NextGenPCR[™]

THERMAL CYCLER | INSTRUCTIONS FOR USE REVISION 2.0

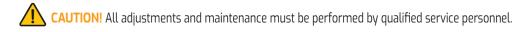




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The user acknowledges that it is their responsibility to ensure that their use of this product complies with all applicable laws and regulations, including patent laws, in their jurisdiction. Molecular Biology Systems B.V. reserves the right to enforce its patent rights to the fullest extent of the law, including through legal action.

Important: Read these instructions for use. Failure to read, understand and follow the instructions in this document may result in damage to the unit, injury to operating personnel, or poor device performance. Contact your local distributor or a Molecular Biology Systems technical specialist if there are any doubts regarding the proper use of your system.



Revision History

Version	Date	Description
1.0	08/02/2020	Development of the IFU – intial version
2.0	09/28/2023	Following parts of the document were updated: Safety symbols, Contact information, Product information, Safety, Footer/Header;

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NextGenPCR[™] THERMAL CYCLER

INSTRUCTIONS FOR USE

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PREFACE

Introduction

Welcome to the user manual for the NextGenPCR[™] Thermal Cycler! We are thrilled to introduce you to our state-of-the-art PCR instrument, designed to revolutionize your DNA amplification workflows. With cutting-edge technology, intuitive controls, and unparalleled performance, the NextGenPCR[™] Thermal Cycler is engineered to meet the demands of both novice researchers and seasoned professionals alike.

This comprehensive manual aims to provide you with all the essential information to maximize the efficiency and accuracy of your PCR experiments. Whether you are conducting routine PCR amplifications or tackling complex genetic analyses, the NextGenPCR™ Thermal Cycler is your ideal companion for achieving reliable and reproducible results. Let's embark on this exciting journey to unlock the potential of your research together!

How to use this manual

This NextGenPCR[™] thermal cycler manual serves as a comprehensive guide for effectively operating and maintaining the NextGenPCR[™] Thermal cycler in molecular biology applications. The manual provides information on the machine's specifications, features, and user interface. It offers detailed instructions for setting up your NextGenPCR[™] machine, including power requirements and environmental conditions. Step-by-step procedures are outlined for operating the machine, such as programming PCR protocols and adjusting temperature and time parameters. The manual includes guidelines for initial device setup, creating and optimizing PCR programs, covering temperature profiles, and cycling parameters.

Maintenance guidelines address routine cleaning, calibration, and the replacement of consumables. Troubleshooting tips and solutions are offered, and contact information for technical support or service assistance is provided. Safety considerations are emphasized.

IMPORTANT! Before operating the NextGenPCR[™] Thermal Cycler, carefully read and familiarize yourself with the user manual provided by the manufacturer. Understand the device's capabilities, limitations, and safety precautions. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

Safety alert words

There are four safety symbols used in this document at points where the user shall be aware of relevant hazards. Each safety word implies a particular level of observation or action.

IMPORTANT! Relates to important information or instructions that are not directly related to safety hazards but are still significant for the proper use and maintenance of the product, as, for example, warranty, maintenance schedules, special considerations.

CAUTION! Indicates a potential hazard or unsafe practice that, if not avoided, could result in minor to moderate injury or damage. It alerts the user to take precautionary measures and follow the specified instructions carefully to prevent accidents.

WARNING! Indicates a potential hazard or unsafe practice that, if not avoided, could cause serious injury, illness, or even death. It signifies a higher level of risk and emphasizes the need for strict adherence to safety guidelines and precautions.

DANGER! Is a strong and urgent safety word used to indicate an immediate and severe risk that, if not avoided, will likely result in serious injury or death. It signifies an extremely hazardous situation that requires immediate attention and adherence to safety procedures.

Reporting

IMPORTANT! Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the Member State in which the user is established.

How to obtain support and more information

Resource	How to contact
Local NextGenPCR™ representatives	Find our representative in your country on our <u>nextgenpcr.com</u> website. If no local representative is present in your country, you can contact us directly by emailing us at <u>info@mbspcr.com</u>
Technical notes and literature	Technical notes and literature can be found on www.nextgenpcr.com
Technical Specialist	Contacting our technical specialist is possible by emailing <u>support@mbspcr.com</u>
Customer support	E: info@mbspcr.com T: +31 113 26 81 10

1. WARRANTY

1.1 Warranty Information

The system's warranty is included in the purchase price. Molecular Biology Systems B.V. warrants this machine to be free from defects in material and workmanship when used under normal laboratory conditions for one (1) year. In the event of a justified claim, Molecular Biology Systems B.V. will replace any defective component or replace the machine free of charge. This warranty does not apply if the damage is caused by fire, accident, misuse, neglect, incorrect adjustment or repair, damage by incorrect installation, adaptation, modification, fitting of non-approved parts, or use or repair by unauthorized personnel. This warranty is in addition to and does not affect any statutory rights.

1.2 Warranty Coverage

The NextGenPCR[™] Thermal Cycler, including the main unit, accessories, and software provided with the system, is covered under this warranty.

1.3 Warranty Duration

Molecular Biology Systems, B.V. warrants this PCR Thermal Cycler against defects in materials and workmanship for a period of 1 year from the date of purchase.

