indicated by changing the color of the cuvette or well positions (Figure 2.23 A; for color codes, see chapter 4).
3. When the experiment is complete, a result file summarizing the Nucleofection® Process will be displayed (Figure 2.23, B).
4. The result file will be saved automatically by the system and can be reopened as described in chapter 2.13.2.2
5. Press “Restart” to start the experiment or “Finish” to return to home screen.



**Figure 2.23:** (A) Nucleofection® Progress (Y Unit). (B) Result Screen. Select wells to receive result details.



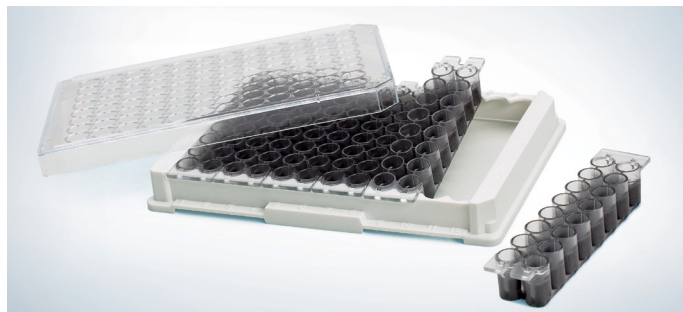
## 2.11 4D-Nucleofector® 96-well Unit

The 4D-Nucleofector® 96-well Unit is designed to simultaneously run up to 96 Nucleofection® Reactions with cell numbers ranging from  $2 \times 10^4$  to  $1 \times 10^6$  cells per reaction.

A



B



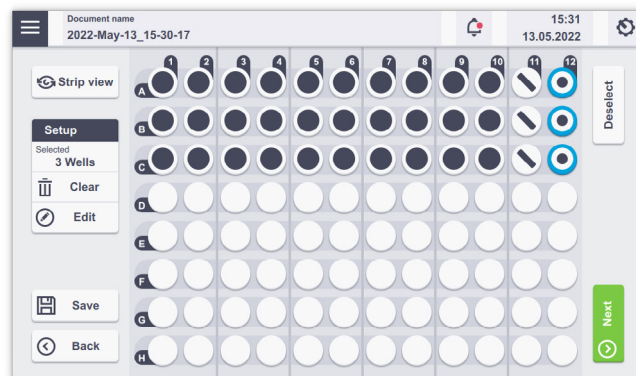
**Figure 2.24:** 4D-Nucleofector® Core Unit and 96-well Unit (A). 96-well Nucleocuvette® Plate (B).

### 2.11.1 Defining a new experiment

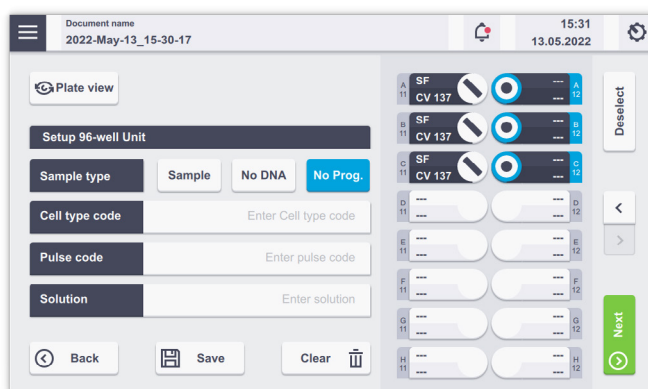
After selecting a vessel type (see chapter 2.8.2), you can either select a predefined experiment (see chapter 2.8.4) or create a new experiment.

If you want to create a new experiment, you may tap in the field "Experiment name" and enter a specific name for the experiment or accept the suggested name generated with date and time stamp. Tap on the checkmark at the bottom to get to the Experiment setup screen (Figure 2.8).

A



B



**Figure 2.25:** Experiment definition (96-well Unit, plate view (A) and strip view (B))

#### 1. Select well(s) to define Nucleofection® Conditions.

By tapping on a well position one sample/well is selected, marked by a blue circle around the well position. Multiple samples/wells can be selected by dragging across the wells.

Additional wells can be added to the pre-selected wells by tapping on them.

Remove wells from your selection by tapping on the selected well again. Dragging across all selected wells will deselect them. Dragging across selected and deselected wells again will toggle your selection.

Switching from whole plate view (Figure 2.25 A) to strip view (Figure 2.25 B) is possible by tapping on the strip view/ plate view button. In plate view mode select wells and tap on edit to enter experiment conditions (Figure 2.26 A). In strip view further information on the well position and applied pulses are visible for each well. The selected positions are highlighted in blue (Figure 2.26 B).



Tapping on the “Clear” button will delete all settings from the selected wells after confirmation.

## 2. Choose your sample type

Choose “Sample” for substrate-containing samples that will be pulsed.

Select “No DNA” for samples that do not contain any substrate (pulse control).

Select “No Prog” for samples with substrate not being subjected to an electrical pulse (no-pulse control)

## 3. Enter Cell type code (optional)

Press the field “Cell type code” to choose predefined Nucleofection® Conditions from a Cell type list (Figure 2.15). A list with all available Cell type codes (defined by Lonza or custom) will appear. Select the code that shall be used by scrolling through the menu or by starting to type in the search field. Enter the cell name in the field Cell type code.

To confirm your selection press “Apply”. If you pick a Cell type code the next two steps do not apply

**NOTE:** Instead of defining solution and program code via the Cell type code, both parameters can also be specified independently of each other, e.g. if no predefined Cell type code is available. For adding new Cell type codes, please refer to chapter 2.13.2.3.

## 4. Enter Pulse code

Modify Pulse code if necessary by pressing the Pulse code field. A keyboard will appear, enabling you to change settings. Press the check mark field to confirm the selection.

## 5. Enter Solution

The Solution code can be modified by selecting the Solution field and clicking on the desired solution. Then press “Apply” to confirm the selection.

## 6. Optional:

At this stage, you can save your defined experiment or only the pulse-solution code combination (as custom Cell type code) for future use by pressing the “Save” button. Select whether the Cell type code or the experiment shall be saved. A keyboard will appear, allowing you to define a name (max. length: 26 characters) for either the Cell type code or the experiment.

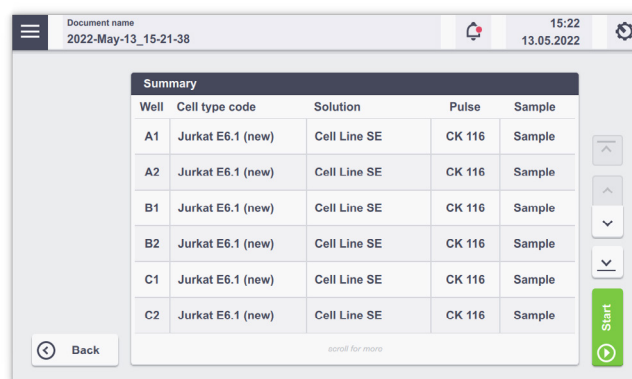
Confirm and save the experiment parameters by pressing “Next” (saves the experiment and opens the drawer) or “Save” (saves the experiment for later use).

A summary of the defined settings will be displayed (Figure 2.16). Before loading samples and pressing “Start” (see chapter 2.9.3), please double-check for accuracy.



**Figure 2.26 A:** Experiment settings after well selection and tapping on “Edit” button in plate view (A) and Sample definition in strip view (B).

For further explanation about parameters, please refer to chapter 2.8.3.



**Figure 2.27:** Summary screen after experiment setup.

### 2.11.2 Loading samples

Load the 96-well Nucleocuvette® Plate (Figure 2.24) into the sample tray (Figure 2.28)



**Figure 2.28:** 96-well Unit sample tray. Watch the orientation of the plate. (Well A1 is placed in the upper left position).

Make sure that the plate is oriented properly: the plate needs to be oriented in a way that well A1 is placed in the upper left corner.

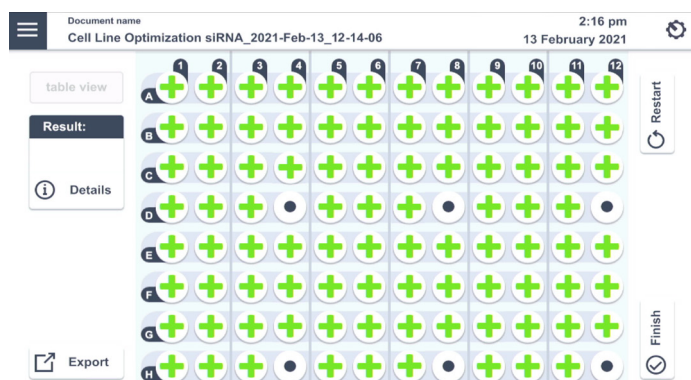
Please note that all Nucleofection® Strips must be inserted into the plate frame to run an experiment – even if only a fraction of the wells are used or defined.

