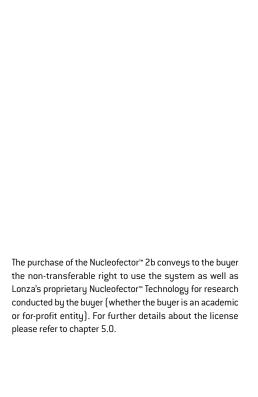
Lonza

Nucleofector™ 2b Manual For Research Use Only





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1 The Nucleofector™ Technology

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

How it works

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

Transfection of any cell

With over 160 protocols optimized by Lonza's R&D team for cell lines and primary cells and more than 690 entries in the online cell database, Nucleofector™ Technology is clearly the transfection method-of-choice for any difficult-to-transfect cell type.

Transfection of any substrate

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

The Nucleofector™ 2b depicts an excellent tool for transfection of hard-to-transfect cell lines and primary cells. As the device uses standard aluminum cuvettes transformation of bacteria is also possible. Based on the well established Nucleofector™ Technology this systems comes with 160 optimized protocols for various cell lines and primary cells. For further details visit our online database.



www.lonza.com/cell-database

The Nucleofector™ 2b Device



If no Optimized Protocol for your cell line of interest is available, we offer a Cell Line Optimization Nucleofector™ Kit enabling you to easily adapt the Nucleofector™ Technology for your specific cell line.



For fine-tuning after the optimization process, please contact our Scientific Support Teams

Europe

Phone +32 87 321 611

E-mail scientific.support.eu@lonza.com

North America

Phone +1 800 521 0390 (toll free) E-mail scientific.support@lonza.com

2 Operating Instructions

2.1 Restrictions

Medical use restrictions

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

License statement

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

Purchasers are granted a non-exclusive, non-transferable license for a limited use of Lonza's Nucleofector™ Technology and related software for research purposes, the terms of which are disclosed in detail in chapter 5.0. Certain commercial application is allowed under Lonza's license for for-profit-entities.



For license information contact Lonza Cologne GmbH:

Phone +32 87 321 611 E-mail ip.cologne@lonza.com

2.2 Maintenance

The Nucleofector™ 2b Device requires very little maintenance to assure reliable operation. To clean and disinfect the case, first unplug the power supply. Use a damp cloth to wipe down the outer case (water or 70-80% ethanol). Avoid wetting the cuvette holder within the cuvette carousel of the device.

The Nucleofector™ 2b Device has been designed for use under a sterile hood with or without UV radiation source. Prolonged exposure of the outer casing to UV light will lead to discoloration with no functional impairment of the Nucleofector™ Device. When laminar flows are sterilized on a regular basis by UV radiation overnight we recommend protection of the device by appropriate shielding or removal during extended UV exposure.

The Nucleofector™ 2b Device is protected by two main fuses. Both are inside a receptacle incorporated in the inner power socket (see Figure 2, page 8). In case of a blown fuse, you can easily replace it. Disconnect the Nucleofector™ 2b Device from power and insert a small flat screwdriver into the slot on the right-hand side of the receptacle to pull it open. To function, both the upper and lower part of the receptable must hold working fuses in the inner positions. Blown fuses can usually be identified by molten interrupted wires inside the glass tube. Only use T630mA or L250V fuses to substitute 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 Nucleofector™ 2b Device has been certified by international safety standards and is safe to use when operated in accordance with this manual.

This device is designed to deliver variable high voltage electrical impulses for the purpose of introducing nucleic acids into eukaryotic and prokaryotic cells.

These electrical impulses can be deadly.