1.4 Warranty Conditions

To ensure that your warranty remains valid, please adhere to the following conditions:

PROPER USE

This warranty is valid only if the NextGenPCR[™] Thermal Cycler is used in accordance with the instructions provided in the manual and for its intended purpose of performing polymerase chain reactions (PCR) and related molecular biology applications.

UNAUTHORIZED MODIFICATIONS

The warranty does not cover any damage caused by unauthorized modifications or repairs performed on the NextGenPCR[™] Thermal Cycler.

EXTERNAL FACTORS

Damages resulting from accidents, misuse, negligence, natural disasters, or other external factors beyond the control of Molecular Biology Systems, B.V. are not covered under this warranty.

ROUTINE MAINTENANCE

Please note that routine maintenance and consumable parts, such as fans, belts (stopper motor), fuses, or other parts subject to normal wear and tear, may have separate warranty terms or may not be covered under the main warranty. Refer to the specific documentation provided for further information.

FORCE MAJEURE

In the event of unforeseeable circumstances beyond the control of either party, including natural disasters, pandemics, or government actions, the affected party's obligations under this agreement shall be temporarily suspended. The parties will work together to find a resolution, and if the event persists, the agreement may be terminated without penalty.

1.5 Warranty Claim Process

In the event that you need to make a warranty claim, please follow these steps:

Contact Molecular Biology Systems, B.V. customer support by phone or email, using the contact details provided in the manual. Provide proof of purchase, such as the original sales receipt or invoice, indicating the date of purchase. Molecular Biology Systems, B.V.'s customer support team will guide you through the necessary steps to assess the issue and initiate the warranty claim process.

1.6 Return of the instrument

If there is a need to return the instrument either for servicing or replacement, please use the original packaging materials, if available. The manufacturer's packaging is designed to provide the best protection during shipping. If the original packaging is unavailable, use sturdy, cushioned packaging materials to keep the instrument stable and secure during transit.

The user is responsible for properly decontaminating the thermal cycler to eliminate any residual DNA molecules or other biological contaminants before returning the instrument. For decontamination and cleaning instructions refer to section 10.3 Maintenance.

After cleaning, allow the thermal cycler to dry completely to avoid any moisture-related damage during transportation. Include all relevant documentation, such as the original purchase receipt, warranty information, and any notes about the instrument's condition or issues you encountered.

Mark the package correctly. Before shipping, communicate with the manufacturer or distributor and let them know the instrument is being returned. They may provide specific instructions or arrange for the return process.

1.7 Limitations of Liability

Molecular Biology Systems, B.V. shall not be held liable for any consequential damages, or data loss resulting from the use or inability to use the PCR Thermal Cycler. In case of a justified claim, the maximum monetary value that can be claimed under this warranty is limited to the original purchase price of the PCR Thermal Cycler.

1.8 Jurisdiction

This warranty is governed by the laws of the Netherlands. Any disputes arising from this warranty shall be subject to the exclusive jurisdiction of the courts in that jurisdiction. Please note that the warranty terms specified here are subject to change. For the most up-to-date and accurate information, please consult the warranty documentation provided by Molecular Biology Systems, B.V. at the time of your purchase.

If you have any further questions or concerns regarding the warranty, please do not hesitate to contact our customer support team.

2. PRODUCT INFORMATION

2.1 Device identification

The NextGenPCR[™] Thermal Cycler is a stand-alone, electrically powered, automated, non-sterile laboratory instrument intended to amplify target deoxyribonucleic acid (DNA) in a clinical specimen using heat-stable polymerase enzymes and cycles of repeated heating and cooling (i.e., thermal cycling) to replicate a product (i.e., a polymerase chain reaction (PCR)). (GMDN 48031). The instrument is not intended for self-testing procedures, nor is it intended for use to directly diagnose or detect the presence of a pathogenic molecule or infectious agent.

For research use only. Not for use in diagnostic procedures. For professional use only.

List of Device Identifiers	
MBS Product Catalog Code	#10001
GMDN	48031 (July 5/2023)

2.2 Intended purpose and intended users

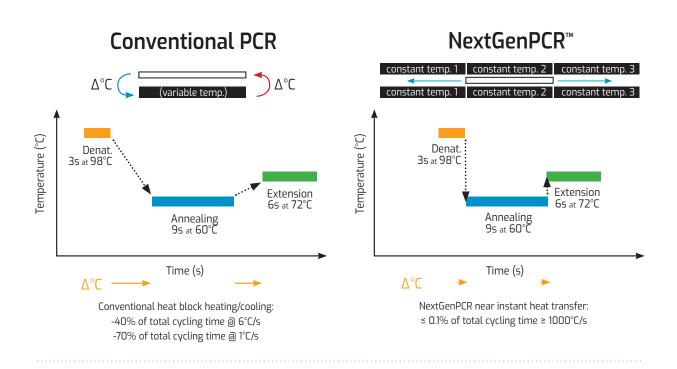
The NextGenPCR[™] Thermal Cycler is a laboratory instrument intended to cycle the temperature of samples in a microtiter plate between two or more temperatures in an ultrafast way in order to perform a polymerase chain reaction. Intended users are qualified and trained laboratory personnel.