### 2.11.3 Running the experiment

1. After loading the samples, press “Start” to run the experiment (Figure 2.27).

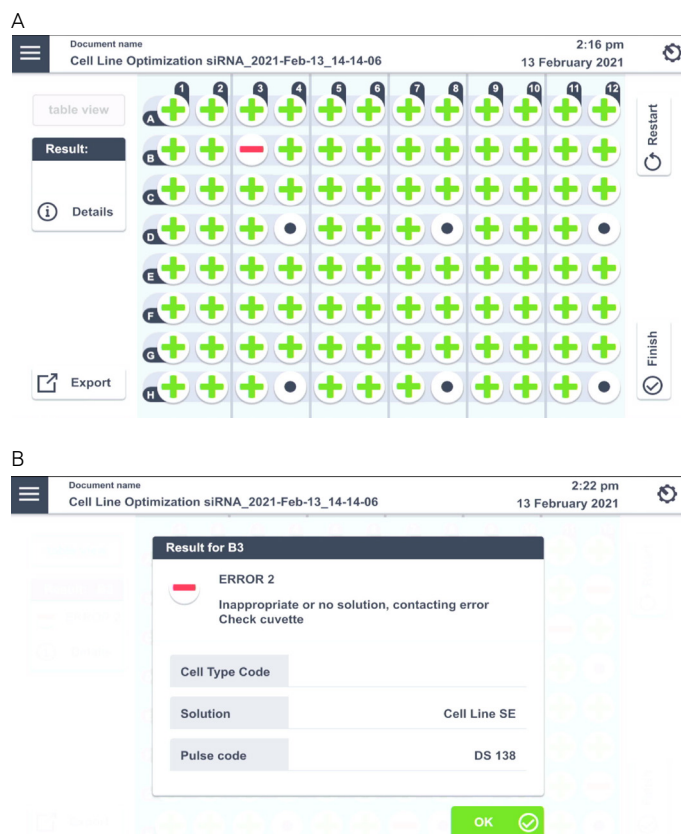
The progress of the experiment is indicated by changing the symbols of the well positions. (for color codes, see chapter 4).

2. When the experiment is complete, a result file summarizing the Nucleofection® Process will be displayed (Figure 2.29).



**Figure 2.29:** Example of a result file.

3. The result file will be saved automatically by the system and can be reopened as described in chapter 2.13.2.2. Selecting a well will display further information of the result. Tapping the “Details” button will open a description for this well, including details about the setup and result.



**Figure 2.30:** Detail screen for experiment results. (A) Selection of well is indicated by blue circle. (B) Clicking on “Details” button reveals further information on the well.

4. Press “Start” to start the same experiment again or “Back” to return to the “Set up Experiment” screen and define a new experiment.

## 2.12 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 flow-through LV Nucleocuvette® Cartridge for cell numbers up to  $1 \times 10^9$  cells.

### Components of 1 mL Nucleocuvette® Cartridge (Figure 2.31, A)

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

### Components of the LV Nucleocuvette® Cartridge (Figure 2.31, B, C)

1. Cartridge
2. Venting tube with 0.2  $\mu$ 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.

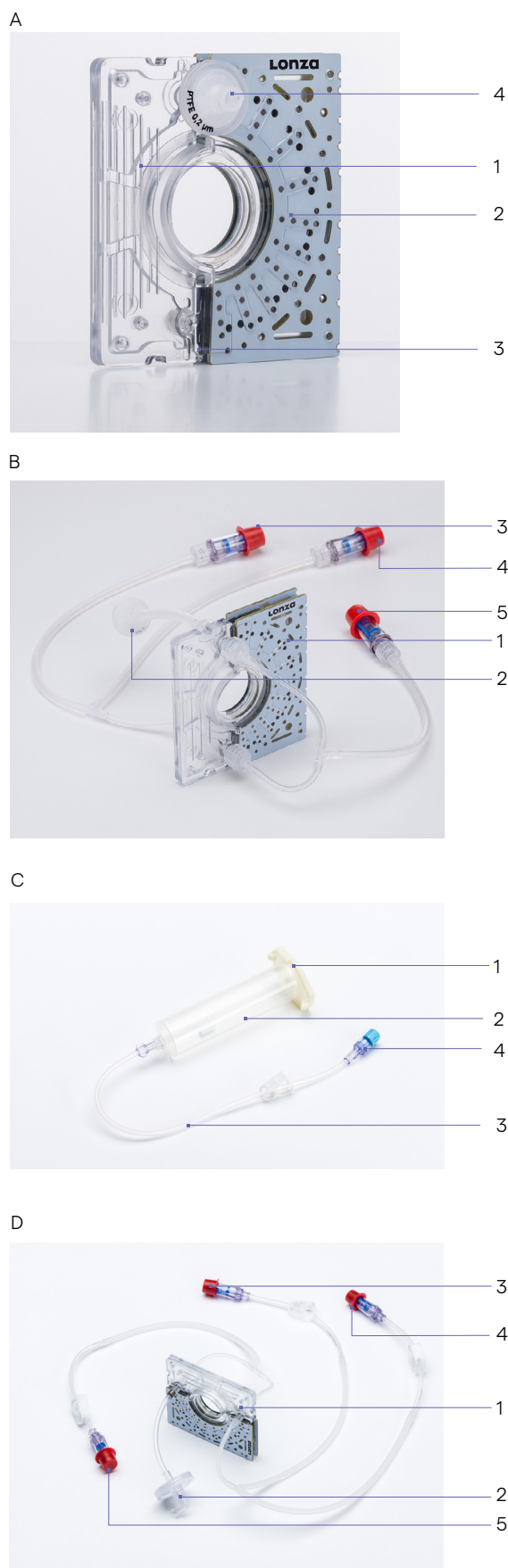
### Components of the 4D-Nucleofector® LV Reservoir (Figure 2.31, C)

1. Reservoir cap
2. Reservoir body
3. Outlet tube (weldable)
4. Outlet port (swabbable injection port (with male Luer cap))

### Components of the LV Nucleocuvette® Cartridge, weldable tubing (Figure 2.31, D)

1. Cartridge
2. Venting tube with 0.2  $\mu$ 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. PVC tube attachments with clamps (for welding)

**Figure 2.31:** 4D-Nucleofector® LV Unit Consumables

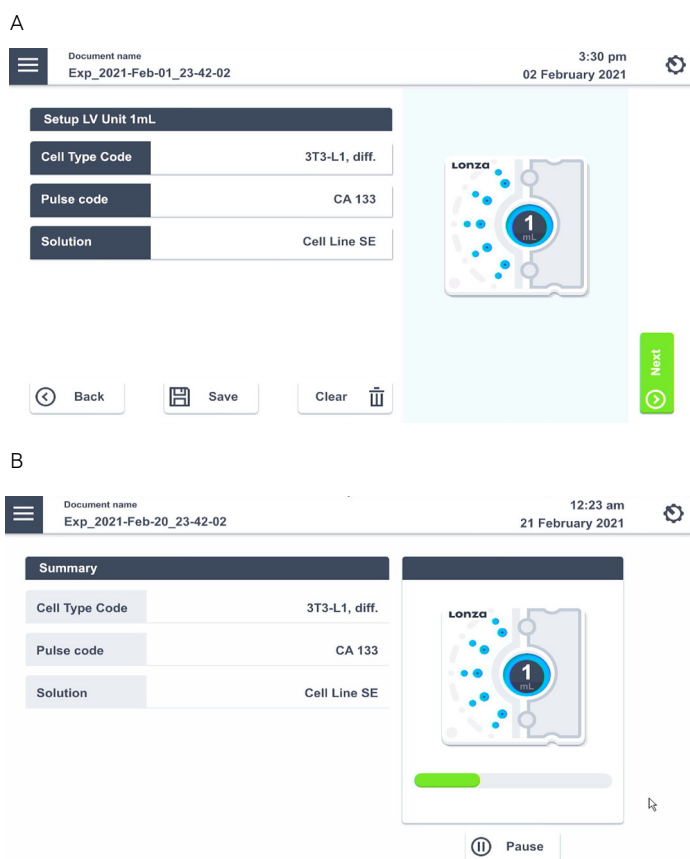


## 2.12.1 Using the 1 mL Nucleocuvette® Cartridge (Fixed volume)

### 2.12.1.1 Defining a new experiment

Following vessel type selection (see chapter 2.8.2) you can now either select a predefined experiment (see chapter 2.8.4) or define an experiment from the beginning.

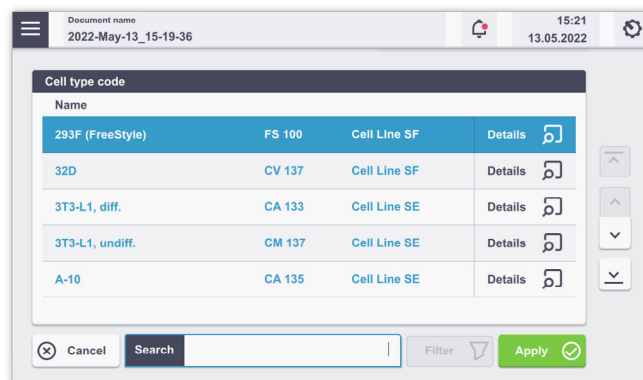
If you want to create a new experiment, you may tap in the field “Experiment name” and enter a specific name for the experiment or accept the suggested name generated with date and time stamp (see chapter 2.8.4). Tap on the check mark button to get to the Experiment setup screen. (Figure 2.8).



**Figure 2.32:** (A) Experiment setup screen for 4D-Nucleofector® LV Unit using 1 mL Nucleocuvette® Cartridge. (B) Progress screen during experiment.

A screen appears with the title “Setup LV 1 mL” (Figure 2.32, A).

1. Press the field “Cell Type Code” to choose predefined Nucleofection® Conditions from a Cell type list (Figure 2.33). A list with all available Cell type codes (defined by Lonza or custom) will appear.