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

- Only use the device once you have read and understood the Nucleofector™ 2b Manual. The manual should be accessible for all users. Make sure that each potential user reads and understands it.
- Do not open the device. The device does not contain user-serviceable parts. Under no circumstances should circuit components be interfered with, as they can deliver an electric shock even when system is not in operation.
- Do not alter the device in any manner.
- Do not obstruct the movement of the carousel of the Nucleofector™
 Device
- Only use the device when it is set on top of a safe, plain and stable table or bench.
- Place the device such that easy removal of the power cord is possible at any time.
- Do not expose the device to a humid environment.
- The device shall not be exposed to direct sunlight nor be placed in a hot environment.
- The device is not approved for use in fire or explosion endangered areas, nor for use with inflammable or explosive media.
- Employ precautions against great impact and vibration in moving and transporting the Nucleofector™ 2b Device.
- Use the device with Lonza's certified Nucleofector™ Solutions and Lonza certified cuvettes only. Use of any other solution or cuvettes from any other source than Lonza will preclude all warranty and liability claims.

- Standard Nucleofector™ Solutions and 96-well Nucleofector™ Solutions respectively 4D-Nucleofector™ Solutions or HT-Nucleofector™ Solutions are not compatible. Please do not use the Nucleofector™ 2b Device with these solutions. Unpack the cuvettes just prior to the experiment. Make sure that the outer contact areas are dry.
- If any fluid has been spilled in the close vicinity of the Nucleofector™ 2b
 Device, safety may be compromised. Ensure that no fluid has been in contact with or entered the device.
- If any fluid has been spilled at the device, the safety may be compromised. To confirm that the use of the device is safe, contact Lonza Scientific Support for actions or precautions.
- Never place any foreign object on the device to avoid the risk of equipment damage.
- Do not enter or place foreign objects in the carousel area of the Nucleofector™ 2b Device.
- If any foreign object has entered the Nucleofector™ 2b Device, safety may be compromised. To confirm that the use of the device is safe, contact Lonza Scientific Support for actions or precautions.
- If the Nucleofector™ 2b Device has been damaged, ensure that the device can not be used by any personnel and contact Lonza Scientific Support for assistance.
- All service shall be performed by Lonza authorized personnel only.
- Handling of device parts that has the possible risk of sample contamination shall always be performed with protective gloves and any disposal of such parts must be according to Federal, state or local procedures for clinical waste handling and disposal. Use secure leak proof containers and avoid unprotected handling of such parts.

Lonza Cologne disclaims all warranties and shall in no event be liable for any kind of damages caused by or arising out of any operation or use in violation of the above safety and handling instructions.

2.4 Waste Disposal

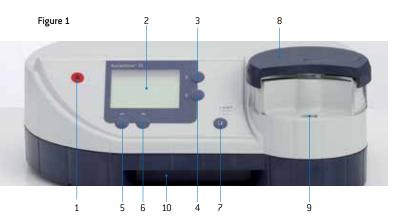
Disposal of consumables from Nucleofector™ Kits (cuvettes, pipettes and Nucleofector™ Solutions): Discard cuvettes, pipettes and expired Nucleofector™ Solution residuals in a biohazard container. Refer to your local waste management organization and to the relevant laboratory safety instructions for proper disposal practices.

2.5 Nucleofector™ 2b Device Components

The Nucleofector™ 2b Device is delivered with the following components:

- 1 Nucleofector™ 2b Device
- 1 Power cord
- 1 Nucleofector™ 2b Manual

Front panel of the Nucleofector™ 2b Device



- 1 Power button
- 2 Graphic display
- 3 "Up" button
- 4 "Down" button
- 5 Functional button "menu"
- 6 Functional button "options"
- 7 "X" button with start, exit or enter function
- 8 Automatic cuvette carousel
- 9 Cuvette holder
- 10 Chipcard slot

Rear side of Nucleofector™ 2b Device



- 11 Fuse
- 12 Power cord receptacle

2.6 Set-up Instructions

- 1. Remove all packing material.
- 2. Attach the power cord to the receptacle on the rear side of the Nucleofector™ 2b Device (12) and plug it into an appropriate electrical outlet. The device will automatically be in the stand-by mode. Turn on the device by pressing the power button in the upper left corner of the front panel (1). The device performs a self test accompanied by a short intro in the graphic display (2). After powering up, the display shows the main menu "Free Program Choice" (Figure 3.). The start mode can be changed to the last program used as described in chapter 2.8.5.