2.3 What is included?

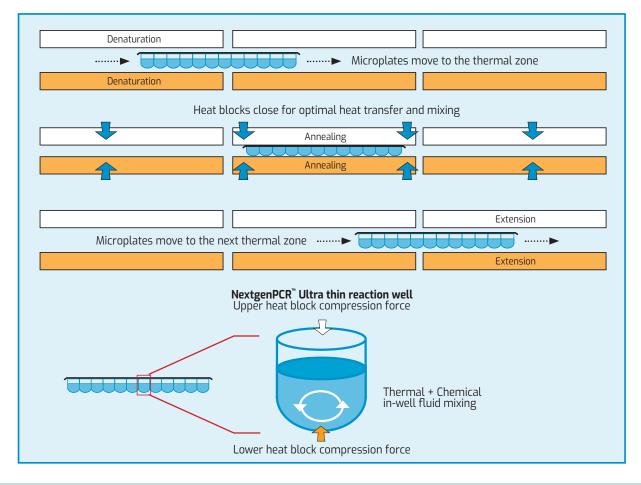
The NextGenPCR[™] Thermal Cycler system includes the following components: NextGenPCR[™] Thermal Cycler, power cable (EU 220V or USA 110V) and the Instructions for Use.

2.4 About

Since the inception of the polymerase chain reaction (PCR) technique in 1983, laboratory PCR instruments have been reliant on slow Peltier technology to transfer heat from an active thermal element to a sample container. The fastest Peltier element-based thermal cycling instruments available on the market can ramp temperatures by 5°C/sec to 6°C/sec. Time spent at intermediate temperatures results in substantially inflated overall cycling times and makes current PCR methodology generally inefficient and time-consuming.



NextGenPCR[™] thermal cycling technology radically improves PCR cycling times by eliminating temperature ramping times from any cycling program, owing to its innovative sample heating technology. In a NextGenPCR[™] reaction, an ultrathin plastic microplate sample container is swiftly moved between three distinct heating zones, each set to a constant temperature. Via compression of two heating blocks per heating zone when the microplate container is in place, samples are heated or cooled nearly instantaneously resulting in much shorter cycling times as compared to regular PCR reactions and instruments.



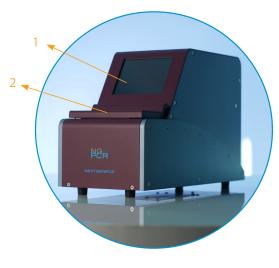
The NextGenPCR[™] Thermal Cycler has universal heating zones that accept multiple sizes of microplate container formats from 24- to 384 wells and 5 to 50 µl reaction volumes with no mechanical modifications required to the interior of the instrument.

Note: For compatibility information please refer to Chapter 8 Device Compatibility

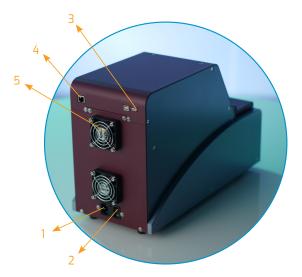
2.5 Instrument Features

Features

Interface	5" HD Touch Screen
Lid*	Manual operation
Connectivity	1× USB-A port (used for service and calibration only)
	1× USB-B port (used for service and calibration only)
	1× ethernet port
	1× C14 power inlet (110V/220V)
	1× Power switch
Serial number	See the ID plate on the bottom of the machine
MAC address	See the ID plate on the bottom of the machine
Operating system	Linux
Internal data storage	SD card 64GB
Storage capacity	106 entries for users or protocols



Front view: (1) Touch screen interface(2) Microplate loading station/lid



Rear view: (1) Outlet (2) Power switch on/off (3) USB type A, USB type B (for servicing and calibration only) (4) Ethernet port (5) Fans

***Note:** For use with a robotic system the cycler can be operated without a lid. Please inquire about details if required. The firmware will be adapted.

2.6 Technical Specifications

DIMENSIONS

Height	240 mm / 9.5"
Width	196 mm / 7.7"
Depth	445 mm / 17.5"
Weight	18 kg / 39.7 lb

TEMPERATURE PROFILE

Position*	Calibrated range** (min-max)
Heat Block #1 (annealing zone)	48°C – 74°C
Heat Block #2 (extension zone)	68°C – 74°C
Heat Block #3 (denaturation zone)	94°C – 100°C

* Orientation from the front side to the back side of the machine.

IMPORTANT! The calibrated temperature control specifications of the NextGenPCR[™] Thermal Cycler are quoted at an ambient temperature of 20°C ± 5°C.

TEMPERATURE CONTROL

Calibrator	PT-100
Accuracy	± 0.2°C of displayed temperature
Heating/cooling rate	≥ 100°C/sec
Heat Block positions	3
Heat Block dwell time (min/max)	0.5 – 999 sec

POWER

Voltage	90-260V, 50/60Hz
Power (max/avg)	680W/150W
Fuse ratings	Т 6.3А

3. OPERATIONAL SAFETY

WARNING! Physical Injury Hazard. Using the instrument in a manner not specified by this manual may result in personal injury or damage to the instrument.