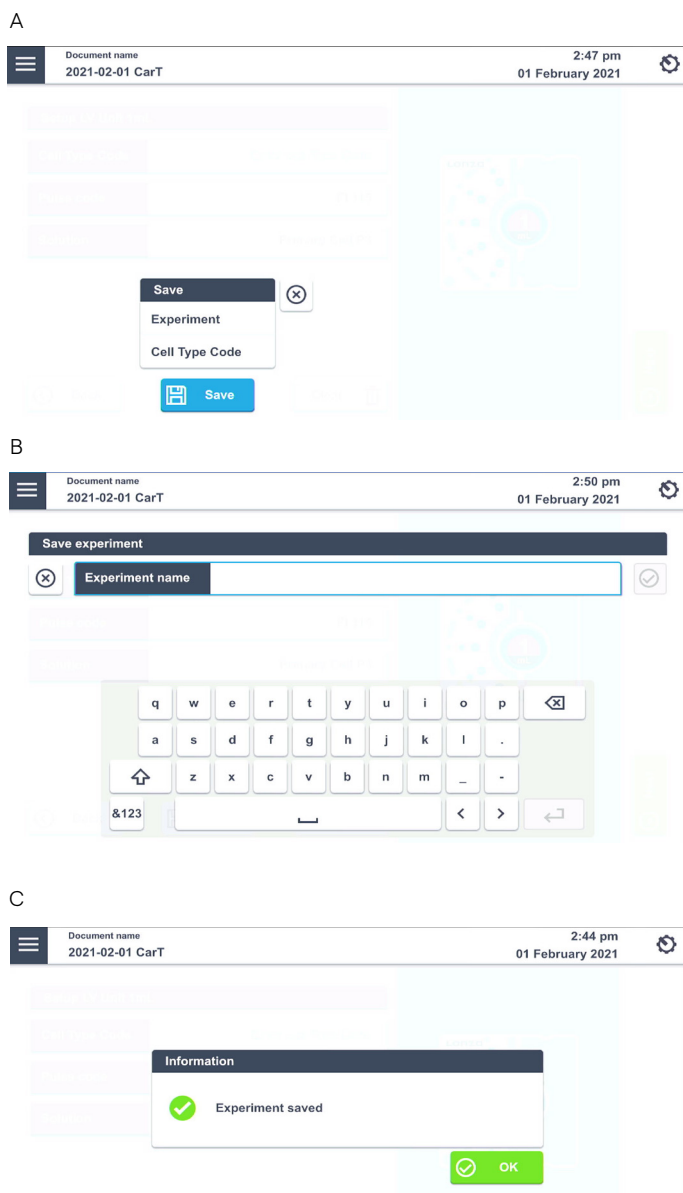


**Figure 2.33:** List of predefined Cell type codes. Select the Code by either typing in a name in the Search Field, scrolling the screen or using the arrows on the right of the screen.

2. Select the code that shall be used by scrolling through the menu or start typing in the search field (Figure 2.33). To confirm your selection press “Apply”. For additional information about the cell type selected press “i”.
3. 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.
4. Instead of defining solution and program code via the Cell type code, both parameters can also be selected manually, e.g. in case no predefined Cell type code is available. For adding new Cell type codes, please refer to chapter 2.13.2.3.

**Optional:** At this stage, you can save your defined experiment or only the pulse-solution code combination (as custom Cell type code) for future use by pressing the “Save” button. Select whether the Cell type code or the experiment shall be saved. A keyboard will appear allowing you to define a name (max. length: 26 characters) for either the Cell type code or the experiment depending on your previous selection.



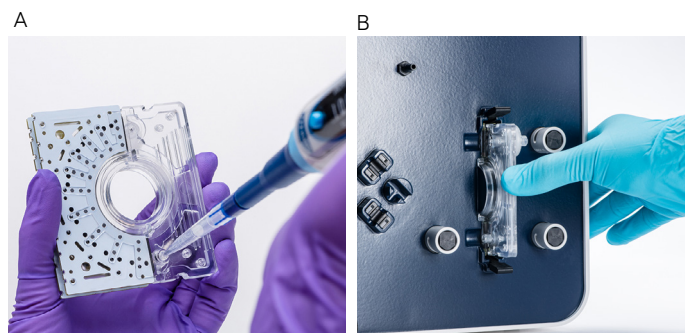


**Figure 2.34:** Saving an experiment. (A) after tapping the “Save” icon in the setup screen. Choose between Cell type code or Experiment. (B) Type in the name for Either Experiment or Cell type code. (C) Click “OK” to proceed.

5. Confirm and save the experiment parameters by pressing “OK” or “SAVE”.
6. Press “OK” to continue (Figure 2.34, C)
7. A summary of the defined settings will be displayed. Before loading samples and pressing “Start” (see chapter 2.12.13), please double-check for accuracy.

### 2.12.1.2 Loading Samples

1. Fill the 1 mL Nucleocuvette® Cartridge with 1 mL sample. For filling hold cartridge in a slight angle as shown in (figure 2.35 A). The sterile filter on the rear side should be at the top, the injection port should be at the bottom (upon pipetting cartridge will be filled from bottom to top). Insert the pipette tip approximately in a 90° angle.



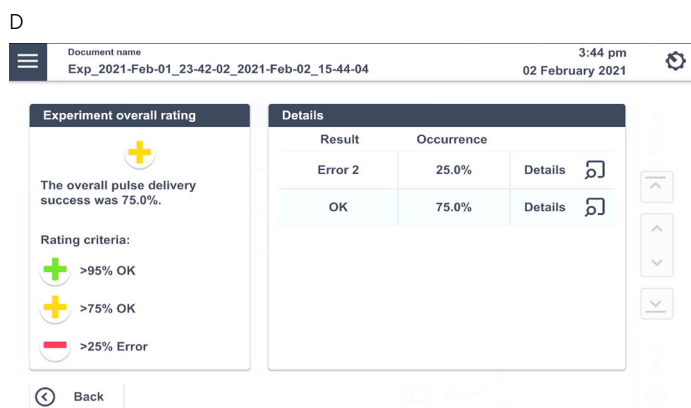
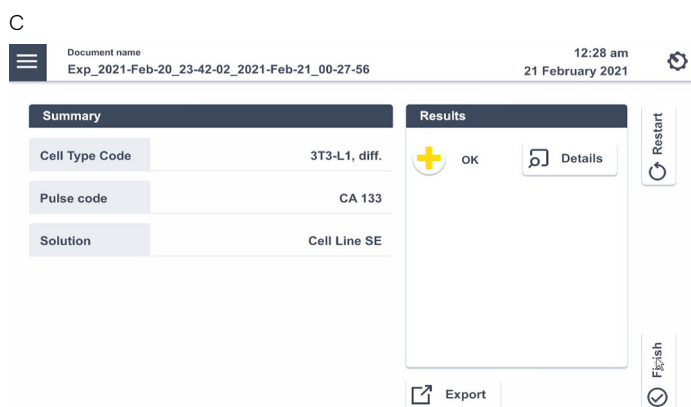
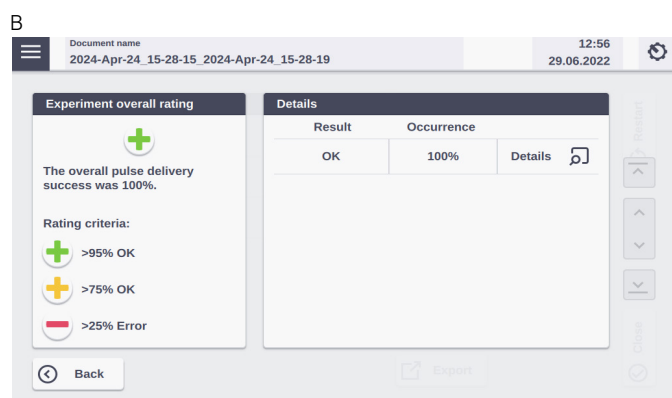
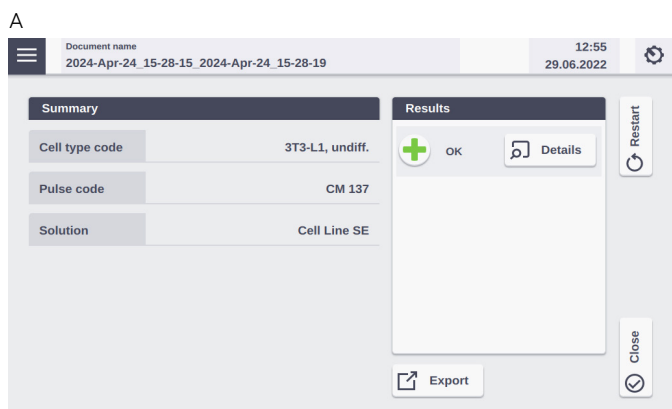
**Figure 2.35:** A volume of 1 mL is required to completely fill the cuvette. Using a higher or lower volume may lead to errors.

2. Insert the filled cartridge into the slot until it snaps in (Figure 2.35, B). Make sure that upper and lower snap mechanism audibly engages to allow for proper contacting.