Figure 3



2.7 Instructions for Using the Nucleofector™ 2b Device

2.7.1 Main menu and program execution

The Nucleofector™ 2b Device offers two separate ways for program selection. Programs can either be chosen by direct selection of the program code, e.g., A-023, in the "Free Program Choice" mode (Figure 3). As a convenient alternative, programs can also be selected by cell type code using the "Cell Type List" (Figure 4). This list is divided in three consecutive sections:

- 1. All programs from currently available Optimized Protocols are filed under the specific cell type name in alphabetical order (Figure 4).
- This alphabetical list for eukaryotic cells is followed by a list of programs for the transformation of bacteria. For details please contact Lonza's Scientific Support Teams and ask for the latest developments and experimental support.
- 3. The last section comprises custom programs (see 2.7.4.) in chronological order. In addition, custom programs can be found in the "Custom Programs" mode. You can switch program modes by pressing the functional button "menu" (5) several times until the required program mode appears and confirm by pressing the functional button "go" (6).

Note

Only custom programs can be stored or deleted by the user. The program codes of the Nucleofector™ 2b Device consist of two parts: a one-digit alphabetical code followed by a three-digit numerical code, e.g., U-025 (Figure 4).

| Mode | Program can be | |
|-----------------------------------|----------------|---------|
| | edited | deleted |
| "Free Program Choice" | Yes | No |
| "Cell Type List" (pre-programmed) | No | No |
| "Custom programs" | Yes | Yes |

2.7.2 Selection of Nucleofector™ Programs by program code — "Free Program Choice"

- Turn on the Nucleofector™ 2b Device by using the power button (1).
 Nucleofector™ 2b Device starts in the "Free Program Choice" mode (Figure 3). If it does not start in the "Free Program Choice" press the functional button "menu" (5) repeatedly until "Go to Free Program Choice" appears and confirm this pressing the functional button "go" (6).
- 2. The alphabetical first digit blinks. Choose the appropriate letter of the program code by using the "Up" and "Down" buttons (3,4). Then move the cursor with the functional buttons "<<" and ">>" (5,6) to the numerical three-digit code. Select the appropriate number of the program code with the "Up" and "Down" buttons (3,4). Continual pressing of the "Up" and "Down" buttons allows for fast scrolling of choices. Your Nucleofector™ 2b Device is now ready for program execution (see 2.7.5).
- 3. Exit this mode by pressing the "X" button.

2.7.3 Selection of Nucleofector™ Programs by name of cell type – "Cell Type List"

- Turn on the Nucleofector™ 2b Device by using the power button [1]. If
 the Nucleofector™ 2b Device starts in the "Free Program Choice" mode
 (Figure 3), exit this mode by pressing the "X" button (7). Press the
 functional button "menu" (5) several times until "Go to Cell Type List"
 appears and confirm this choice by pressing the functional button
 "go" (6).
- 2. Choose the appropriate program for your cell type (Figure 4) by navigating through the pre-programed list using the "Up" and "Down" buttons (3, 4). The programs are displayed in alphabetical order. The list is organized by first the cell name (e.g., T cells), descriptions of the species and further details of the cell type are given in line three. Line five shows the corresponding program itself. In cases where two or more programs are available, the fourth line indicates the specificity of the program such as for higher transfection efficiency or for higher cell viability. Your Nucleofector™ 2b Device is now ready for program execution (see 2.7.5).

Figure 4



2.7.4 Selection and storage of Nucleofector™ Custom Programs

The Nucleofector™ 2b Software provides the option to add programs to the Cell Type List by storing the appropriate program under a new individual name. In this way information about an optimized program for a particular cell type can be stored directly in the Nucleofector™ 2b Device.

- Turn on the Nucleofector™ Device by using the power button (1).
 If the Nucleofector™ 2b Device starts in the "Free Program Choice" mode (Figure 3), exit this mode by pressing the "X" button (7).
- Press the functional button "options" [6]. Select "new" from the popup menu by using the "Up" and "Down" buttons [3, 4] and confirm with "X" button [7]. The menu jumps to a free custom program memory location (Figure 5).