3.1 General safety

Before operating the NextGenPCR[™] Thermal Cycler, carefully read and familiarize yourself with the user manual provided by the manufacturer. Understand the device's capabilities, limitations, and safety precautions. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

IMPORTANT! It is essential to make certain that individuals operating the instrument have received comprehensive instructions in both general laboratory safety practices and specific safety practices pertaining to the instrument.

WARNING! The device's surface must never become hot during operation. If this does happen, disconnect the device immediately and notify the manufacturer of the issue.

3.2 Use of Personal Protective Equipment (PPE)

Always remember to wear the appropriate Personal Protective Equipment (PPE) while using the thermal cycler. This includes, but is not limited to, lab coats, safety goggles, and disposable gloves. PPE serves as a barrier to protect you from potential chemical splashes, biohazards, or any accidental spills that may occur during operation. By wearing the recommended PPE, you not only safeguard yourself but also maintain the integrity of your experiments.

3.3 Visual inspection

Visually inspect the thermal cycler before each use. This includes checking for any visible damage, loose cables, or irregularities.

WARNING! If any issues are found, refrain from using the equipment and contact the manufacturer or authorized service personnel.

3.4 Moving and lifting the device

CAUTION! The NextGenPCR[™] instrument weighs approximately 18kg. The movement and positioning of the instrument should only be carried out by authorized personnel or the designated vendor. If you need to relocate or lift the instrument after installation, it is essential to refrain from doing so without assistance from others, utilization of suitable moving equipment, and the application of proper lifting techniques. It is crucial to note that improper lifting can result in severe and lasting back injuries. Depending on the weight involved, the relocation or lifting of an instrument may necessitate the involvement of two or more individuals. Never move or carry the instrument when used or connected to the power/machine electricity supply.

3.5 Cleaning or decontaminating the device

CAUTION! Prior to utilizing a cleaning or decontamination method that deviates from the manufacturer's recommended procedures, verify with the manufacturer whether the proposed method will cause any damage to the equipment.

3.6 Symbols on the device

3.6.1 ENVIRONMENTAL SYMBOLS ON THE DEVICE

Symbol	Description
X	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).

3.6.2 INFORMATION SYMBOL ON THE DEVICE

Symbol	Description
	A QR code readable by a digital device leads the customer to the Instructions for Use (TBD)

3.6.3 ELECTRIC COMPATIBILITY (EMC) SYMBOL ON THE DEVICE

Symbol	Description	
	European safety and EMC standards.	
CE	Safety	
~ ~	The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by the manual.	
	This device meets European requirements for safety (Low Voltage Directive 2006/95/EC).	
	Directive 2014/30/EU – Electromagnetic Compatibility (EMC) Directive Directive 2014/35/EU – Low Voltage Directive (LVD) Overvoltage category II (IEC 60664-1)	

4. ELECTRICAL SAFETY

4.1 Electric shock hazard

DANGER! Severe electrical shock can result from operating the NextGenPCR[™] Thermal Cycler without its side panels in place. Do not remove the side panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

4.2 Fire hazard – fuses

CAUTION! Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

CAUTION! For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

4.3 Electrical hazard – power

DANGER! Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected. Use properly configured and approved line cords for the voltage supply in your facility. Plug the system into a properly grounded receptacle with adequate current capacity.

5. GETTING STARTED

5.1 System placement requirements

IMPORTANT! The NextGenPCR[™] Thermal Cycler shall be placed in the control laboratory environment.

Place the NextGenPCR[™] Thermal Cycler on a smooth level surface before use. Ensure that the surface can support the weight of the instrument without wobbling or tipping over. Avoid placing the instrument adjacent to heaters, cooling ducts, or in direct sunlight. Fluctuations between day and night temperatures can cause system instability. Place the device away from any equipment that vibrates, such as a refrigerator or centrifuge.

5.2 Environmental requirements

The NextGenPCR™ Thermal Cycler is designed to operate optimally under the following environmental conditions:

Room temperature	20°C ± 5°C
Relative humidity, non-condensing	60% - 80%

IMPORTANT! Make sure the NextGenPCR[™] Thermal Cycler is at ambient temperature for at least 30 minutes before switching on for the first time.

5.3 Unpacking and Installation

For the proper installation of the device, the following steps are necessary:

Verify if the package contains all the necessary content to set up the system:

- · NextGenPCR™ Thermal Cycler
- Power cable (EU 220V or USA 110V)

Remove all contents from the packaging.

IMPORTANT! Ensure that the air inlet and outlet vents on the bottom and rear of the system are free from obstruction.

IMPORTANT! Avoid overhanging any part of the system over the bench edge. Make sure there is some space between the NextGenPCR[™] Thermal Cycler and any objects placed in proximity (at least 5 centimeters / 2 inches).

IMPORTANT! Store the original packaging in a safe location. In the event of returning the machine for servicing or repairs, the machine is required to be packed in the original casing (see Section 1.6 Return of instrument).

DO NOT TURN ON THE INSTRUMENT IMMEDIATELY AFTER CONNECTING. DIRECTLY PROCEED WITH THE NEXT STEPS IN ORDER.

Connect the supplied power cable to the NextGenPCR[™] Thermal Cycler and then to the electrical supply. It is advised to use a grounded electrical supply source to ensure the safety of the end user.

