BioResearch

Lonza

Amaxa™ HT Nucleofector™ hardware manual

For research use only

The purchase of the HT Nucleofector™ conveys to the buyer the non-transferable right to use the system as well as Lonza's proprietary Nucleofector™ technology only 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 5.0.

Content

1 The Amaxa™ Nucleofector™ technology

Since its introduction in 2001, Amaxa™ Nucleofector™ technology has transformed transfection. Nucleofection™ has proven to be a reliable and reproducible easy-to-use technology that is suited to a wide variety of applications and provides an answer to almost every transfection challenge. 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.

How it works

Nucleofector™ technology is based on two unique components: the Nucleofector™ system, which 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 for cell lines and primary cells optimized by Lonza's R&D team and more than 680 entries in the online cell data base, Nucleofector™ technology is clearly the transfection method-of-choice for any difficult-to-transfect cell type. Moreover, Nucleofection™ is the only electroporation based technology allowing reliable transfection of adherent cells and cells in suspension.

Transfection of any substrate

Nucleofector™ technology offers high flexibility within applications, since the same transfection parameters apply for almost all substrates. DNA vectors, RNA duplexes, and peptides can be transfected using our sophisticated transfection protocols. This makes Nucleofection™ the 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.

www.lonza.com/cell-database

The HT Nucleofector™ system

The HT Nucleofector™ — designed for high-throughput approaches

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

Automation-compatible – full integration into liquid handling platforms
Fast – process 384 samples in one minute
Screening-compatible – low cell number required per sample

For fine-tuning of the optimization process, please contact our 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. Note that the Nucleofector™ technology is not intended to be used for diagnostic purposes, or for testing or treatment in humans.

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For license information contact Lonza Cologne GmbH:
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2.2 Maintenance

The HT Nucleofector™ system requires minimal maintenance for reliable operation.

To clean the plate handler, switch off the power supply and use a damp cloth to clean the outer casing with water or 70% ethanol.

NOTE: Avoid wetting the 384-well Nucleocuvette™ plate carousel, especially the surface of the retainer and the connectors located on the left side of the device.

To remove dust from the HT Nucleofector™ power supply, switch off the device and remove the power cable from the power inlet. Use a dry cloth to remove dust from the surface of the power supply.

NOTE: Do not use any liquid for cleaning the power supply. Liquids entering the unit may cause severe damage.

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 HT Nucleofector™ system has been certified by international safety standards and is safe to use when operated in accordance with this manual.