### 2.12.1.3 Running the experiment

1. When the cartridge is inserted, press “Start” to initiate the Nucleofection® Process.
2. The progress of the experiment is indicated by a progress bar (Figure 2.32, B).
3. When the experiment is complete, a result file summarizing the Nucleofection® Process will be displayed (Figure 2.36).
4. The result file will be saved automatically by the system (either as Result + Date + Time or in case you saved the experiment under an individual name as “Experiment name + Date + Time) and can be reopened as described in chapter 2.13.2.2.
5. The overall result is indicated by a color code:
  - a. Green cross for “OK” (Figure 2.36, A)
  - b. Yellow cross in case of few errors (Figure 2.36, B)
  - c. Red dash in case of multiple errors (not shown).





**Figure 2.36:** Result screens for LV Unit. (A/B) Result screen for no errors in experiment. (C/D) Result screen for experiment with few errors. Tapping the “Details” icon will provide further information on the pulse delivery.

If errors occurred you can check for more details by pressing “Details”. The result details (Figure 2.36 B or D) will display the type of error and its’ percent occurrence in the processed volume. For additional information on the error press “Details”.

6. Once the process is completed take out the cartridge by pressing the black levers.

7. Aspirate the transfected sample via the port (see figure 2.35, A) and transfer cells into culture. Optionally you may wash the cartridge with medium to aspirate residual liquid.

8. You can repeat the same experiment by pressing “Restart”. Press “Finish” to return to home screen.

## 2.12.2 Using the LV Nucleocuvette® Cartridge (Scalable volume)

### 2.12.2.1 Overview

For running an experiment with the LV Nucleocuvette® Cartridge the recommended order of steps is:

Step 1: Start defining the experiment (see 2.12.2.2)

Step 2: Mount the LV Nucleocuvette® Cartridge including all tubes onto the 4D-Nucleofector® LV Unit (see 2.12.2.2).

**NOTE:** In case you prefer establishing the connection of the LV Nucleocuvette® Cartridge to the input and output reservoirs prior to cartridge mounting at the device, please make sure the tubings are clamped at a position close to the reservoir body to avoid liquid movement.

Step 3: Connect LV Nucleocuvette® Cartridge and output LV Reservoir (see 2.12.2.3). Add medium to the output reservoir, insert it into a 4D-Nucleofector® LV Rack and connect it to the outlet tube of the LV Nucleocuvette® Cartridge either by manual connection or by welding (when using the LV Nucleocuvette® Cartridge with weldable tubing)

Step 4: Prepare your cell sample and substrate using the input LV Reservoirs (see 2.12.2.4)

Step 5: Connect the input LV Reservoirs to the LV Nucleocuvette® Cartridge (see 2.12.2.4)

Step 6: Verify volume settings and execute experiment (see 2.12.2.4)

**NOTE:** In case you use bags and not Lonza LV Reservoirs for sample input and/or collection, please contact Lonza Scientific Support for maximum tube volumes.

### 2.12.2.2 Defining a new experiment

Following vessel type selection (see chapter 2.8.2) you can now either select a predefined experiment (see chapter 2.8.4 or create a new experiment).

If you want to create a new Experiment, tap in the field "Experiment name" and enter a specific experiment name or accept the suggested name generated with date and time stamp. Press "Submit" to get to Experiment setup screen (Figure 2.32).

#### 1. Enter Cell Type Code (optional)

Press the field "Cell type code" to choose predefined Nucleofection® Conditions from a cell type list (Figure 2.33). A list with all available Cell type codes (defined by Lonza or custom) will appear. Select the code that shall be used by scrolling through the menu or start typing in the search field. To confirm your selection press "Apply". If you pick a preset Cell type codes the next two steps do not apply.

**NOTE:** Instead of defining solution and program code via the Cell type code, both parameters can also be selected manually (step 2 and 3), e.g. in case no predefined Cell type code is available. For adding new Cell type code, please refer to chapter 2.13.2.3.

#### 2. Enter Pulse code

Modify pulse code if necessary by pressing the "Pulse code" field. A keyboard will appear, enabling you to change settings. To confirm your choice, click "Apply".

#### 3. Enter Solution

The solution code can be modified via selecting the "Solution" field and click on the desired solution. Then press "Apply" to confirm the selection.

#### 4. Enter the volume you plan to process

Press on the field "Volume" (Cell suspension volume) to define the planned volume of your cell suspension (up to 20 mL are possible).

- 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 separate". A second volume field will show up for "Substrate" to define the volume of substrate independently. In addition a field will appear named "Bubble detection":

- If bubble detection is de-activated (default setting), the liquid sensor is only used for priming of the substrate line (detection of liquid start). Afterwards the system would ignore any air fractions within the substrate feed and continue processing until the end of liquid according to volume entry is reached or if multiple errors occur that point to missing liquid. However, the air fraction may lead to an increased error frequency. In addition larger fractions without substrate may lead to a decrease in transfection efficiency, as the sample is pulsed without substrate.
- If bubble detection is activated, the system will interrupt the pulsing process in case the substrate line contains a larger bubble (>7 mm). A message will appear and the user can decide whether the process shall be continued or aborted. In case "continue" is selected, the system will continue the process like in the "de-activated" setting, i.e. it would ignore any further air fractions in the substrate feed and continue processing until the end of liquid according to volume entry is reached or if multiple errors occur that point to missing liquid.

**NOTE:** The maximum total volume that can be entered is 20 mL and the ratio between Cell suspension and Substrate volume should be between 1:1 (e.g. 10 mL cell suspension and 10 mL substrate) and 9:1 (e.g. 18 mL cell suspension and 2 mL substrate)

#### 5. Save your experiment (optional)

At this stage, you can save your defined experiment or only the pulse-solution code combination (as custom Cell type code) for future use by pressing the "Save" button. Select whether the Cell type code or the experiment shall be saved. A keyboard will appear allowing you to define a name (max. length: 26 characters) for either the Cell type code or the experiment depending on your previous selection.

#### 6. Continue with mounting the LV Nucleocuvette® Cartridge (see 2.12.2.3)

### 2.12.2.3 Mounting an LV Nucleocuvette® Cartridge

1. Insert the LV Nucleocuvette® Cartridge into the slot. Make sure that upper and lower snap mechanism audibly engages to allow for proper contacting.
2. Attach the venting tube with the filter (upper left of the cartridge) to the male Luer connector on the front plate (Figure 2.37, A).
3. If you started defining your experiment setup already (see 2.12.2.1), the software can support you in inserting the tubes into the pinch valves by automatic opening of the valves. On the right side of the experiment screen you can select "Yes" if you want to insert the tubes of the LV Nucleocuvette® Cartridge into the pinch valves now (Figure 2.37, B). Alternatively, you can open the pinch valves manually by pushing the black button on the valves.
4. Upon pressing "Yes" the pinch valves will automatically open for 30 seconds allowing to conveniently insert the tubes into the three pinch valves (Figure 2.37, C).

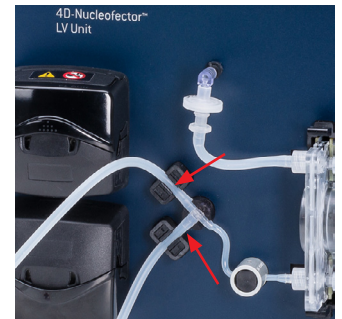
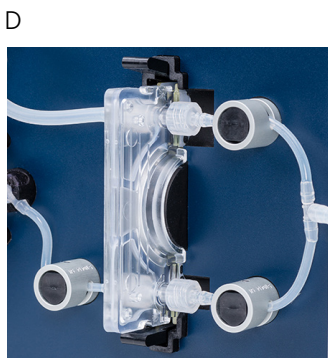
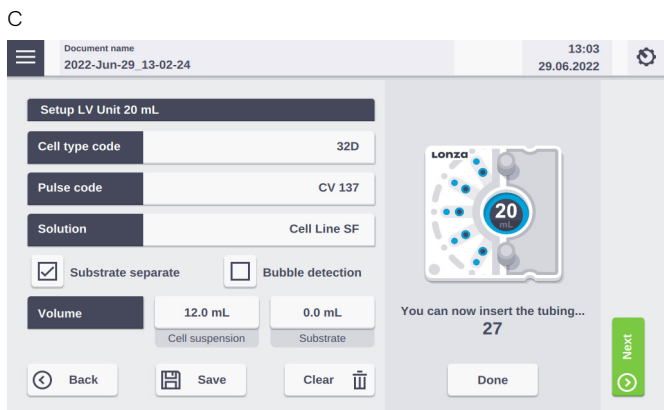
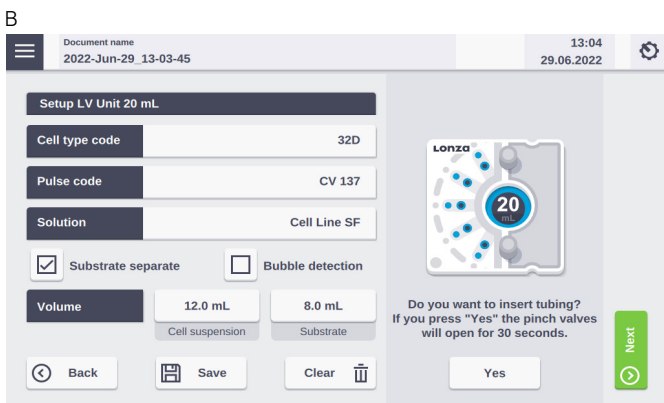
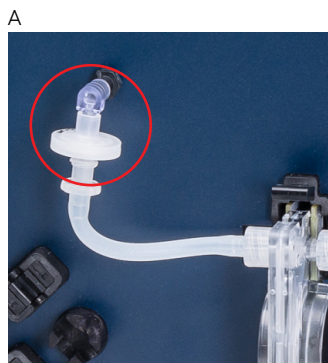
**NOTE:** The open time of the pinch valves is limited to 30 seconds to avoid overloading of the electronics.