IMPORTANT! Remove the foam block inserted into the sample loading tray before switching it on for the first time.

To access the sample loading tray, lift the lid at the front of the system. After removing all packaging materials, switch on the machine using the power switch at the back of the machine. The display will light up, and the microplate holder will initialize its position.

The NextGenPCR[™] Thermal Cycler will start heating to a baseline state of 94°C for the denaturation zone, 50°C for the annealing zone, and 72°C for the extension zone and is ready for use.

Note: The baseline temperature settings will be automatically adapted to the temperature settings of the last run.

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5.4 Temperature calibration and verification

The NextGenPCR[™] Thermal Cycler undergoes calibration and temperature verification before customer delivery. Upon purchasing the device, customers receive a Certificate of Calibration. To ensure ongoing optimal performance, it is recommended to perform an annual calibration as part of routine maintenance. See Section 10 Maintenance and Servicing.

6. OPERATING THE INSTRUMENT'S FIRMWARE

6.1 Connecting the instrument for remote monitoring

The NextGenPCR[™] Thermal Cycler instrument firmware can be accessed directly by using the touch screen or remotely via Windows on a desktop or laptop. This feature allows users to, for example, check the status of the cycler from outside of the laboratory or incorporate the NextGenPCR[™] Thermal Cycler in an automated laboratory workflow.

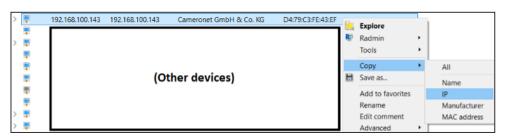
Note: To use the remote function, both the cycler and the laptop must share the same network.

The following components are needed:

- PC (desktop, laptop) with preinstalled software.
- OS Windows 7 or later
- Advanced IP Scanner v2.5.1 or later
- · Firefox v114.0 or later, or an equivalent internet browser
- RJ45 ethernet cable.

To access your NextGenPCR™ Thermal Cycler using your PC follow these steps:

- Connect the NextGenPCR[™] Thermal Cycler to the network access point such as a router or WiFi hub (avoid direct connections to a PC or laptop) with an ethernet cable.
 The socket is located at the back of the NextGenPCR[™] Thermal Cycler, near the power inlet.
- Turn on the NextGenPCR™ Thermal Cycler using the power switch.
- · Start the Advanced IP Scanner software.
- · Click the Scan button at the top left of the screen.
- In the list of identified devices, there will be an access point manufactured by 'Cameronet GmbH & Co. KG':



- Copy the IP address associated with the 'Cameronet GmbH & Co. KG' device (right click, then 'Copy' 'IP') to the address bar of your internet browser and press enter. You will now log in to the NextGenPCR[™] Thermal Cycler software.
- The NextGenPCR[™] Thermal Cycler software currently running on your instrument will be displayed in the browser window.
- The instrument can now be controlled using keyboard and mouse inputs.
- · Close the browser window to disconnect your computer from the instrument when finished.

Note: Recommended IP range should be as large as possible

6.2 Home screen



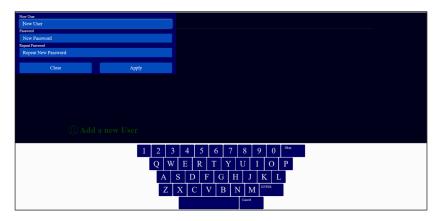
This screen serves as a login point where users can log into their existing account or add a new user if needed. During the first start, either a servicing account called 'GB' or 'MBS', or no users will be shown.

	EMB	s												
	0	GB												
	0	MBS												×
	\bigcirc Ad	ld a new User	r											
Insert Password for ME	3S													
			1	2	3	4	5	6	7	8	9)	0	Bkop
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By tapping the username of choice, a keyboard will pop up, and you will be prompted to input your password:

CREATE A USER PROFILE

To add a new user, press "Add User" and the following screen will appear:



Press on the New User field and input your desired username. Then choose a password, enter it twice and hit Apply.

IMPORTANT! Users without a specified password are unable to save protocols.

The new User can now be accessed from the home screen.

WARNING! To prevent unauthorized access to the information portfolio, ensure that the users' information is stored securely.

DELETE A USER PROFILE

To remove a user from the user list, click the red cross icon next to the user:



Note: As this feature is not typically required by end users (the NextGenPCR[™] Thermal Cycler can store up to 106 unique user profiles with the available storage space), the Remove User feature is locked behind a superuser password. Please contact support if you want to reconfigure the user list of your NextGenPCR[™] instrument.

6.3 Protocol menu

LOGIN TO THE DESIRED USER PROFILE.

∃MBS≁		User name: MBS
There are no	protocols for this user	
All Users	Add a new Protocol 🖹	

You will be taken to the Program Menu where the desired PCR protocol can be defined including initial denaturation and/or final extension steps by filling out the relevant fields of the screen.

