Mastercycler® / Mastercycler® gradient

Authorized Thermal Cycler

This instrument, Serial No. , is an Authorized Thermal Cycler.

Its purchase price includes the up-front fee component of a license under the foreign counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188, owned by F. Hoffmann-La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that

Contents

1 Introduction

The Eppendorf Mastercycler® and Mastercycler® gradient are PCR devices for molecular biological and biochemical research laboratories.

Temperature control is carried out with Peltier elements, which enable extremely rapid changes in temperature of up to 3 °C per second. This allows the temperature of the samples to be controlled extremely rapidly between 4 °C and 99 °C.

A special "tube control" mode has been developed for temperature-controlling samples in various different tubes with various different filling volumes.

The thermoblock has been constructed as a universal block which can accommodate microtiter plates in the 8 x 12 format, 96 Eppendorf PCR test tubes of 0.2 ml, 77 thin-walled Eppendorf PCR test tubes of 0.5 ml or other suitable tubes – without the block having to be exchanged.

With the aid of the *in situ* Adapter, *in situ* reactions may be carried out on up to four glass slides.

To prevent the formation of condensation, the devices have a heated lid which has been perfectly tailored to suit all tubes.

The devices are easy to operate, with user guidance available in the integrated, eight-line display. Experiments and tests can be documented with the aid of a printer which may be connected up to the devices.

One particular advantage of the Mastercycler® gradient is the Gradient function, which greatly facilitates the optimization of PCR experiments. The gradient enables the temperature across the entire block to be varied within a range of up to 20 °C; it can be programmed with every temperature step.

The Mastercyclers are authorized cyclers licensed by the Perkin-Elmer Corporation.

Before using the Mastercycler® or Mastercycler® gradient for the first time, please read the complete operating manual. The Mastercycler® or Mastercycler®

Transfer

If the device is passed on to someone else, please include the instruction manual.

Disposal

In case the product is to be disposed of, the relevant legal regulations are to be observed.

The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC on waste electrical and electronic equipment (WEEE).

According to these regulations, any devices supplied after 13.08.05 in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. They are marked with the following symbol to indicate this.

As disposal regulations within the EU may vary from country to country, please contact your supplier if necessary.

3 Installation

3.1 Delivery package

The delivery package contains the following items:

1 Mastercycler® or Mastercycler® gradient
1 Main power cable
1 Operating manual
1 Personal card
1 bag of 0.2 ml PCR test tubes (100 pieces)
1 bag of 0.5 ml PCR test tubes (100 pieces)

3.2 Setting up the device

When setting up the device, please ensure that enough space is available to allow the ventilation slit to remain uncovered and to allow air to flow under the device for cooling purposes. Please ensure that no objects are under the device (e.g. lab bench paper).

No special equipment is required for transporting the device. It can be lifted up and carried by being held on both sides.

Space requirements:	Width:	26 cm
	Depth:	41 cm
	Height:	27 cm
Mains connection:	1 shockproof socket for the Mastercycler® / Mastercycler® gradient. If a printer is to be connected up, a second mains connection must be used.	

The delivery packaging should be stored in a safe place to enable the device to be shippeded in the event that repairs are necessary.

3.3 Starting up the device

Remove the adhesive strip on the heated lid and take out the bubble-wrap from below the heated lid.

The Mastercycler® / Mastercycler® gradient is connected to the mains supply using the mains cable.
Before starting up the device, please compare the power supply with the voltage requirements listed on the identification plate (see Sec. 4.1, Fig. 3).

The procedure for connecting and starting up a printer is described in Sec. 10.1.