Only use the HT Nucleofector™ power supply in connection with the HT Nucleofector™ plate handler. Under **no** circumstances should it be connected to any other device delivering high-voltage electrical impulses. The system is designed to deliver variable high-voltage 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 and understood the HT Nucleofector™ manual. The manual should be accessible to all users. Make sure that each potential user reads and understands it.
- Set up the system in a dry place. Avoid spilling liquid onto or into the HT Nucleofector™ power supply or the HT Nucleofector™ plate handler. Do **not** use the system if any components are wet.
- Place the HT Nucleofector™ system components on top of a safe, level and stable table or bench.
- Before connecting/disconnecting any of the interface cables between the HT Nucleofector™ plate handler and the HT Nucleofector™ power supply, ensure that the HT Nucleofector™ power supply is switched off and the power cable is removed from the power inlet.
- Prevent sudden impacts and vibration while moving and transporting system components.
- Only use HT Nucleofector™ devices in conjunction with the HT Nucleofector™ software.
- Standard Nucleofector™ solutions and the Nucleofector™ solutions used for the HT Nucleofector™ are not compatible.
- Do not use solutions or plates from any source other than Lonza.
- Unpack the 384-well Nucleocuvette™ plates immediately before the experiment. Make sure that the outer contact areas are dry.
- Always place the lid onto the 384-well Nucleocuvette™ plate before transferring it to the HT Nucleofector™ plate handler.
- Safety may be compromised if any fluid has been spilled in the close vicinity of the HT Nucleofector™ system. Ensure that no fluid comes into contact with or enters the system components. If any fluid has been spilled in the close vicinity of or onto the HT Nucleofector™ plate system, contact Lonza scientific support for advice on precautions to be taken before further use.
- Do **not** open the device housing. 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 device in any manner.
- Do **not** use the device if the insulation of the high voltage cable connecting the plate handler and the power supply is damaged.
- Do **not** use the device if any of the housing parts or covers are missing.
- Do **not** detach any of the housing parts or covers.
- Do **not** expose the device to a humid environment.
- Do **not** expose the device to direct sunlight or place the instrument in a hot environment.
- Under **no circumstances** plug the high-voltage cables of the HT Nucleofector™ plate handler into external electrode sockets of any device other than the HT Nucleofector™ power supply. Use of a device from any source other than Lonza will invalidate all warranty and liability claims.
- Do **not** use lids other than those provided with the 384-well Nucleofector™ kits as the system is calibrated to the supplied lids. Other lids may cause damage to the system.
- Do **not** obstruct any moving parts, e.g. the plate carousel of the HT Nucleofector™ plate handler.
- Do **not** move the HT Nucleofector™ plate handler if a 384-well Nucleocuvette™ plate is inside the device. Moving the plate handler may cause sample fluid from the plate to spill into the device.
- Never place any foreign object onto the device or onto the plate carousel. If a foreign object has entered the HT Nucleofector™ plate handler, the safety of the device may be compromised. Contact Lonza scientific support for advice on precautions to be taken before further use.
- The device is not approved for use in fire- or explosion-endangered areas, or for use with inflammable or explosive media.
- If any component of the HT Nucleofector™ system has been damaged, ensure that the HT Nucleofector™ system cannot be used (e.g. by disconnecting the HT Nucleofector™ power supply), and contact Lonza scientific support for assistance.
- Service shall only be performed by personnel authorized by Lonza.
- Handling of device parts that may be contaminated by samples 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.

Note: Lonza disclaims all warranties expressed or implied 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 safety and handling instructions above.

2.4 Waste disposal

Disposal of consumables from HT Nucleofector™ kits (cuvettes, and Nucleofector™ solutions): dispose of cuvettes and expired 384-well 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 HT Nucleofector™ system components

The HT Nucleofector™ system comprises two hardware modules (the power supply and plate handler), HT Nucleofector™ software preinstalled on a Windows®-based laptop, and a set of cables to connect the units.

Figure 2.1: HT Nucleofector system components

1. HT Nucleofector™ power supply
2. HT Nucleofector™ plate handler

Figure 2.2: HT Nucleofector™ set of cables

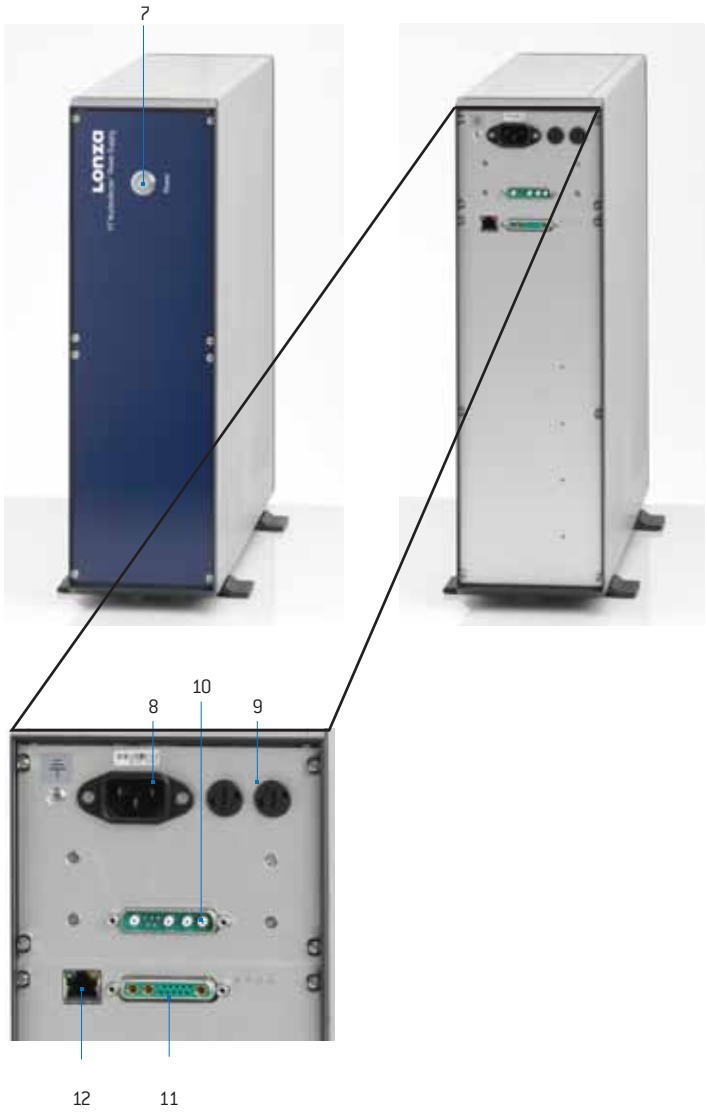
3. Power cable
4. Lonza interface data cable
5. Lonza interface HV cable
6. USB cable