Figure 5



- 3. The cursor blinks at the starting position. Scroll through the character set by using the "Up" and "Down" button (3,4) to enter the desired text and move to the consecutive character positions with the functional button ">>" (6). By using the functional button "<<" (5) the cursor moves one step back and deletes the entered character.
- 4. After entering the desired name for the program, confirm the name by pressing the "X" button [7]. The cursor automatically jumps to the program code position. Then choose the appropriate letter of the program code, e.g., C-013 by using the "Up" and "Down" buttons [3,4]. Then move the cursor with the functional button ">>" [6] to the numerical three-digit code. Select the appropriate program number with "Up" and "Down" buttons [3,4]. In order to save the custom program press the "X" button [7] and choose "yes" in the arising window by pressing the left functional button [5]. Exit and save by pressing the "X" button [7].
- 5. Saved custom programs can be selected by navigating through the "Cell Type List" using the "Up" and "Down" buttons (3, 4). Custom programs are listed after the pre-programmed cell-type list, in chronological order. Additionally, for faster access the programs are listed separately in the "Custom Programs" mode.

6. In order to modify or delete a saved custom program, navigate to the corresponding program in the "Cell Type List" or "Custom Program" mode using the "Up" and "Down" buttons (3, 4) and subsequently press the functional button "options" (6). Move to "Edit" or "Delete" in the popup menu (Figure 6) using the "Up" and "Down" buttons (3, 4) and select it with the "X" button (7). In case you want to edit your program, you may now modify any position of the custom program entry by the procedure described above for custom program entry. To leave the popup menu without any action press the functional button "options" (6) again.

Figure 6



- 7. Leave the edit or delete mode, save the changes, and return to the main menu by pressing the "X" button (7).
- 8. Your Nucleofector™ 2b Device is now ready for program execution [see 2.7.5].

2.8 Additional Nucleofector™ 2b Settings and Options

2.7.5 Program execution

After you have chosen the correct program, place the closed cuvette filled with 100 μL Nucleofection Sample in the cuvette holder (9). For program execution press the "X" button (7). The cuvette carousel (8) closes automatically (see 2.7.2) and the program is executed. Subsequently, the carousel automatically rotates back to reveal the cuvette. "OK" will appear on the display if the program has been successfully completed. After addition of pre-warmed medium (see Optimized Protocols for further details) remove the Nucleofection Sample from the cuvette with the plastic pipette provided in the Nucleofector™ Kit. Discard cuvettes after one use.

Note

Only use fresh Lonza certified cuvettes, which have passed rigorous quality tests. Use of both uncertified cuvettes and re-use of Lonza certified cuvettes (after even one use), impairs experimental results, proper function of the device and risks damaging of the Nucleofector™ 2b Device itself.

- 2. Press the "X" button (7) to acknowledge. The device can be re-used immediately after the last program execution.
- 3. In case the device was unable to execute a program or the program was not completed successfully, the display will indicate the error type and error code. For more information on specific errors, see chapter 3
- 4. To continue working, press the "X" button (7) to acknowledge the error message. The device can then be re-used immediately.
- 5. When you have finished working, please remove the last cuvette from the cuvette holder (9) and switch off the Nucleofector™ 2b Device by pressing the power button (1).

Note

The Nucleofector™ Programs available correspond to the list of Optimized Protocols that are provided with the Nucleofector™ Kits which can be downloaded at www.lonza.com/protocols. If the Nucleofector™ Program you want to apply is not available on your device, please contact the Lonza Scientific Support Team to receive a chipcard to update the program software.

2.8.1 Change of standard settings

In order to change the settings of your Nucleofector[™] 2b Device, press the functional button "menu" (5). By repeatedly pressing the button "menu" (5) you will first pass the three program mode options "Go to Free Program Choice", "Go to Cell Type List" and "Go to Custom Programs" and then be guided through the settings. To return from any sub-menu to the main menu press the "X" button (7).