4 Technical description

4.1 Device construction

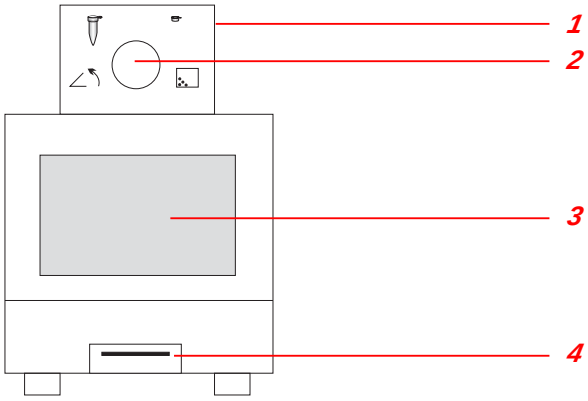


Fig. 1: Front view

1 Heated lid
2 Locking button
3 Display and control panel
4 Personal card reader

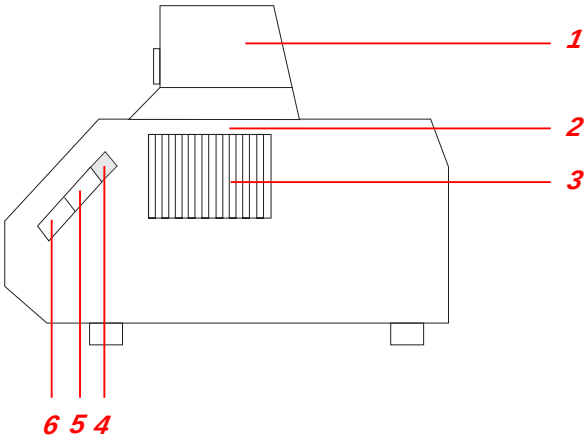


Fig. 2: Side view

1 Heated lid
2 Thermoblock
(not visible in this picture)
3 Ventilation slit
4 Brightness regulator
5 PC connection socket
6 Printer connection socket

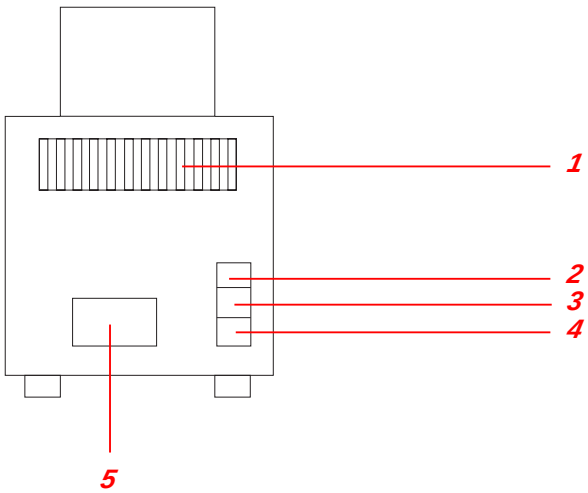


Fig. 3: Rear view

1 Ventilation slit
2 Main power switch
3 Fuses
4 Main power socket
5 Identification plate

Fig. 4: Display and control panel

1 Programming keys
2 Cursor keys
3 Control keys
4 Digit entry block, decimal point and reverse sign key
5 Display

4.2 Keys

Programming keys

– When this key is pressed, the approximate remaining run time of a program and the time of the expected end of the program appears in the display for approximately five seconds.
– To select programming options for the "Temperature" command (ramp, gradient, increment).

– To delete program lines, digits or letters or to reset parameters. Holding down the key deletes not only individual letters but also a complete name.

– To select a program command or to select from a list.
– A menu item can be selected using the key instead of the keys.
– The key can also be used to select from a list and to enter the letters of program names. The direction of selection of the letters can be changed by pressing the key.

5 Description of menus

Main-Menu

Start	***(1)***		***To start programs***
FILES . .	(2)		To program
	Edit	(1)	To change a program in the processing level
	Load	(2)	To load and change any program
	Standard	(3)	Example program
	New	(4)	To create new programs
	Delete	(5)	To delete programs

OPTIONS . .

5 Description of menus

5.2 Start

Only programs can be started from the "Start" menu.

The program displayed in the processing level can be started directly by pressing the Start Stop key (1).

If a program which has been stored has to be started, it is selected with the cursor keys from the displayed list after E has been pressed (2) and cofirmed by pressing .

The "Start" menu has no sub-programs.

5.3.2 Load

"Load" is used to call up a list of existing programs. These programs may be taken from the internal memory or from a personal card. The selected program is loaded into the "Edit" processing level. If the "Edit" processing level contains a program which has not been saved, a safety check appears in the display, which then enables the program to be saved.

If the user does not want to save the program, it is overwritten when the new program is loaded. As the "Edit" processing level is called up automatically, the program can be changed immediately and can then be tested for test purposes.

The procedure for loading programs from a personal card is described in Sec. 9.3.

5.3.3 Standard

An example program called "Standard" is available for creating new programs. It is not necessary to rewrite a program completely; the example program can be supplemented or changed and then saved under a new name. All programs which are in the internal memory can be used as an example program.

Following a safety check, the example program is loaded into the processing level. The process is described in more detail in Sec. 7.4.