HT Nucleofector™ power supply

The HT Nucleofector™ power supply generates and delivers the electrical programs to the plate handler and mediates the data transfer between the computer and the plate handler. It is connected to the plate handler via two cables.

7. Main switch with status LED
8. Main power inlet
9. Fuse holders with fuses
10. Lonza interface (HV port)
11. Lonza interface (data port)
12. Ethernet port

Figure 2.3: HT Nucleofector™ power supply



HT Nucleofector™ plate handler

The HT Nucleofector™ plate handler serves as the contacting unit of the system. Transfection of the samples occurs in 384-well Nucleocuvette™ plates that are loaded onto the carousel of the plate handler.

13. Lonza interface (HV port)
14. USB host (for firmware update)
15. USB port for PC connection
16. Ethernet port
17. Lonza interface (data port)

Figure 2.4: Plate handler connections

Plate handler front

18. Status LED from the left: power on (green); boot status (green); error (red); plate status (blue)
19. Plate carousel
20. Plate retainer position

Figure 2.5: Plate handler front view

The Nucleofector™ software start screen

The HT Nucleofector™ operating software provides the graphical user interface for operating the HT Nucleofector™ system. It controls all processes during an experiment and manages data transfer to the host PC.

Figure 2.6: Login screen

384-well Nucleocuvette™ plate – solution and supplement

High-throughput Nucleofection™ occurs in unique 384-well Nucleocuvette™ plates supplied with HT Nucleofector™ kits. The plates meet SBS standards and can therefore be used in liquid handling workstations.

Figure 2.7: HT Nucleofector consumables

2.6 System set-up instructions

The HT Nucleofector™ system will be installed and checked by a Lonza technical specialist. Follow the instructions below if the instrument is moved from its original location.

- Connect the HV port of the HT Nucleofector™ power supply (10) to the HV port on the plate handler Unit (13) using the cable marked "Lonza interface HV cable."
- Connect the data port of the HT Nucleofector™ power supply (11) to the data port on the plate handler (17) using the cable marked "Lonza interface data cable."
- Connect the USB slave port of the HT Nucleofector™ plate handler (15) to a USB port on your personal computer using the supplied USB cable.
- If required install, the HT Nucleofector™ operating software on your personal computer, following the instructions in the HT Nucleofector™ software manual.
- Connect the main power plug of the HT Nucleofector™ power supply (8) to a wall outlet with protective grounding conforming to the power rating specified in Chapter 6.
- Start the system as described in Chapter 3.1 of the HT Nucleofector™ software manual.

2.7 Instructions for using the HT Nucleofector™ system

The table below gives a brief overview of the steps required for a typical Nucleofection™ workflow.