5. Insert the tubes into the three pinch valves (Figure 2.37, D). Ensure that they are inserted completely (Figure 2.37, E). After 30 seconds, the pinch valves will close automatically. In case you did not finalize insertion within 30 seconds, you may re-open the valves by pressing "YES" again or manually (see "Note" above).
6. Insert the T connector on the left hand side of the cartridge into the T connector holder (Figure 2.37, F, left).
7. Insert the two larger diameter tubes into the black holders (figure 2.37, F, right). Ensure the tubes are inserted completely into the black holders. The lower holder comprises a light sensor for liquid detection in the lower tubing line.
8. Insert the upper tube into the upper pump and close the flap (Figure 2.37, G). When closing the flap, gently hold the tube without pulling it.
9. Repeat with the lower tube and the lower peristaltic pump (optional, if working with 2 input reservoirs).
10. Fill the output reservoir with medium (for detailed recommendations, please refer to cell type-specific protocol) and mount it into a 4D-Nucleofector® LV Reservoir Rack (Figure 2.37, H).

11. 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 output reservoir (or any other reservoir with female Luer connector, figure 2.37, I). Alternatively, when working with the weldable LV cartridge version, you may establish the connection by welding.
12. Before continuing check setup status: At this point steps 1–6 and 8 of the arrangement shown in figure 2.31 should be done.
13. Continue with sample loading (see 2.12.2.4).

### 2.12.2.4 Loading Samples

1. Prepare cell suspension under the sterile hood (for detailed recommendations, please refer to cell type-specific protocol).
2. Fill a defined volume of cells and substrate into the input reservoir(s) mounted on a 4D-Nucleofector® LV Reservoir Rack.
3. 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.
4. 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 output reservoirs and connect both (Figure 2.37, G). Alternatively, when working with the weldable LV cartridge version, you may establish the connection by welding.
5. The system is now fully assembled. Check correct assembly as shown in figure 2.38.



**Figure 2.37: Mounting the 4D-Nucleofector® LV Nucleocuvette® Cartridge.**  
 (A) LV Cartridge inserted into slot and venting tube attached.  
 (B) Setup screen for LV Cartridge.  
 (C) Remaining time for tube insertion is shown. If the time is not sufficient, you can start the process again after the time ran up.  
 (D) Insertion into three pinch valves.  
 (E) Detail  
 (F) Insertion into the T connector holder and liquid sensors.  
 (G) Insertion in upper pump.  
 (H) Reservoir filled with medium and mounted into a 4D Nucleofector® LV Reservoir Rack.  
 (I) Manual connection with LV Cartridge.  
 The pictures reflect the first version of the consumables, later versions may show slight modifications. However, the mounting procedure stays the same.



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 2.38:** Setup Checklist for LV Nucleocuvette® Cartridge. The picture reflects the GMP-grade TheraPEAK® Consumables, research-grade versions may slightly differ but the mounting procedure stays the same.



### 2.12.2.5 Running the experiment

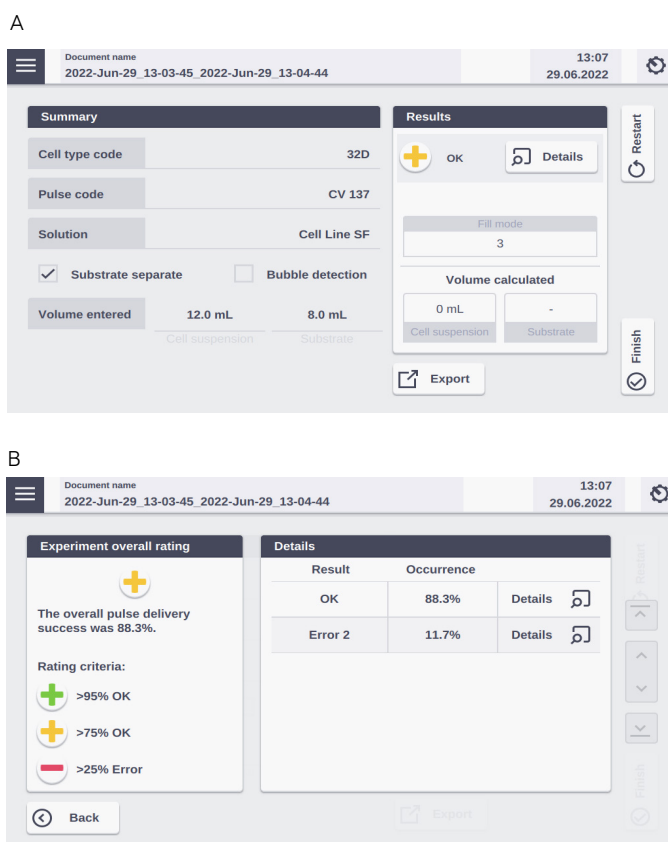
1. Verify again that the volumes you have entered before (see 2.12.2.2, step 5) correctly reflects the volumes you have prepared. If not, adapt volumes again.
2. Upon pressing “Next” a summary of the defined settings will be displayed. Please check for correctness before pressing “Start” to initiate the Nucleofection® Process.
3. Press “Start”. The system will remind you to open the clamps (Figure 2.39, A).



**Figure 2.39:** (A) Prompt before starting the actual experiment to check tubing and open clamps. (B) Progress of the experiment.

4. Before starting the liquid priming process, the system performs a calibration step.
5. The progress of the experiment is indicated by a progress bar (Figure 2.39, B).
6. Once the required cycles to process the sample are completed, a Result screen summarizing the Nucleofection® Process will be displayed (Figure 2.40, A). Further details are displayed when pressing on “Details” (Figure 2.40 B).

**NOTE:** The volume calculation may not exactly match the entered volume as it is just an approximation based on the number of pump steps and not based on real volume monitoring. As such, it is dependent on the tubing diameter which may slightly vary due to raw material variations and/or tubing tension.



**Figure 2.40:** (A) Result screen. (B) Result details after tapping on the “Details” Button (Figure 2.32 A)

7. The result file will be saved automatically by the system (either as Result + Date + Time or in case you saved the experiment under an individual name as “Experiment name + Date + Time) and can be reopened as described in chapter 2.13.2.
8. The overall result is indicated by a color code:
  - Green cross for “OK”
  - Yellow cross in case of few errors (Figure 2.40, A)
  - Red dash in case of multiple errors

If errors occurred you can check for more details by pressing “Details”. The result details (Figure 2.40, B) will display the type of error and its’ percent occurrence in the processed volume. For additional information on the error, press “Details”.

9. Once the process is complete, disconnect the output reservoir and transfer transfected cell suspension into cell culture vessels.
10. Disassemble the tubing, take out the LV Nucleocuvette® Cartridge by pressing the black levers (Figure 2.37, I) and discard it.
11. You can repeat the same experiment by pressing “Restart”.

12. Press “Start” to start the experiment again, or “Back” and return to the “Experiment set up” screen.

## 2.13 Additional settings and options

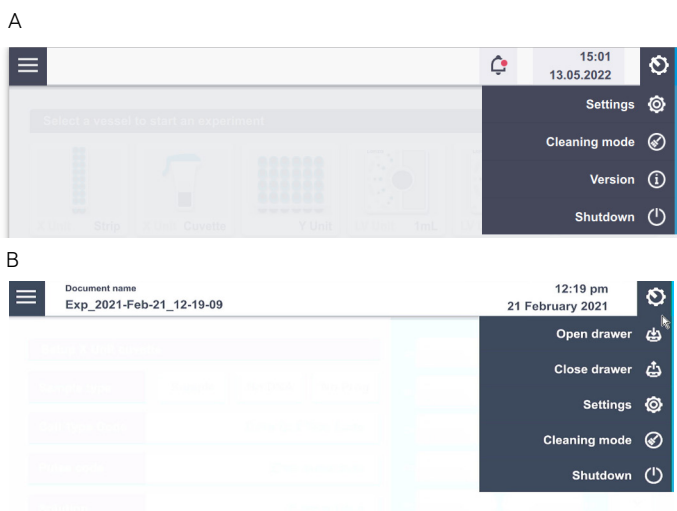
This chapter explains how to change settings like date and time and how to organize and manage your stored experiments, results and custom programs.

### 2.13.1 Settings menu on the right

Activate the “Settings” menu by tapping on the gear icon on the right on the home screen. A screen appears displaying a list of setting options (Figure 2.41).

Depending on the available units, the available menu items may differ (Figure 2.41 A or B).

To open one of the setting menus, touch the appropriate field. Most of the menus are intuitive. The menus requiring additional information are described below.



**Figure 2.41:** Settings menu without (A) or with (B) upfront choice of functional unit.



**Figure 2.42:** System settings screen

### 2.13.1.1 System settings

Tap on “Settings” to open the system settings. Here you can adapt various general settings and also access the software version.