Although the "Standard" example program cannot be deleted, it can be changed to suit individual requirements. After the changes have been made, the example program is saved under the name "Standard". To restore the original example program, the "Standard" program must be deleted in the"Delete" sub-menu. When "Standard" is called up again, it is the original example program which is then loaded into the processing level.

5.3.4 New

"New" can be used to call up the processing level automatically. To create a new program, an existing program is deleted.

The essential input CNTRL BLOCK, Lid =0°, NOWAIT AUTO, end is simulated automatically and the program is given the provisional title UNNAMED.

5.3.5 Delete

Programs can be deleted from the internal memory or from a personal card. After "Delete" has been selected, a list of program names appears in the display. A program can be selected from this list using the cursor key and can be deleted by pressing Enter. The program in the processing level cannot be deleted.

The procedure for deleting programs from a personal card is described in Sec. 9.3.

5.4 OPTIONS

The "OPTIONS" menu can be used to define general system settings for the program editor, printer, time/date, etc.

Selection of YES/NO, ON/OFF and other settings can be carried out using the key.

The menu is exited by pressing the key.

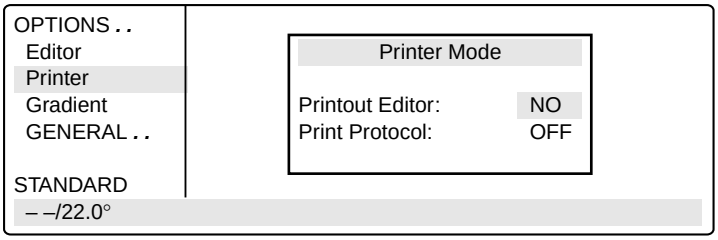
5.4.1 Editor

Show Ext. Steps To display temperature options.

YES All options (temperature increment, time increment, ramp, ramp increment, gradient) for each step appear in the display.

NO Only the nominal temperature and the cycle time appear in the display. If the options have been defined, the command is marked with an "*" for information purposes when the program is called up. Nonetheless, the options can be called up by pressing the key and can then be entered or displayed.

5.4.2 Printer



Entries are necessary only when a printer is connected.

Printout Editor

YES: The program in the "Edit" processing level is printed out.
If "YES" has been selected, but no printer is accessible, the error message "Printer: No Response" appears in the display.

NO: Appears automatically when the program printout has finished.

Print Protocol

ON: When the program starts, the commands are printed out; during the run, every completed command is printed out together with the time.

OFF: No printout is made.

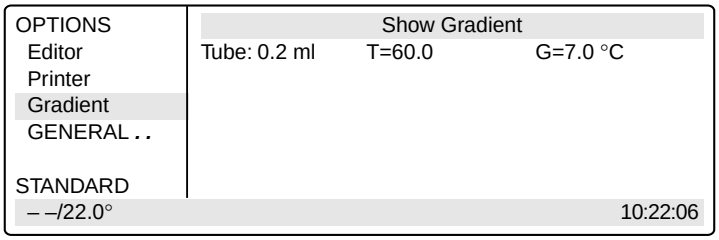
5.4.3 Gradient

The Gradient function can be selected with the Mastercycler® gradient only.

The Gradient function can be used to vary the temperature distribution across the block.

In every temperature step, the thermoblock can be programmed so that each column has a different temperature. The lowest temperature is in the cavities on the left-hand side. It then rises gradually across the block so that the highest temperature is on the right-hand side.

Show Gradient The "Show Gradient" menu can be used to show which temperature is in which column:



The tube type "0.2 ml, 0.5 ml, 0.5 ml thin, plate" is selected by and confirmed by pressing .

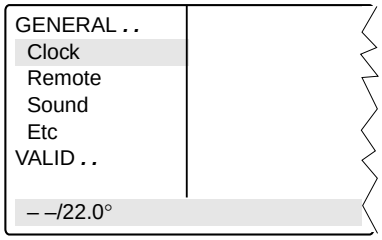
After a temperature and a gradient have been entered, the temperature distribution across the block appears in the display:

The gradient may be a maximum of ± 10 °C with a resolution of 0.1 °C. This means that the overall temperature span is 20 °C. The selected temperature is in the middle of the block.

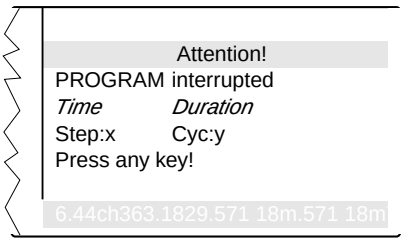
The left-hand side is temperature-controlled to nominal temperature – gradient
The right-hand side is temperature-controlled to nominal temperature + gradient.