Step	Description	For more information see
1	Switch on the HT Nucleofector™ system.	This chapter
2	Start the HT Nucleofector™ software on the computer and log in.	Software manual
3	Check your user settings (optional).	Software manual
4	Check that all connections are active / reconnected	Software manual
5	Open an existing experiment or create a new experiment.	Software manual
6	Prepare and transfer samples into the 384-well Nucleocuvette™ plate.	Optimized protocol
7	Insert the 384-well Nucleocuvette™ plate into the HT Nucleofector™ plate handler.	Chapter 3.1
8	Start Nucleofection™ process from the computer.	Software manual
9	Monitor Nucleofection™ process during program execution (optional).	Software manual
10	Remove 384-well Nucleocuvette™ plate from the plate handler and transfer samples to appropriate culture plates.	Optimized protocol
11	Check result file on the computer for potential errors.	Software manual
12	Close files, exit HT Nucleofector™ software and switch off all components.	Software manual

The HT Nucleofector™ system enables Nucleofection™ experiments to be conveniently controlled and monitored from a computer. Only a few steps are required to initialize the system.

- Switch on the HT Nucleofector™ system via the main switch located on the front panel of the power supply. LED 1 (green) on the plate handler front panel is lit.
- Start the HT Nucleofector™ software on the personal computer and log in. LED 2 (green) on the plate handler front panel blinks during the boot sequence and remains lit when the process is successfully completed. The system is now initialized.
- If LED 3 (red) is lit, an error occurred during the boot process. Refer to the HT Nucleofector™ software manual for troubleshooting.

3 Running an experiment

This chapter explains how to run a Nucleofection™ experiment on the HT Nucleofector™ system. Because correct cell handling before and after the Nucleofection™ process is crucial for successful experiments, refer to the dedicated optimized protocol to find detailed guidelines for these steps.

3.1 Processing a 384-well Nucleocuvette™ plate

This chapter describes loading and processing of the 384-well Nucleocuvette™ plate using a predefined experiment. See the HT Nucleofector™ software manual for further details on defining and modifying experiments.

Figure 3.1: 384-well Nucleocuvette™ plate handler work area

The plate handler carousel has two plate retainer positions for 384-well Nucleocuvette™ plates. Load plates into the retainer position that is accessible at the front of the plate handler. Do **not** turn the plate handler carousel manually to access the second position. To load prepared plates:

- Make sure that the plate is covered with an appropriate lid.
- Make sure that plate position A01 is in the upper left corner.
- Place the plate gently into the plate handler retainer position (1)

After loading the 384-well Nucleocuvette™ plate, start the experiment from the computer. The 384-well Nucleocuvette™ plate will be automatically rotated into the plate handler to be processed. It will be rotated back when the experiment is completed. You can follow the progress of the experiment by checking the blue progress LEDs on the front panel of the plate handler.

If the HT Nucleofector™ is integrated into an automated liquid handling system follow the appropriate instructions in the HT Nucleofector™ software manual for plate handling.

NOTE: Do not attempt to turn the carousel manually when the system is active! This will compromise system integrity.

NOTE: Do not use lids other than those provided in the 384-well Nucleofector™ kits. Other lids may cause damage to the HT Nucleofector™ plate handler.

After completion of a run, remove the processed 384-well Nucleocuvette™ plate from the plate handler and process it according to the relevant optimized protocol.

3.2 System shutdown

Close all parameter and result files, exit the HT Nucleofector™ operating software, and switch off the system using the main switch on the HT Nucleofector™ power supply front panel.

4 Troubleshooting

The following troubleshooting guide may be helpful if experiments using the HT Nucleofector™ system do not provide the expected results. The comments are intended to help optimize experimental conditions.

Should you have any questions regarding the HT Nucleofector™ device, please do not hesitate to contact Lonza's scientific support team