As an example, a PCR program with 10 minutes of initial denaturation at 98°C, followed by 35 cycles of 30 seconds of denaturation at 98°C, 30 seconds of annealing at 60°C and 30 seconds of extension at 72°C, followed by 10 minutes of final extension at 72°C can be programmed as follows:

•	Ritter M85	× +						~		-	5	×
\leftarrow	\rightarrow C	O 🔒 192	168.100.143/table.html				\$		0	<u>m</u>	Û	=
							Protocol name: 3	e .				
			Initial Denaturing	Denaturing	Annealing	Extension	Final Extension					
		Temperature (*C)	98	98	60	72						
		Time (s)	300	30	30	30	600					
		Cycles 35		Teach		Step Dewa						
				Advanced Modus		Reverse Transcription						
			Save			Run						

6.4 Custom PCR settings

The NextGenPCR[™] Thermal Cycler is capable of multiple different types of PCR programs, including reverse transcription, touchdown, step-down and advanced cycling (Advanced Mode) setups.

REVERSE TRANSCRIPTION

If reverse transcription is required prior to continuing with the main cycling program, first press the Reverse Transcription checkbox. **A new window will open:**

- Configure the reverse transcription program by entering the incubation temperature ("RT-Step") and denaturation temperature and length in seconds.
- Press Apply.
- If the Reverse Transcription box is checked and the program details entered, the two programs with be executed sequentially starting with reverse transcription.

TOUCH DOWN & STEP DOWN

Both 'touch down' (linear decrease in annealing temperature per cycle) and 'step down' (binary decrease in annealing temperature per cycle) options can be powerful tools to increase the specificity of amplicon generation during the PCR reaction.

 If multiple annealing temperatures are required for the cycling program, select either Touch Down or Step-Down options on the NextGenPCR™ Thermal Cycler:



Using Touch Down mode, the user can specify a linear decrease in annealing temperature with increasing cycle number. In the example above, the target annealing temperature will decrease by 0.2°C every cycle for each of the 50 total cycles in the program.

Touch Down				Delta T/Cycle
From Cycle	0	to	50	0.2
From Cycle	0	to	0	0
From Cycle	0	to	0	0
From Cycle	0	to	0	0
From Cycle	0	to	0	0
	Close			Apply

Using Step Down mode, the annealing temperature can be changed by a set number for a range of cycles. In the example above, the first 15 cycles are performed at 60°C, the second set of 15 cycles at 59°C, the third set of 15 cycles at 58°C and the last five cycles at 57°C.

ADVANCED MODE

With Advanced Mode, the user can define two sets of cycling conditions within the same program that will be carried out consecutively. This allows specification of 'precycles'

(initial few cycles where amplification conditions are more lenient) and main cycles separately:

	Initial Departuring	Denaturing	Annealing	Extension	Final Extension
Temperature (°C)	98	98	60	72	
Time 1 (5)	300	30	30	30	600
# Cycles		5	5	5	
Time 2 (s)		10	10	10	
# Cycles		30		30	
Cycles 35		Teach Down		Step Down	
		Advanced Mindus		Reverse Transcription	
	Save			Run	

The use of precycles is recommended for amplification of difficult templates, such as GC-rich templates or templates with high repeat numbers.

7. MICROPLATE PREPARATION

LIST OF REQUIRED MATERIALS:

- pipetting anvil
- sealing anvil
- microplates
- seals
- sealer
- copper plate (if applicable)
- applicable master mix
- pipetting tips and pipets

LOADING SAMPLES

- Place the Pipetting Anvil in a PCR Cabinet or equivalent.
- Place an empty Microplate on the Pipetting Anvil.
- Thaw your reagents and prepare your PCR master mix according to the manufacturer's instructions.
- · Pipette the sample volumes into the Microplate wells.

Note: Include at least two positive control samples and two non-template controls (NTC) to allow for quality control after cycling has been completed.

SEALING

Note: If using a NextGenPCR[™] Semiautomatic Heat Sealer (#10102), make sure that the metal spacer provided with the instruments is inserted into the loading tray before sealing. If using an off-brand microplate heat sealer, verify whether the distance between the Sealing Anvil, Microplate and top heating element is adequate. Additional accessories such as spacers might be required.

- Place the Sealing Anvil on top of the spacer in the sample loading tray.
- · Place the Microplate containing your sample volumes on top of the Sealing Anvil.
- Place a Heat Seal on top of your Microplate, taking careful note that the indicated side of the seal is facing the correct direction.

Note: If using a Clear Heat Seal, place the Copper Sealing Plate on top of the Microplate with the unheated seal in place. If using an Aluminum Heat Seal, proceed to the next step directly.

Set up the Semiautomatic Heat Sealer to use the following parameters:

Temperature	175°C
Duration	2 sec

PERFORMING A RUN

IMPORTANT! Make sure the NextGenPCR[™] Thermal Cycler is switched on before continuing with sample preparation.

Define and save a PCR cycling program using the instructions in Section 6.

Note: It is recommended to wait for 30 minutes after the machine is turned on to provide initial heat block warm-up time before starting the first cycling program.

Verify if the correct accessories (Pipetting Anvil, Sealing Anvil) and consumables (Microplates, Seals) are available for the experiment according to the List of Required Materials.

MONITORING A RUN

- · Insert the sealed Microplate in the loading tray of the NextGenPCR[™] Thermal Cycler and close the lid.
- Start your PCR cycling program by selecting it from the list of preprogrammed PCR cycling programs and pressing 'Run'.
- The instrument will now start and run the indicated PCR cycling program.
- During cycling, the temperature of the heating zones is indicated on the screen and is displayed in GREEN when the temperature specified by the user is equal to the measured temperature in the heat block.