5.4.4 GENERAL

To enter general settings and to test the device.

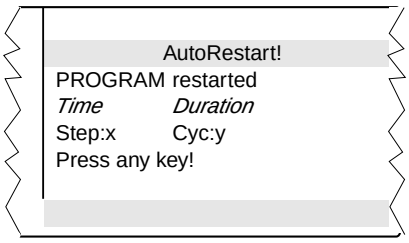


Clock	Format:		The time in the 24-(military) or 12-hour-clock format (choice of PM or AM) can be selected using the Sel key.
	Time:		To enter the current time in hours:minutes:seconds.
	Date:		To enter the current date as day/month/year. The month can be selected using the Sel key.
Remote	Baud rate:		The transmission rate of 19,200, 9,600, 4,800, 2,400 or 1,200 for a PC connection can be selected using the Sel key.
Sound	KeyClick:	ON:	Every keyclick is confirmed by an acoustic signal.
		OFF:	The acoustic signal for keyclicks is switched off.
	Warnings:	ON:	In addition to the warning text, warnings are acknowledged by an acoustic signal.
		OFF:	No acoustic signal is emitted to acknowledge a warning.
			Note: Errors are always acknowledged by an acoustic signal, irrespective of this setting.
Etc	Start with:	MAIN:	After the device has been switched on, the main menu appears in the display.
		LAST:	After the device has been switched on, the most-recently used menu appears in the display.
	Auto Restart:	NO:	A program interrupted by a power failure is not continued.



YES: Depending on the duration of a power failure, an interrupted program will automatically continue or can be manually continued or stopped.

***Power failure < 3 min**:*
A currently running program automatically continues if power is restored within 3 minutes of the commencement of the power failure. A display indicates when, how long and at which step the program was interrupted:



When any key is pressed, the display jumps back to the program view. If the program is to be cancelled, press the S key and confirm by .

***Power failure > 3 min**:*
In the event of a longer power failure, an aucistic warning signal is given as soon as the power supply returns, and the program can be manually continued or stopped.

If the interrupted program is to be continued, select "YES" with and confirm with . Press any key to return to the program view.

If the program is to be discontinued, select "NO" with and confirm with .

VALID . . These programs can be used to test the device. The measurements can be documented by connecting a printer to the device.
The "Validate" and "Calibrate" menus require the temperature-validation system (order no.: 5331 222.005). This system consists of a special temperature sensor and a personal card with the corresponding software.
For precise information on operating the "Validate" and "Calibrate" programs, see the operating manual for the temperature-validation system.

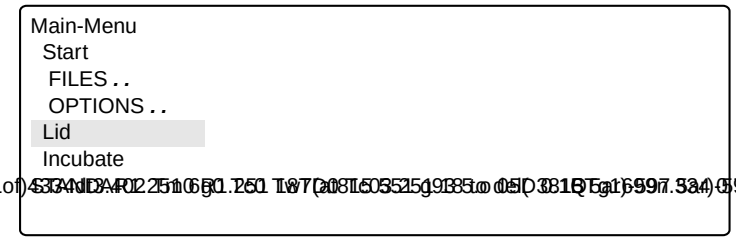
Validate: To check the device temperature with the aid of a temperature-measuring device. This measuring device forms part of the temperature-validation system and is connected via the serial interface of the Mastercycler.

Calibrate: To align the device temperature with the aid of a temperature-measuring device. This measuring device forms part of the temperature-validation system and is connected via the serial interface of the Mastercycler.

5 Description of menus

5.5 Lid

The "Lid" menu in the main menu can be used to determine the temperature of the heated lid and to switch the heated lid on or off (e.g. to preheat the lid).



LidTemp:	Nominal/actual temperature.To enter the temperature for the heated lid. The lid heating switches off when a temperature value of 0 °C is entered.
Heater is On:	Lid heating is on.
Heater is Off:	Lid heating is off.

When sensitive PCRs are carried out, it may be important to delay the start until the lid temperature has prevented condensation. This can be achieved by programming the "WAIT" setting, which means that the program does not start until the lid reaches the specified temperature.

In addition, the lid can also be preheated. After the device has been switched on, the "Lid" menu is selected, the desired lid temperature is entered and "Heater is On" is selected by pressing the key.

When a program starts, it is the program settings which have priority.

When a program is running, the lid heating cannot be switched on or off.