2.8.2 Motor

The cuvette carousel (8) is connected to a motor which enables automatic movement of cuvette into position by closing and opening the cuvette holder carousel. In the menu settings you can choose between "on" for fully automated turning or "off" for manual opening and closing.

Notes

- In case you have switched off the motor option in the menu, you will
 have to rotate the carousel manually by 180° clockwise. The carousel
 must be turned completely to the blocked position so that the cuvette
 contacts the electrodes.
- For safety reasons, the motor of the Nucleofector™ 2b Device is equipped with a sensitive blockage detection. If closing of the carousel is hindered, e.g., by an inserted finger, the motor stops immediately and returns to its starting position. The display shows the corresponding error code (Err2 = Inappropriate or no cuvette or carousel not closed). Continue working by pressing the "X" button (7) to exit the error mode.

2.8.3 Display contrast

In order to optimize readability of the display, change of the display contrast is possible by pressing the "Up" and "Down" buttons (3,4). The change has to be confirmed by pressing the functional button "set" (5). By leaving the submenu without pressing "set", the change is reversed in order to reduce the risk of modifying the contrast in such way that you may have difficulties reading the display.

2.8.4 Beep on/off

The beep following the switch-on of the device and erroneous program execution can be inactivated by choosing "off" with pressing the "Up" and "Down" buttons (3,4). The default setting is "on".

2.8.5 Change of start mode

The Nucleofector™ 2b Device can either start in the "Free Program Choice" mode or with the last program used. Choose the appropriate option by pressing the "Up" and "Down" buttons (3,4). The default setting is "Free Program Choice".

2.8.6 Language

The Nucleofector™ 2b Device offers a language option. The default setting is English. It can be switched to Japanese (or back to English) by pressing the "Up" and "Down" buttons (3,4). The change has to be confirmed by pressing the functional button "set" (6). By leaving the submenu without pressing "set", the change is reversed in order to reduce the risk of modifying the language by mistake.

2.8.7 Software version

Press the functional button "menu" (6) to view the current software version of the Nucleofector™ 2b Device that comprise the operating programs of your Nucleofector™ 2b Device (for updating the program software see 2.8.8).

2.8.8 Upgrading program software

- In order to upgrade the program list on your Nucleofector™ 2b Device, insert the chipcard with the novel software version provided by Lonza into the chipcard reader slot (10) (Figure 7).
- After the Nucleofector™ 2b Device recognizes the chipcard, confirm loading of the novel Nucleofector™ Software by pressing the "X" button (7).

Figure 7



- 3. The upgrade starts with a program table. Confirm by pressing the "X" button (1).
- 4. Secondly, the list of optimized programs is loaded. Confirm again with the "X" button (1). The stored custom programs will be neither deleted nor overwritten by upgrading novel non-custom optimized programs.
- Confirm the software upgrade and return to the main menu by pressing the "X" button (1) and remove the chipcard from the chipcard reader slot.

2.8.9 Serial number

Press the functional button "menu" (6) to view the serial number of your Nucleofector™ 2b Device.

3 Troubleshooting

The following troubleshooting guide may be helpful if experiments using the Nucleofector™ 2b Device do not work as expected. The listed comments are intended to help optimize experimental conditions.



Should you have any questions regarding the Nucleofector™ 2b Device, please do not hesitate to contact Lonza's Scientific Support Team

Europe

Phone +32 87 321 611 scientific.support.eu@lonza.com www.lonza.com/researchsupport