RETRIEVING SAMPLES FROM A MICROPLATE

- Transfer the Microplate from the NextGenPCR[™] Thermal Cycler loading tray to a microtiter plate centrifuge (or equivalent) and spin down for 30 seconds to concentrate liquids at the bottom of the wells.
- Place the Microplate on your Pipetting Anvil.
- Using a single- or multichannel pipette, carefully pierce through the Aluminum Heat Seal and retrieve the sample volume from the well.
- **Note:** A sample volume loss of up to 10% can occur after a Microplate has been thermally cycled, e.g., when 20 μl reactions are prepared, a maximum of 18 μl can be retrieved from the microplate.
- Transfer the volumes to a clean tube or PCR microplate for storage or downstream use.

8. DEVICE COMPATIBILITY

Accessory	-rrieve "Auminum Fr	(#400 Pipetting Angola) (#20304), #20304)	HALL HILLS	pipertine Anull S	Sealing Annie Staling Annie Hilling Annie	Sealing Annu (#201021) :: 96 * 514	Sealing Ann.	Imaging Anvi	Imaging Anuli (TODSOL) il 96 × 544	Imaging Annu John Zohl	
Microplate Format		104)	96 × 511	* 2011	14 × 5111	16×511	16 * 2011	184 × 5H1	36×511	6* 2011	384 × 541
24 × 20µl (#31212, #31213, #32212, #32213)	~	~		~			~			~	
24 × 50µl (#31222, #31223)	~	~		~			~				
48 × 20μl (#31412, #31413)	~	~		~			~			~	
48 × 50µl (#31422, #31423)	~	~		~			~				
96 × 5µl (#31662, #31663)	~	~	~			~			~		
96 × 12.5µl (#31652, #31653)	~	~		~			~			~	
96 × 20µl (#31612, #31613, #32612, #32613)	~	~		~			✓			~	
384 × 5µl (#31812, #31813, #32812, #32813)	~	~			~			~			~
EZtrieve™ 96 × 20μl (#33602, #33603, #33604, #33605)	•	•		~			~			~	

IMPORTANT! The NextGenPCR[™] Microplate is the only type of microtiter plate that is compatible for use with the NextGenPCR[™] Thermal Cycler. No other microplates can be used due to crucial differences in material composition.

For optimal performance, NextGenPCR™ reagents are recommended.

Reagent	Туре	Catalogue number
NextGenPCR™ Arctic Fox HF Chemistry 2×	DNA polymerase	50050
NextGenPCR™ RT-PCR Chemistry 2×	Reverse transcriptase + DNA polymerase	50005

9. TROUBLESHOOTING

The NextGenPCR[™] device is an instrument designed to amplify DNA samples through the polymerase chain reaction process. While the machine is designed to work efficiently and reliably, occasional issues may arise. This chapter provides troubleshooting steps to help you identify and resolve common problems encountered during PCR experiments.

IMPORTANT! Before proceeding with troubleshooting, ensure that you have followed all the instructions provided in the previous chapters, including proper setup, sample preparation, and reagent handling.

DANGER! Under NO circumstance should the end user remove the outer cover of the instrument by themselves. The NextGenPCR[™] Thermal Cycler should ONLY be dismantled by trained professionals. Removing the outer instrument cover could expose the user to high voltage surges that can be detrimental to personal health.

Possible Issue	POTENTIAL CAUSE	PROPOSED SOLUTION
	Instrument not connected	Connect instrument via a power cable
Instrument does not power on	to power network	Ensure stability of power connection
	Blown fuse	Request service
Instrument screen does	Unresponsive software	Turn the instrument off/on
not function correctly	LCD screen failure	Service request
Instrument displays negative cycling time	User error in program specification	Verify correct PCR cycling conditions in program setup menu
numbers in program setup	Software issue	Request software update
Instrument does not begin cycling after pressing Run after pressing Run	Lid is not closed	Close the lid

Possible Issue	POTENTIAL CAUSE	PROPOSED SOLUTION
Instrument does not	Temperature calibrator does not function correctly	Request calibration
reach set temperatures during a run	Invalid cycling program	Verify correct PCR cycling conditions in program setup menu
	Broken heater	Service request
	Power failure during a run	Service request
Instrument runs successfully but no	User error in reaction setup	Verify reaction set up and whether no degradation of components (template, primers, polymerase) has occurred fragments are produced
fragments or non-specific	Poor assay design	Verify primer specificity to target template or optimize cycling conditions
	Incompatible consumables were used	Use recommended NextGenPCR™ consumables and accessories
Microplate is stuck in instrument after a run or instrument produces grating noise during a run	Microplate jammed in machine interior	Attempt to retrieve microplate from sample loading area. If the problem persists, request service
Microplate sample wells	error in microplate sealing	Verify if microplate sealing was
are empty after a run	Contamination of instrument interior with PCR reagents	Use NextGenPCR™ Cleaning Kit See Section 10.3 Maintenance and Servicing
Microplate surface is dirty/sticky after a run	Mechanical wear or manufacturing error	Service request
	Instrument not connected to network via ethernet cable	Connect instrument via ethernet cable
Cannot remotely connect		Ensure network signal stability
to instrument	Bad ethernet cable	Verify if ethernet cable works correctly or use different cable
	Software issue	Request software update

10. MAINTENANCE AND SERVICING

10.1 Storage

Choose a suitable storage location that is clean, dry, and free from dust, humidity, and direct sunlight. A temperature-controlled room is preferable to avoid extreme temperature fluctuations.