- **Current time:** Set or change the time by tapping on this field.
- **Time format:** Change the time format to 24 hours or AM/PM.
- **Current Date:** Tap on this field to adjust the date
- **Date format:** Tapping on this field allows for setting different date formats
- **Transportation mode:** This field is checked by default and will apply the transportation lock upon shutdown of the system.
- **Brightness:** Use the slider to adjust brightness of the screen.
- **Volume:** Use slider or tap on the speaker icon to adjust or mute sound.
- **Network:** Shows available network connections
- **Version:** Displays software and firmware versions of all connected 4D-Nucleofector® Units.
- **Fill mode** (only available if LV Unit is connected): The “Fill mode” can be adapted when using the flow-through cartridge version, the LV Nucleocuvette® Cartridge. The standard fill mode “1” (default setting) calculates with a slight overfill of the cartridge to compensate for variations in tube diameters and avoid an underfill, as this can lead to arcing when using strong pulses. Since this “overfill” strategy may also lead to some potential dead volume, the filling strategy can be adapted for an individual use case by selecting other fill modes from “2” (moderate reduction of overfill) to “5” (strong reduction of overfill). The user may keep the standard fill mode (1) which safely avoids underfilling and arcing or try different underfill levels to maximize the transfected volume (knowing that this may cause arcing).

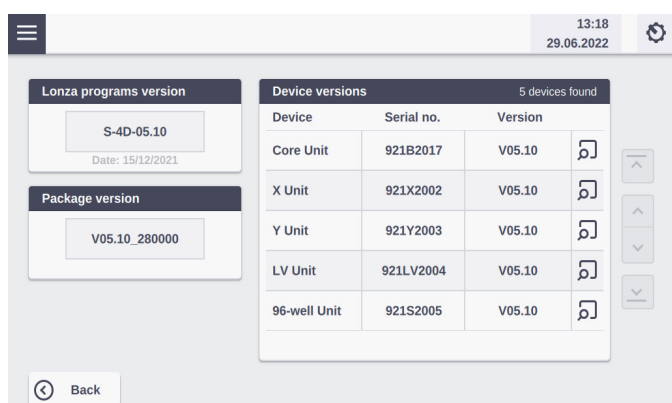
### 2.13.1.2 Cleaning the Nucleofection® Vessel Tray (X, Y, or 96-well Unit)

To allow convenient cleaning, the Nucleofection® Vessel tray can be removed from the X, Y, and 96-well Unit. To dismount the tray please follow the instructions below:

- Open the “Settings” menu after vessel type selection
- Press “Cleaning Mode”.
- 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 sides of the tray.
- Wash the tray with a cloth dampened in water and rinse it with tap water until all leftovers are washed off.
- Dry the tray carefully.
- Remount the dried tray on the support.
- Press “Finish”.
- The drawer will close and the tray will be moved to its home position.

### 2.13.1.3 Version

Tap on “Version” to find out more information about the software and the Lonza program list version. The table “Device Versions” displays the firmware version and the serial number of all functional units. See „2.13.4 Firmware Update” for instructions on updating the firmware.



**Figure 2.43:** The table “Device Versions” displays the firmware version and the serial number of all functional units

### 2.13.2 Left menu

Activate the left menu by tapping on the accordion icon on the left side of the screen (Figure 2.4).



**Figure 2.44:** Accordion menu drop down.

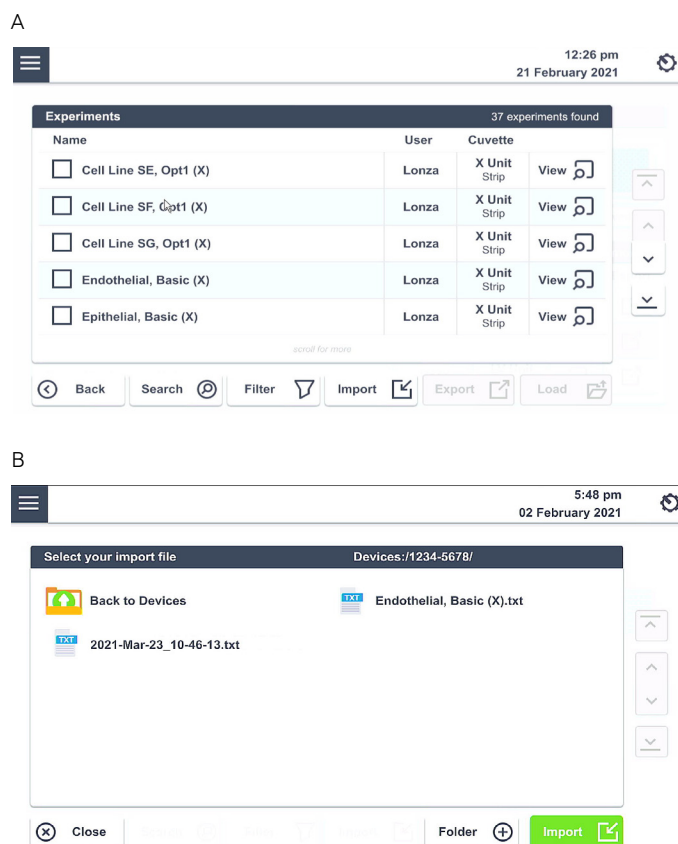
Tapping on home will close this screen and you will return to the home screen (Figure 2.7).

### 2.13.2.1 Experiments

This menu displays a list of all Experiments which are either ready-to-use templates pre-installed by Lonza (i.e. optimization schemes for X, Y, and 96-well Unit only) or previous experiments saved by the user. By pressing “Open” you can access the Experiment setup screen for the respective experiment.

**Experiment import:** The user can also upload an experiment that has been predefined on another system or via the PC Editor Software (see chapter 2.14.2). To load a predefined experiment from a USB drive, insert the drive into the USB port on the front of the Core Unit, press “Import” and follow the instructions displayed on the screen (Figure 2.45, B).

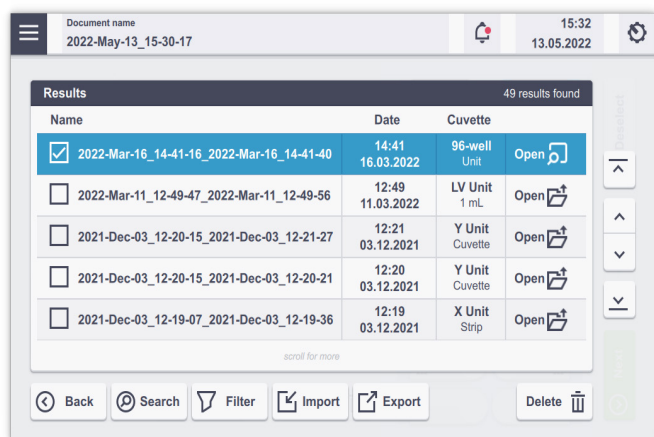
**Experiment export or loading:** Tap “Open” to load experiment or on the checkbox in front of an experiment to export it onto a USB stick. To delete experiments from the list, select the respective experiment and select “Delete”.



**Figure 2.45:** Experiment screen. (A) all available experiments are listed with information on the available cuvette format. Check the box and tap on “Export” to export the experiment file or tap on “Load” to start the experiment. Tap on “View” to see details about the experiment. (B) Tap on “Import” to import an experiment from an USB Stick and follow the instructions.

### 2.13.2.2 Results

This menu will display a list of all generated result files and allow you to manage them. Result files are listed in chronological order with the latest results (date) at the top.



**Figure 2.46:** Result screen. All saved results are displayed. Scroll the list to find the result or use the arrows on the right. Tapping on “View” will display the experiment result.

To open existing result files just select the file you would like to open and press “Open”. To store result files on a USB stick or transfer them into the PC Editor Software (see chapter 2.14.3) for printing, insert the USB device into the USB port on the front of the Core Unit and press “Export” (Figure 2.46).

### 2.13.2.3 Custom Cell type codes

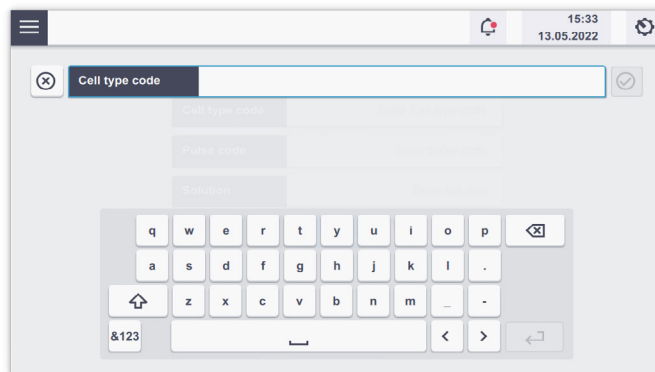
A “Cell type code” encodes a specific combination of Nucleofector® Solution and Nucleofector® Pulse Code. In addition to the available cell type codes that were predefined by Lonza (Lonza optimized conditions), the software offers the option to add custom Cell type code for choosing a defined combination of a Nucleofector® Pulse Code and Solution that has been identified by a user, e.g., after an optimization. Custom Cell type codes are included in alphabetical order and can be distinguished from Lonza defined Pulse codes by color:

Blue: Lonza defined Cell type code  
Black: Customer defined code

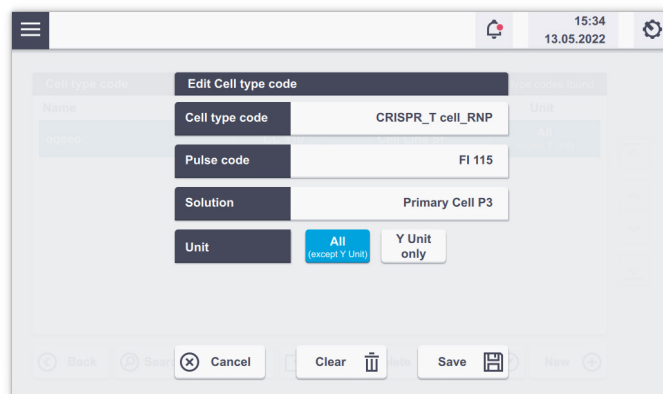
To create a new custom Cell type code or to edit an existing custom Cell type code execute the following steps:

- Press “New” or select an existing Pulse code and press “Edit”.
- A screen will open, allowing to select “Pulse code” and “Solution” field and a “Unit” selection (Figure 2.47, A and B). Alternatively, a new Cell type code can be saved directly from the experiment screen (see unit-specific chapters).