USA

Phone +1 800 521 0390 scientific.support@lonza.com www.lonza.com/researchsupport

| Problem | Possible error | What happened? | |
|-------------------|--|---|--|
| Low survival rate | Cells remained too long in the cuvette. | To avoid damage to the cells, transfer cells immediately into pre-warmed medium (37°C). We recommend removing the sample from the cuvette directly after Nucleofection. | |
| | Cells are damaged by harvesting or through handling. | Avoid severe conditions during harvesting, especially centrifugation at higher speed and overexposure to trypsin Pipette cells smoothly. Use a plastic pipette provided in the kit for removal of cells from the cuvette. Add pre-warmed medium to the cuvette before removal of the cells. | |
| | Cells are stressed by culture conditions. | Cells should be viable and in culture for a certain number of passages. Freshly thawed cells should not be used for Nucleofection. Avoid excessive cell densities or cell confluencies since this may negatively influence the viability of the cells after Nucleofection. For detailed recommendations on passage number, cell density and confluence please refer to the individual Optimized Protocol. | |
| | Cells are stressed by centrifugation. | Centrifuge at lower speed (90×g). | |
| | Multiple use of cuvettes. | We strongly recommend using cuvettes only once, because the electric pulses that are applied drastically reduce their quality and impair their physical integrity. | |
| | Poor DNA quality. | Plasmid DNA used should be of high purity. We strongly recommend the use of high quality products for plasmid purification such as QIAGEN Endo Free" Plasmid Kits. Do not use procedures involving phenol or chloroform treatment. | |
| Low gene transfer | Plasmid amount is too low. | We recommend a plasmid amount between 1—5 μg DNA per sample. If both gene transfer efficiency and cell mortality are low, the plasmid amount can be increased up to 10 μg per sample. Increasing the DNA amount may lead to higher gene transfer efficiencies but at the same time may result in higher cell mortality. | |
| | High cell confluency/density. | Gene transfer efficiency into many cell types is poor if the cells are too dense at the time of harvest. Please follow guidelines in the Optimized Protocols. | |
| | Too high or too low cell number in the cuvette. | We recommend using $1\times10^6-5\times10^6$ cells per sample for cell lines and $2\times10^5-5\times10^6$ cells per sample for primary cells. Please refer to the Optimized Protocol for specific details. | |
| | Poor DNA quality. | Plasmid DNA used should be of high purity. We strongly recommend the use of high quality products for plasmid purification such as QIAGEN Endo Free* Plasmid Kits. Do not use procedures involving phenol or chloroform treatment. | |

4 Nucleofector™ 2b Device Error Codes

The following guide indicates possible Nucleofector™ Error Codes and provides suggestions to solve the problem on your own.



Should these suggestions not resolve the problem, please call Lonza's Scientific Support Team

Europe

Phone +32 87 321 611

E-mail scientific.support.eu@lonza.com

North America

Phone +1 800 521 0390 (toll free) E-mail scientific.support@lonza.com

Internet

www.lonza.com/researchsupport

If the Nucleofector™ 2b Device has to be returned for repair, please contact our Scientific Support Team for shipping and warranty instructions.

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 Nucleofector™ 2b Device is equipped with a hardware safety that immediately detects arc formation at its beginning to protect the cells from damage. After the arc interruption the Nucleofector™ 2b Device resumes program execution. Normally, the program can be completed successfully ("arc discharge correction 1 or 2") and only limited differences in transfection efficiency can be observed. When repeated arc discharges within one program occurs, it might be impossible for the Nucleofector™ 2b Device to complete program execution. In this case ("Err8 - Arc discharge") significant impacts on transfection efficiency might be detected. Discard the possibly damaged cuvette and its contents, reset the device by pressing the "X" button (1), and repeat the experiment with a new cuvette. It is not necessary to switch off the Nucleofector™ 2b Device.