5.6 Incubate

The "Incubate" menu in the main menu is used to determine the block temperature and to switch on/off the temperature control of the block. (e.g. for incubation experiments or to preheat the block). The temperature of the heated lid is aligned to that of the block.

BlockTemp:	Nominal/actual temperature. To enter the temperature for the thermoblock. It is possible to enter values from +4 °C to 99 °C. It is not possible to select a gradient.
Heater is On:	Temperature control of the block is on.
Heater is Off:	Temperature control of the block is off.

Another option is to reduce the program run time if the thermoblock and the heated lid have reached the required temperature before the program starts.

To bring the thermoblock and the heated lid to the desired temperature, select the "Incubate" menu after the device has been switched on, enter the desired block temperature and select "Heater is On" using the key.

If "Incubate" is running, the "Lid" menu cannot be selected.

When a program starts, it is the program settings which have priority.

When a program is running, the temperature control of the block cannot be switched on or off.

To interrupt: If "Heater is On" is in the display, set the device to "Heater is Off" using the key and confirm by pressing .

Caution: If the "Incubate" menu has been selected, the thermoblock and the heated lid may be hot when the tubes are inserted, which means that there is a high burns risk for the user.

6.1.2 Sample volume

T

6.2 Switching on the device

– Switch on the device using the main power switch on the rear of the device.

  The current software version appears briefly in the display.
  The main menu then appears.

  The program which is ready to be started appears in the processing level.

6.3 Starting a program

–

If the program contains the "CNTRL/TUBE" command (temperature control set to sample), it is necessary to enter the tube type and the filling volume after the program has started.

– Select the tube type: 0.2 ml, 0.5 ml (e.g. Safe-Lock), 0.5 ml thin (thin-walled tubes), plate (microtiter plate) or *in situ* using the Sel key and confirm by pressing Ent.

– Enter the filling volume and confirm by pressing .

  Tube type 0.2 ml/plate: 5 to 50 µl
  Tube type 0.5 ml/thin: 5 to 100 µl
  in situ: no entry necessary

  If no entries are made for "Tube" and "Fill.Vol.", the program will not run. "EnterTube" appears in the main menu instead of the program name and a long acoustic signal is emitted until the entry is made.

  After the start of the program and while it is running, the name of the program appears in the main menu in alternation with "Running".

  A flashing temperature display indicates that the nominal temperature has not been attained and that heating/cooling is currently taking place.

– The display of a program can be exited at any time (e.g. to program in the "Edit" processing level) by pressing .

6.4 Displaying the running time of a program

While a program is running, the approximate run time and the expected end time of the program can be shown in the display.

– Press o.

The key is effective only when a program run appears in the display, not in the main menu, with other menus or sub-menus.

6.5 Interrupting a program

A program can be aborted, paused or continued at any point by pressing .

– Press .

Using the key, select between:

Stop	To abort the program
Pause	To interrupt the program
Run	To return to the display of the program sequence

– Using the key, move to "Pause".

– Confirm "Program:PAUSE" by pressing .

The program is interrupted, time counting stops and the last nominal temperature is retained.

The name of the interrupted program appears in the main menu in alternation with "Paused" and the "Start" menu changes to the display "Stop".

6.6 Continuing an interrupted program

The name of the program appears in
the display in alternation with "Paused"

– Press ◯.

Using the key, select between:
Stop To abort the program
Pause To interrupt the program
Resume To continue the program

– Using the key, move to "Resume".

– Confirm "Program:RESUME"
by pressing ;
the program is continued.

6.7 Aborting a program

– Press .

– Confirm "Program:STOP"
by pressing .

6.8 Switching off the device

After a program has been completed,
the following appears in the display:

If a new program has to be started, exit
the old program by pressing .

To switch off the device, move the main
power switch on the rear of the device to .