A



B



**Figure 2.47:** (A) Typing the name of the custom Cell Type Code. (B) Overview of conditions for custom Cell Type Code. Press “Save” to save the conditions, press “Clear” to start again.

- Define a name for your custom cell type code 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 “Apply”.
- Select the Nucleofection® Program by typing in the “Pulse code” — you can determine the best Pulse code by using one of our optimization kits or simply use the code from an existing optimized protocol.
- Select the functional units for which the code should apply (either X, 96-well and LV Unit, or Y Unit).
- Confirm the setting for your custom cell type code program by pressing “Save”.
- The custom cell type code can now be selected for an experiment by pressing the “Cell type code” field during experiment setup (Figure 2.15).

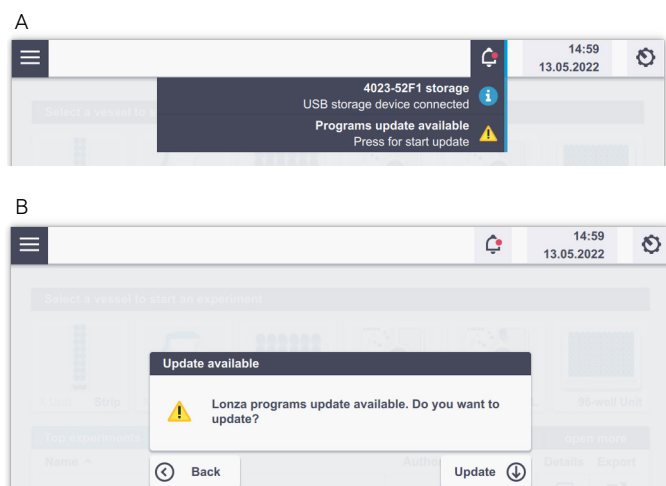
Custom cell type codes will not be overwritten in the event an updated program definition file is installed (see chapter 2.13.3).



### 2.13.3 Lonza programs

The Lonza programs list, comprising all available “Solutions”, “Pulse codes” and “Cell type code” lists can be exchanged independently from a firmware update (see chapter 2.13.4). For uploading a new “Lonza programs list” follow instructions below:

- Insert a USB stick comprising the file you want to upload (\*.pd) at the front or the rear of the Nucleofector® Device.
- The bell symbol at the top of the screen shows a notification (red dot; Figure 2.47A). Tapping on the bell icon shows further information.



**Figure 2.47:** (A) Message upon insertion of USB stick with respective Lonza program file. (B) message after tapping on the program update button.

- Choose “Program updates available” by tapping in the field.
- Tap on “Update”
- The new Lonza program files will be updated and the Nucleofector® Core Unit will shut down. Please restart the system after the update.

### 2.13.4 Firmware update

Due to technical improvements 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 home screen will appear.
- Plug in the USB stick containing the new firmware (as provided by Lonza). The software update will only be recognized if the 4D-Nucleofector® System is switched on.
- The bell symbol at the top of the screen shows a notification (red dot). Tapping on the bell icon shows further information. (Figure 2.48). Please note that the red dot only indicates that a USB stick with a software version is connected to the device. The red dot does not disappear after an update has been performed.

- Follow the instructions on the screen to update the firmware. The Update will be loaded and the Nucleofector® Core Unit needs to be restarted. Each functional unit has to be updated individually. (see chapter 2.13.5)

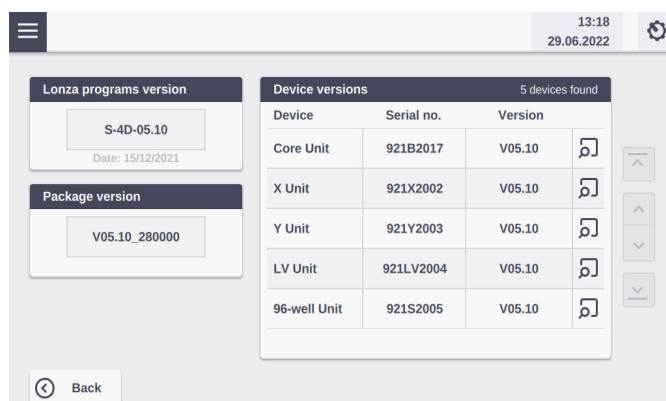
**NOTE:** More comprehensive firmware updates may not be possible via USB upload and require the return of the system to Lonza.

### 2.13.5 Software update of functional units

Click on the “Gear menu” and select “Version” to update the software of a functional unit (Figure 2.42).

Select the magnifying glass icon (Figure 2.49) and press “Install” for the selected software version. Please note that the screen turns black during the upgrade and that it can take up to 30 s until the home screen is available again.

**NOTE:** If newer and older units are combined, the system’s firmware and the software of the functional units may differ. To make sure if an upgrade is advised, please reach out to Scientific Support.



**Figure 2.49:** “Version” shows software version status and serial number of all units.

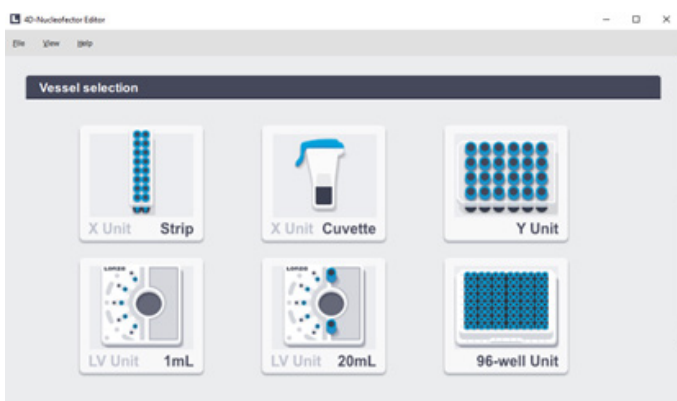
## 2.14 4D-Nucleofector® PC Editor Software

The PC Editor is a PC-compatible version of the 4D-Nucleofector® User interface. It can be used to define experiments on the PC and then transfer them to the 4D-Nucleofector® System via USB stick or view and print result files exported from the 4D-Nucleofector® System. This chapter describes the installation of the 4D-Nucleofector® PC Editor as well as the transfer of experiment files from your PC to the 4D-Nucleofector® System and upload of result files exported from the 4D-Nucleofector® System.

### 2.14.1 Installation of the PC Editor

It is not required to run an installation routine to install the 4D-Nucleofector® PC Editor on your PC. Just follow the steps below to copy the software onto your hard disk drive:

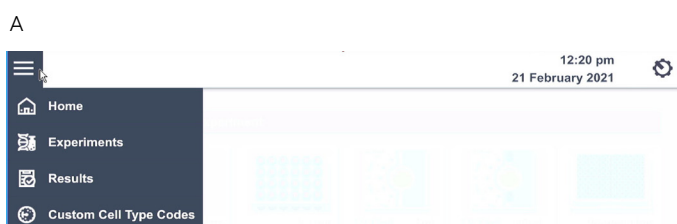
- Insert the 4D-Nucleofector® User information USB stick into the USB port of your PC
- Copy all files from the 4D-Nucleofector® PC Editor software folder to your hard disk – preferably into a folder called “4D-Nucleofector® PC Editor”
- Open the folder, search the file called “4D-Nucleofector® Editor” and double click on it to start the software.
- A window will appear displaying the graphical user interface of the 4D-Nucleofector® PC Editor. The functionality of the PC Editor matches with the operation of the 4D-Nucleofector® Software.
- Use the PC Editor to set up and save your experiments or view and print your result files.



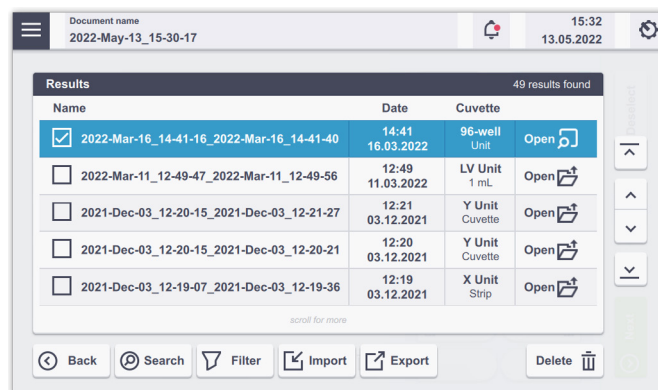
**Figure 2.48:** Graphical user interface of the 4D-Nucleofector® PC Editor

### 2.14.2 Transfer of experiments from the PC Editor

- Plug USB drive into a USB port of your PC.
- Load/Open your newly defined experiment.
- Select “Save” and “Export experiment” and confirm by clicking on the green checkmark.
- Select the desired storage location on the USB drive and wait until the “Export finished successfully” message appears.
- Unplug the USB drive from your PC and turn on the 4D-Nucleofector® System.
- Plug the USB stick with the new experiment into one of the USB ports at the front of the Core Unit.
- Go to “Experiments” via the accordion menu (Figure 2.50) and select and import the required experiment. A message “Import completed successfully” will appear to verify that your new experiment is now saved onto the 4D-Nucleofector® System.