| | | | _ |
|---|--|---|--|
| Code | Possible error | What happened? | Procedure |
| Arc discharge correction 1 or 2 | Inappropriate cuvette or Nucleofector™ Solution or inappropriate volume in the cuvette or device possibly defective. | Device possibly generated an arc discharge. Program was automatically resumed and successfully completed. A limited impairment on efficiency and vitality has to be presumed. | Reset device by pressing any button. Utilize sample if maximum efficiency is not essential or try again with a new sample. For further samples check if the volume in the cuvette is 100 µl. |
| WEAK – Re-used cuvette or inappropriate solution | Re-used cuvette or inappropriate Nucleofector™ Solution. | A weak pulse occurred. Only sub-optimal efficiency might be achieved. | Acknowledge the error message by pressing any button; utilize the sample if maximum efficiency is not essential. Otherwise, try a new sample. |
| Err1A – Internal communication failure | Internal error or device possibly defective. | No pulse generated. | Acknowledge the error message by pressing any button; try to apply program a second time. If Err1A re-occurs, switch off the device, wait for 2 seconds and switch on again. |
| Err1B — Internal communication failure | Internal error or device possibly defective. | Pulse generated with unclear outcome. | Acknowledge the error message by pressing any button; utilize sample if maximum efficiency is not essential. Otherwise, try with a new sample. If Err1B re-occurs, switch off the device, wait for 2 seconds and switch on again. |
| Err2 – Inappropriate or no cuvette or carousel not closed | Inappropriate or no cuvette. Inappropriate Nucleofector™ Solution or inappropriate volume. Carousel motor is switched off. | No pulse generated. | Check cuvette. Check type and volume of Nucleofector" Solution. Cuvette can be re-applied. If carousel is not closed, close it manually or change motor option to "on" (see 2.7.2). |
| Err3 – Device possibly defective | Device overheated or possibly defective. | Very weak pulse generated. | Switch off the device. Wait for 2 minutes, switch on again; repeat the program with a new sample. |
| Err7 — Supply voltage too low | Supply voltage insufficient or device has been switched off for a long time. | No pulse generated. | Acknowledge the error message by pressing any button. Try again with a new cuvette. |
| Err8 – Arc discharge | Inappropriate cuvette or Nucleofector™ Solution or inappropriate volume in the cuvette or device possibly defective. | Device possibly generated an arc discharge leading to incomplete program execution. A substantial impairment on efficiency and viability has to be presumed. | Acknowledge the error message by pressing any button. Check the volume of Nucleofector™ Solution. Try again with a new cuvette. |
| Err9 – Inappropriate solution, volume or cuvette | Inappropriate cuvette or Nucleofector [™] Solution or volume or device possibly defective. | Device possibly generated an overcurrent. | Acknowledge the error message by pressing any button. Try again with new cuvette. |
| Err11 – DSP error | Internal error or device possibly defective. | Pulse possibly omitted. | Switch the device off and on to reset. Try again with a new sample. |
| Err12 — Motor error | Carousel motor failure. | Motor blocked, or motor control communication defective, or device possibly defective. | Acknowledge the error message by pressing any button. Try again. |

5 Purchaser Notification

The purchase of the Nucleofector™ 2b conveys to the buyer a nontransferable, non-exclusive license without the right to grant sublicenses limited for the use of Lonza's proprietary Nucleofector™ Process and Method.

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6 Appendix

| | Technical data | |
|--------------------|--|--|
| Power supply | 100–240 VAC 50–60 Hz self-regulating | |
| Power consumption | 50 VA | |
| Fuse Protection | T630 mA, L250V | |
| Temperature range | +15°C to +40°C, non-condensing | |
| Altitude | < 2000 m above sea level | |
| Safety class | EN 61010-1 UL 61010A-1 IP 20 | |
| Weight | 2.8 kg 6.2 lb | |
| Dimensions (w×d×h) | 30×23×11 cm 11.8×9.6×4.3 in | |
| Manufacturing date | uring date The manufacturing year is encoded by the second and third digit of the serial number, e.g., serial number x11xxxxxx was manufactured in 201 | |

| Notes |
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For research use only. Not for use in diagnostic procedures.

Manufacturer and distributor information: Nucleofector™ 2b Device is manufactured by Lonza Cologne GmbH, Nattermannallee 1, 50829 Cologne, Germany and distributed in the US by Lonza Walkersville, Inc. (8830 Biggs Ford Road, Walkersville, MD 21793).

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CD-MN029-1 03/15

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