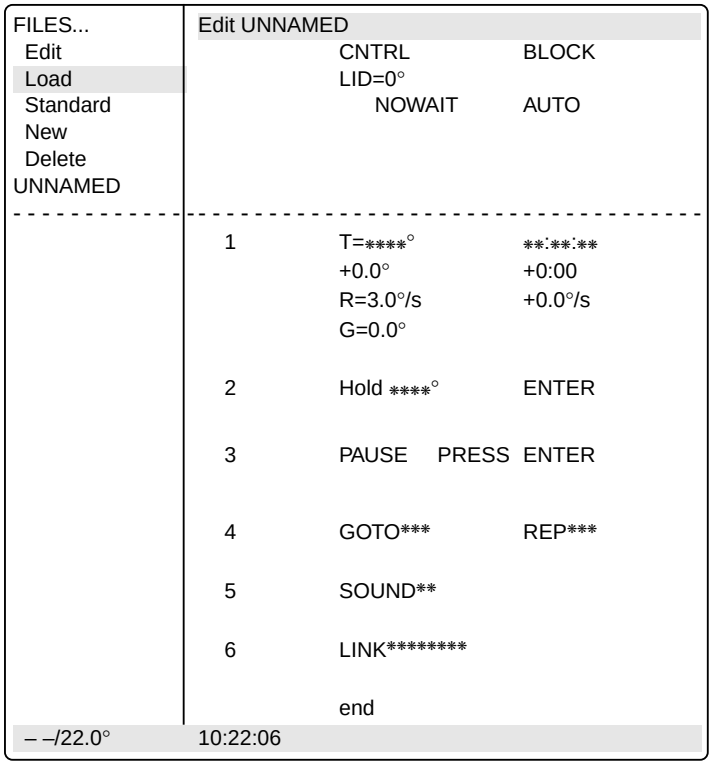
7.1 Command description

A program contains two basic commands for controlling the temperature of the thermoblock and of the heated lid as well as six different commands for programming.

A program can contain up to 40 program lines. The commands may be repeated as desired.

Digits or letters may be entered or selections are made using the Sel key.

When digits are entered, the message "Value out of range" appears when values are entered which are not within the given specifications. The permitted limit value is then entered automatically.



7.1.1 CNTRL

With this command is determined the type of temperature control for the block.

BLOCK The temperature is measured on the thermoblock and the nominal temperature is then set.

TUBE The software adapts the temperature of the thermoblock to the temperature of the tubes and the sample quantity. For this reason, the tube type and the filling volume must be entered immediately after the start of the program. No further entries are necessary when *in situ* is selected.

Tube type	0.2 ml or plate:	5 to 50 µl filling volume
	0.5 ml or 0.5 ml thin:	5 to 100 µl filling volume

7.1.2 Lid

To determine the temperature of the heated lid. If 0 °C is selected, the heated lid remains switched off.

Permitted values	0 to 110 °C
Entry increments	1 °C

In addition to the entry for the temperature of the heated lid, it is also possible to determine the behavior of the lid temperature at the start and at the end of a program:

At the start

NOWAIT The program is started, independent of the lid temperature.

WAIT The program is not started until the programmed lid temperature has been attained.
Note: To accelerate the start of the program, the lid may be preheated using the "Lid" menu.

At the end

FIX The lid temperature does not depend on the block temperature. The lid heating is switched off at the end of the program.

AUTO The lid temperature depends on the block temperature. If 22 °C is maintained for longer than five minutes, the lid heating switches off; this is also applicable in the case of a Hold command at temperatures < 22 °C.

The six commands for programming can be called up directly using the allotted number.

7.1.3 T Selection also possible using 1

To enter the temperature and the cycle time as well as the accompanying specific options.

The options are entered by positioning the cursor on the program line number and pressing the key.

The options may be entered for every temperature command.

T = *°***	*****:**:*****	Nominal temperature, cycle time
+ 0.0 °	***+ 0:00***	Temperature increment, time increment
R = 3 °/s	***+ 0.0 °/s***	Ramp, ramp increment
G = 0.0		Gradient (for Mastercycler® gradient only)

T = *°*** The nominal temperature is entered in degrees Celsius (°C).

Permitted values	4 to 99.0 °C
Entry increments	0.1 °C

::** The cycle time is entered in "hours:minutes:seconds".

Permitted values	0:00:01 to 9:59:59
Entry increments	1 s

± 0.0 ° Temperature increment (

7.1.4 HOLD Selection also possible using 2

The program holds the temperature at the value which was entered. The program is continued or ended by pressing .

Note

menu
key
ing Enter.

u using

Enter.

.

, keys.
d "Auto" (key)
ing .

and T (Temperature)
key (it may be
to press the key
nes)
firm by pressing .

er the temperature,
ess the , keys
and confirm by pressing .

Main-Menu	
Start	
FILES . .	
OPTIONS . .	
Lid	
Incubate	
STANDARD	
– –/22.0°	10:22:06

FILES . .	Edit UNNAMED		
Edit		CNTRL	BLOCK
Load		LID=0°	
Standard		NOWAIT	AUTO
New		end	
Delete			
UNNAMED			
– –/22.0°	10:22:06		

– Enter the time (seconds:minutes:hours).