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4.1 Nucleofection™ problems

Problem	Possible cause	Solution
Low survival rate	Cells remained too long in the Nucleocuvette™ plate	If a 10 minute post incubation step is not recommended for your specific protocol, add pre-warmed medium to the cells and remove samples immediately after the Nucleocuvette™ plate has been processed.
	Cells are damaged by harvesting or through inappropriate handling	Avoid severe conditions during harvesting, especially centrifugation at high speeds and overexposure to trypsin. Pipette cells smoothly. After Nucleofection™, add the appropriate amount of pre-warmed medium to the wells and resuspend cells gently before removing them.
	Cells are stressed by culture conditions	Cells should be viable and have been in culture for a certain number of passages. Freshly thawed cells should not be used for Nucleofection™. Avoid high cell densities or confluencies since this may negatively influence the viability of the cells after Nucleofection™. For detailed recommendations on passage number, cell density and confluency refer to the relevant optimized protocol.
Low transfection efficieny	Cells are stressed by centrifugation	Centrifuge at lower speed (max. 90 x *g*).
	Multiple use of 384-well Nucleocuvette™ plates	We strongly recommend using 384-well Nucleocuvette™ plates only once, because the electric pulses that are applied impair their physical integrity. The performance of plates that are used more than once will be dramatically reduced .
	Cells may be contaminated with mycoplasmas	Test cultures for mycoplasma contamination. Contact our scientific support team for further advice.
	Poor DNA quality	Use high-purity plasmid DNA. We strongly recommend the use of high-quality products for plasmid purification such as the QIAGEN EndoFree® plasmid kit. Do not use procedures involving phenol or chloroform treatment for DNA purification.
	Plasmid amount is too low	We recommend a plasmid amount between 0.2–1 µg DNA per sample. If both gene transfer efficiency and cell mortality are low, the plasmid amount can be increased up to 2 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 or density	In many cell types, gene transfer efficiency is poor if the cell density has been too high at the time of harvest. Follow the guidelines in the relevant optimized protocol.
	Cell number too high or too low	We recommend using $2 \times 10^4 - 1 \times 10^6$ cells per sample for cell lines and primary cells. Refer to the relevant optimized protocol for specific details.

4.2 Mechanical failures

Great care has been taken to ensure that mechanical failures will not occur as long as the system is operated using the guidelines presented in this manual and in the HT Nucleofector™ software manual.
In case of a system failure while a 384-well Nucleocuvette™ plate is still inside the plate handler unit, proceed as follows to recover your samples:

1. Turn off the HT Nucleofector™ using the main switch of the power supply.
2. Disconnect the main power cable from the power supply power inlet.
3. Remove the small plastic cap on top of the plate handler housing (see Figure 4.1) to access the emergency lift screw of the contact bridge.
4. Using a screwdriver, turn the screw clockwise as far as possible.
5. The plate handler carousel can be turned manually and samples can be recovered.
6. To recover a 384-well Nucleocuvette™ plate loaded on plate retainer position 1 (position 2 is visible at the front of the plate handler) turn the carousel counterclockwise to recover your samples. To recover samples from plate retainer position 2 (position 1 is visible), turn the carousel clockwise.
7. Remove the 384-well Nucleocuvette™ plate from the plate handler carousel.
8. Contact Lonza's technical service for analyzing the error and for repair of the system.

Figure 4.1: Plastic cap covering the emergency release of the contact bridge

Figure 4.2: Lift contact bridge by turning the screw clockwise

Figure 4.3: Turn carousel manually to recover samples

5 Purchaser notification

The purchase of the HT Nucleofector™ conveys to the buyer a non-transferable, non-exclusive license without the right to grant sublicenses, limited for solely the use of Lonza Cologne's proprietary Nucleofector™ process and method.

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6 Appendix

Technical data power supply

Power supply	100–110 VAC or 230 VAC 50–60 Hz Self-regulating Attention: Only use power outlets with protective earth/ground Only use the power cable supplied with the HT Nucleofector™ system
Power consumption	750 VA / fuse T10A H250VP
Operation temperature range	+15°C to +40°C, non-condensing
Safety class	EN 61010-1 UL 61010-1 IP 20
Weight	14 kg 31 lb
Dimensions (w × d × h)	13.5 × 50 × 45 cm 5.3× 19.6 × 17.7 in
Manufacturing date	The year of manufacture is indicated by the second and third digit of the serial number, e.g. a device with the serial number x11xxxxx was manufactured in 2011.

Technical data plate handler

Power supply	24 V supplied by HT Nucleofector™ power supply
Operation temperature range	+15°C to +40°C, non-condensing
Safety class	EN 61010-1 UL 61010-1 IP 20
Weight	10.5 kg 23.1 lb
Dimensions (w × d × h)	40 × 40 × 15 cm 15.7 × 15.7 × 5.9 in
Manufacturing date	The year of manufacture is indicated by the second and third digit of the serial number, e.g. a device with the serial number x11xxxxx was manufactured in 2011.

Notes

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www.lonza.com
www.lonza.com/specifications

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