B



**Figure 2.50:** (A) To import experiments go to “Experiments” and (B) select “Import” to transfer pre-defined experiments to the 4D-Nucleofector® System.

### 2.14.3 Viewing and printing result files

To generate a printable result report with the PC Editor the following steps are required:

#### a) Export from 4D-Nucleofector® System

- Plug in a USB stick
- Open the left menu and select “Results”
- Select the result to be printed (Figure 2.50) and press “Export”
- Navigate to the desired folder on the USB stick and press “Export”

#### b) Import into PC Editor

- Start the 4D-Nucleofector® PC Editor
- The “Home” screen will appear
- Open the left menu and select “Results”
- Select “Import” and navigate to the directory where the desired result file has been stored
- Select the desired result and press “Open”.
- The result file is now copied to the PC editor
- Select “View” to view the result “Print” to print the result or “Load” to load another exported result file from the USB stick

# 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® Procedure. For further details, please refer to the dedicated optimized protocol.
	Nucleocuvettes® Vessels were reused	It is strongly recommended to use 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® Experiments 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	Depending on cell type and Nucleofection® Vessel a certain DNA amount per sample is recommended (for details, please refer to the respective optimized protocol). If both, transfer efficiency and cell mortality are low, the DNA amount could be increased. Increasing the amount of DNA may result in increased transfection efficiency but also in increased 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® Experiments should be of high purity. We strongly recommend endotoxin-free preparation of the DNA. Do not use procedures involving phenol /chloroform treatment.
System unable to start	Buffer battery too low to start the system	If the device is not used for longer periods, it will require occasional recharging to avoid battery discharge and ensure functionality. To avoid a deep discharge of the battery and loss of data, switch on the system at least once a month.

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

### Europe

Phone: +49 221 99199 400

E-mail: [scientific.support.eu@lonza.com](mailto:scientific.support.eu@lonza.com)

### North America

Phone: 800 521 0390 (toll free)

E-mail: [scientific.support@lonza.com](mailto:scientific.support@lonza.com)

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

This chapter describes how to rescue your samples from the X or Y Unit in the event of a system failure or power outage.

- Unplug system from the power source.
- Remove the terminator cap or cable connection to next unit (Figure 3.1, A).
- Insert the hexagon screw driver supplied with the 4D-Nucleofector® System in the hole. (Figure 3.1, see arrow)
- Turn the screw manually counter-clockwise until the screw blocks (may require > 60 turns). Turn back clockwise one full turn.

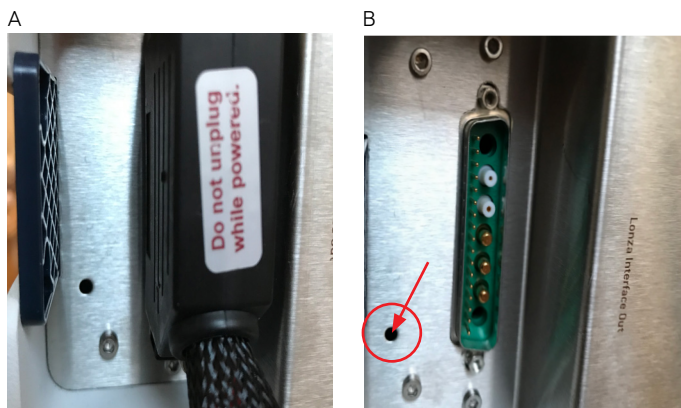


Figure 3.1: (A/B) Rescue of samples

#### Further steps

- Manually open the front flap.
- Carefully pull the tray./
- Remove the Nucleocuvette® Vessels and rescue your cells.
- Contact your Lonza representative to solve potential technical issues.



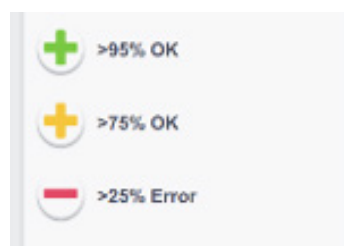
# 4 Error Codes of the 4D-Nucleofector® System

## 4.1 Color coding of wells (X, Y and 96-well Unit)

Well symbols and colors differ depending on the status of the well. In the “Result” screen, the color of a well indicates the completion status of each well (Table 4.1). In case of an error or warning, the respective error code can be displayed by clicking on the well in the result file.

## 4.2 Result rating (LV Unit)

For 4D-Nucleofector® LV Unit experiments no well coding exists. Since a Nucleocuvette® Cartridge consists of several segments symbols are used to provide a summary rating for the whole sample based on the percentage of segments which were pulsed properly (OK) and segments where an error occurred. Following thresholds were empirically defined by Lonza.



## 4.3 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.

**Do not re-use consumables.**

Table 4.1: Color code of wells

	Experiment	Result, OK	Result, Warning	Result, Error	Result. Skipped Well
Undefined					
Sample					
No Program					
No DNA					

## 4.4 4D-Nucleofector® System Error Codes

The 4D-Nucleofector® System detects and reports different types of errors. Some are just error messages, others might be indicated by a code.

The table below explains the result codes (errors or results) that can be indicated in the next generation 4D-Nucleofector® System. For most codes this information is also shown in the software. This list does not contain LV Unit specific error codes yet

Model Code	X Unit	Y Unit	96-well Unit	LV Unit	What Happened?	Possible Error	Procedure
Err 1A	x	x	x	x	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
Err 2	x	x	x	x	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.
Err 3	x	x	x	x	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	x	x	x	x	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.
Err 7	x	x	x	-	No pulse generated	No lid detected on the cuvette or plate	Clear the error message by pressing any button. Check proper placement of the lid and rerun experiment with the same cuvette
Err 8	x	x	x	x	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.
Err 11	x	x	-	-	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 of first generation.
Err 12	x	x	x	-	Pulse possibly omitted. No pulse generated.	Internal device temperature too high for pulsing.	Switch off the device, wait for 2 minutes and run experiment with a new cuvette.
Err 13	x	x	x	-	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	-	x	-	-	Used well: Multiple use of well. Nucleofection® Process 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.

Model Code	X Unit	Y Unit	96-well Unit	LV Unit	What Happened?	Possible Error	Procedure
Err 22	-	x	-	-	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	-	x	-	-	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 24	-	x	-	-	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	-	x	-	-	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	-	x	-	-	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	-	x	-	-	Used well: Well was skipped	User confirmed to skip the well as it had been used before	-

[www.lonza.com/nucleofection-error-codes](http://www.lonza.com/nucleofection-error-codes)

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

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E-mail: [scientific.support@lonza.com](mailto:scientific.support@lonza.com)

# 5 Purchaser Notification

## Purchaser notification

The purchase of the 4D-Nucleofector® System (the “Purchased Device”) conveys to the buyer a limited non-transferable, non-exclusive license (the “License”) for the use of the Purchased Device and Lonza’s proprietary Nucleofector® Process and methods (the “Licensed Process”) without the right to sublicense.

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## 6 Technical Specifications

Technische Daten, Specifications, Spécification	
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 / environment	+15°C to +40°C, (59°F - 104°F) non-condensing
Transfektionen pro Minute / transfections per minute / transfections par minute Nucleocuvette® Strips)	<b>X Unit:</b> 16–32 reactions using 16-well Nucleocuvette® Strips 20–26 reactions using 100 µL Nucleocuvette® Vessels
	<b>Y Unit:</b> 8–10 reactions using Dipping Electrodes
	<b>LV Unit:</b> 2–3 mL using LV Nucleocuvette® Cartridges
	<b>96-well Unit:</b> 32–44 reactions using 96-well Nucleocuvette® Plate
Schnittstellen / interfaces / interface	USB network connection
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 × h × d)	Core Unit: 4.4 kg (9.7 lb) 24.5 x 14.5 x 28 (cm)/ 9.7 x 5.7 x 11(in)
Gewicht / weight / poids	X Unit: 4.7 kg (10.4 lb) 24.5 x 10.5 x 28 (cm)/ 9.7 x 4.1 x 11 (in)
	Y Unit: 5.0 kg (11.0 lb) 24.5 x 10.5 x 28 (cm)/ 9.7 x 4.1 x 11 (in)
	LV Unit: 5.7 kg (12.5 lb) 24.5x 20.7 x 28 (cm)/
	96-well Unit: 4.7 kg (10.4 lb) 24.5 x 10.5 x 28 (cm)/ 9.7 x 4.1 x 11 (in)/ 9.7 x 8.1x 11
Hersteller / supplier / manufacture	Lonza Cologne GmbH Nattermannallee 1 D-50829 Cologne, Germany
Herstellungsdatum / date of manufacture / date de production number	The manufacturing year is encoded by the second and third digit of the serial number, e.g., serial number x16xxxxx was manufactured in 2016.

## Contact Us

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Brazil	+55 11 4028 8000
China	+86 21 6305 8866
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 124 6052941
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)

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