  Press the , , keys and confirm by pressing .

Denaturation

T=95.0° 0:00:45

Programming: see page 30.

Annealing

0:00:45

key for options for the
e command (temperature- and
nent, ramp, ramp increment,

r must be on the program line
n front of the temperature
d.

ne , keys and confirm by pressing .

he keys and confirm by pressing .

to proceed to Gradient or Elongation.

with Mastercycler® gradient only.

er the gradient,
ss the , keys and
nfirm by pressing .

gation

2.0° 0:00:45

ct the command T.

Press the , keys and
confirm by pressing .

Press the keys and
confirm by pressing .

Cycle

GOTO 2 REP 19

– Select the GOTO command using the key (it may be necessary to press the key several times)
Confirm by pressing .

– Enter the line number for the beginning of the program section which is to be repeated.
Confirm by pressing .
Enter the number of repeats (REP).
Confirm by pressing .

Note: The total number of cycles is REP + 1.

Final elongation

T=72.0° 0:02:00

Programming see page 30.

Cooling of samples

HOLD 22.0° ENTER

– Select the HOLD command using the key (it may be necessary to press the key several times) and confirm by pressing .

– Enter the temperature. Confirm by pressing .

7.2.2 Saving a program

– Exit the "New" menu using the key.

– To save, press the key; the device asks for the program name.

In the case of a new program, the device suggests the name "UNNAMED".

– Confirm the name by pressing or – if an other name is desired – delete by pressing (if the key is held down, the entire name is deleted; if the keys is pressed briefly, individual letters only are deleted).

– Enter the new program name. Select letters using the key. Press the key to move to the next position etc. and confirm by pressing .

If the program is to be saved under an existing name, the question appears:

– "Overwrite:YES"
Confirm overwriting by pressing
Enter.

If you do not want to overwrite the existing program, select "NO" using the S key, press and enter a new name. Confirm the new name by pressing .

If a program is not saved ("Save:NO"), it remains in the processing level and can be supplemented or modified by calling up "Edit". Safety checks in other sub-items prevent a program which has not been saved from being accidentally overwritten.

The internal memory can accommodate a maximum of 99 programs. The number possible depends on the length of the programs.

The program in the processing level can be started by pressing the key.

Note: The procedure for saving programs on a personal card is described in Sec. 9.3.

7.3 Modifying a program

A program which is already in the internal memory or on a personal card can be modified by being loaded into the processing level. The program resident in the "Edit" processing level is thus deleted. If a program which has not yet been saved is in the processing level, it can be saved following a safety check prior to deletion.

– Select the "FILES" menu using the or key and confirm by pressing .

– Select the "Load" menu using the or key and call up by pressing .

A list of existing programs appears in the display.

– Select the program using the or key and load by pressing .

– Using the , , , keys or , move the flashing cursor onto the parameter which is to be changed.

– Enter data using the numeric keypad
or select between settings using the key
or enter letters using the key.

Inserting a program line

– Move the flashing cursor onto the number of the program line in front of which a new command is to be inserted.

– Press the key. A new line is inserted above the cursor.

– Select the wanted command using the key.

Note: Press the key for entering options regarding the temperature command.
In this case, the cursor must be positioned on the program line number in front of the temperature command.

Deleting a program line

– Move the flashing cursor to the program line number.

– Press the key. The line is deleted.

– After a program has been changed, exit the "Edit" processing level by pressing the key.

– Confirm the safety check "Save:YES" by pressing .

– The modified program can be saved using the same name, in which the old program is overwritten.

7.4 Creating programs using an example program

It is possible to program quickly with the aid of the "Standard" example program. A program does not need to be completely rewritten; the sample can be supplemented, modified and then saved under a new name.

– Select the "FILES" menu and call up by pressing .

– Select "Standard" and call up by pressing .