Avoid placing the instrument near heat sources or in areas prone to moisture.

Ambient storage temperature shall not exceed -5°C to +30°C.

Before storing the thermal cycler, make sure it is powered off and unplugged. This helps protect the internal components and extends the lifespan of the machine.

Dust cover or protecting lid is advisable, to protect the cycler from dust and potential spills.

10.2 Handling

LIFTING AND CARRYING

When lifting the thermal cycler, use proper lifting techniques to avoid strain or injury. Bend your knees and keep your back straight while lifting. Use both hands to hold the instrument securely. If the thermal cycler is particularly heavy, consider using a trolley or seeking assistance from another person.

AVOID DROPPING OR BUMPING

The thermal cycler is a sensitive laboratory instrument with delicate internal components. Avoid dropping or bumping the instrument to prevent damage.

TRANSPORTATION

If you need to move the thermal cycler to a different location, ensure that it is adequately secured during transportation. Use padded or cushioned materials to protect the instrument from any potential impacts. It is recommended that the original packaging is stored upon purchase and used during any subsequent transportation.

10.3 Maintenance

WARNING! Before cleaning the outer case of your instrument, disconnect it from the power supply.

The outer case of the NextGenPCR[™] machine may be cleaned with a cloth dipped in water with some soap. Use mild, non-abrasive cleaning agents, e.g., soap, multipurpose cleaner, or ethanol (max. 70% v/v). Avoid using harsh chemicals (i.e., acetone, bleach) that may damage the surface of the instrument. Pay particular attention to removing any spilled reagents, dust, or residues.

IMPORTANT! Do not use aggressive solvents such as acetone or abrasive cleaners. No part of the case or cover should be immersed in solvents.

INTERIOR CLEANING

The inside of the instrument shall be cleaned by running a special cleaning protocol and a dedicated MBS NextGenPCR[™] Cleaning Kit.

(Product Number #35001 https://www.nextgenpcr.com/product/cleaning-kit/)

Before using any cleaning or decontamination method except those recommended here, the person responsible should check with MBS that the proposed method will not damage the equipment.

IMPORTANT! Do not use any cleaning agents or methods other than the NextGenPCR[™] Cleaning Kit to clean the interior of the instrument. The Cleaning Kit contains specialized chemical cleaning buffers to remove contaminants from the active elements inside the NextGenPCR[™] instrument with minimal risks of adversely influencing the instrument.

After cleaning, allow the thermal cycler to dry completely to avoid any moisture-related damage.

The frequency of interior cleaning depends on the instrument's use with a suggested cleaning interval of once every three months for optimal performance.

Both external and internal cleaning are configured for client-friendly execution.

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10.4 Scheduled Maintenance and Servicing

A client who purchased the NextGenPCR[™] Thermal Cycler has the following options to address the maintenance and servicing:

- 1. Pay-as-you-go program, where the client chooses from the list of available services according to the need.
- 2. Annual preventive maintenance program as a single package one time per year.

The maintenance program includes the following:

- · General Inspection: Thorough inspection of the Equipment.
- Temperature calibration: Ensuring the Equipment's calibration accuracy.
- Temperature verification: Validating the Equipment's performance.
- Cleaning of the interior
- Cleaning of the exterior
- · Condition and tension of belt drive check
- Lubrication of moving parts
- · Software upgrades (if applicable)

Any replacement parts or materials required for the instrument maintenance or repair are not included in the program.

For more detailed information about the available Maintenance program, contact support at supportambspcr.com

CAUTION! All adjustments and maintenance must be performed by qualified service personnel.

RETURNING THE INSTRUMENT FOR SERVICING

In the event of returning the instrument for servicing, please see the instructions in Section 1.6 Return of the instrument.

FUSE REPLACEMENT

IMPORTANT! Fuse shall be replaced only by a qualified electrician.

Power of and unplug the instrument. Remove the fuse drawer. Use the spare fuse as a replacement.

The anti–surge fuse is located at the rear of the machine, beside the power switch. The value of the fuse is T 6.3A, 250V.

Exchange the faulty fuse in the fuse holder for a working fuse of the correct value. Finally, replace the fuse drawer in the fuse compartment and push the drawer shut.

IMPORTANT! Fuses which blow repeatedly may indicate a serious fault, and you should contact your local distributor for repair.

10.5 Device disposal

Device disposal: Before disposing of the thermal cycler, unplug it from the power source and remove any consumables or accessories. Contact your local electronic waste recycling facility.

Consumable disposal: Dispose of consumables, including PCR plates and chemical reagents, in accordance with the guidelines provided in their respective product documentation. Adhere to local regulations regarding the disposal of hazardous materials.

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