The example program is loaded into the processing level. It is structured in the following way:

	CNTRL		BLOCK	Temperature control for the block.
	LID=105°			Sets heated lid to 105 °C.
	NOWAIT		AUTO	NOWAIT = Program is started immediately. AUTO = Lid heating is switched off automatically when temperature (22 °C) is maintained for more than 5 minutes.
1	T=94.0°		0:02:00	Maintains 94 °C for 2 min. (initial denaturation)
2	T=94.0°		0:00:15	Maintains 94 °C for 15 secs. (denaturation)
3	T=44.0°		0:00:15	Maintains 44 °C for 15 secs. (annealing)
4	T=72.0°		0:00:30	Maintains 72 °C for 30 secs. (elongation)
5	GOTO 2		REP 24	Repeats 24 times from program step 2 onwards. The total number of cycles is 25.
6	T=72.0°		0:00:30	Maintains 72 °C for 30 secs. (final elongation)
7	HOLD	22.0°	ENTER	Cools down to 22

Although the example program cannot be deleted, individual changes can be made. After changes have been made, the example program must be saved under the name "Standard". To restore the original example program, the "Standard" program in the "Delete" sub-menu must be deleted. When "Standard" ís called up, the original example program is reloaded into the processing level.

7.5 Deleting a program

– Select the "FILES" menu and call up by pressing .

– Select the program name.

– Confirm by pressing . The program is deleted.

The procedure for deleting programs on a personal card is described in Sec. 9.3.

7.6 Examples of programming

The following passage contains several examples of the versatility of the commands and functions of the Mastercyclers.

7.6.1 Gradient PCR (for Mastercycler® gradient only)

The Gradient PCR is used to optimize temperatures in a PCR experiment. The gradient may be programmed with a temperature range of up to 20 °C with every temperature command.

The most common application is the determination of the optimal annealing temperature (see example), for which a gradient of, for example, ± 10 °C is built up. The recommended mean gradient is 5 °C higher than the calculated annealing temperature.

	Gradient PCR		
	Temperature control of tubes	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	94°	2 min
2	Denaturation	94°	15 sec
3	Annealing	60°	15 sec
	Gradient	***G = 10°***	
4	Elongation	72°	30 sec
5	Number of cycles	go to 2	Rep 29
6	Final elongation	72°	2 min
7	Cooling and storage	Hold 20°	enter
	End	end	

7.6.2 Using the "Pause" and "Hold" commands (Example: Hot Start PCR)

The "Pause" command is used to interrupt the program. The most-recently entered temperature is maintained. A text explaining the reason for the interruption may be entered as required. After confirmation by pressing Enter, the program is continued.

The "Hold" command enables all possible temperatures to be maintained until the program is continued by pressing Enter.

In the example of a Hot Start PCR listed below, the program is stopped folllowing initial denaturation in order to add a reaction component. After the final elongation, the block is cooled down to room temperature.

	Hot Start PCR		
	Temperature control of tubes	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	94°	2 min
2	***Pause for adding substance***	***Pause***	***Adding***
3	Denaturation	94°	15 sec
4	Annealing	55°	15 sec
5	Elongation	72°	30 sec
6	Number of cycles	go to 3	Rep 29
7	Final elongation	72°	2 min
8	Cooling and storage	Hold 20°	enter
	End	end	

7.6.3 The temperature increment (Example: Touch Down PCR)

The temperature increment, which can be programmed as desired with every temperature command, allows the temperature to be reduced or increased by a defined value with every cycle.

Touch Down PCR can be used to increase the specificity of the PCR by changing the the annealing temperature in succession from higher to lower temperatures.

In the example given, the annealing temperature is reduced by 1 °C per cycle for the first 16 cycles, until 50 °C has been attained. At this annealing temperature,14 additional cycles are carried out.

7.6.5 Regullating the temperature-control speed (Example: RAPD-PCR)

Variable heating and cooling rates, which can be programmed as desired with each temperature command, enable the temperature-control speed to be aligned to the temperature.

With the RAPD-PCR process or with PCR with A/T-rich primer/template hybrids, it may be necessary to heat up carefully after annealing.

In the example given, the elongation temperature is attained at a speed of 1 K/s.

	RAPD-PCR		
	Temperature control of tube	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	94°	2 min
2	Denaturation	94°	15 sec
3	Annealing	55°	15 sec
4	Elongation	72°	30 sec
	Heating rate	***R = 1 °/s***	
5	Number of cycles	go to 2	Rep 29
6	Final elongation	72°	2 min
7	Cooling and storage	Hold 20°	enter
	End	end	

Temperature-control speeds, which may be as slow as the user desires, can be selected by means of program loops, with which a gradual change in temperature is obtained via temperature increments.

In the following example, the elongation temperature is raised slowly to 72 °C after annealing with the aid of a program loop.

	RAPD-PCR		
	Temperature control of tube	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	94°	2 min
2	Denaturation	94°	15 sec
3	Annealing	55°	15 sec
4	Elongation	56°	7 sec

7.6.6 Sample cooling and subsequent PCR

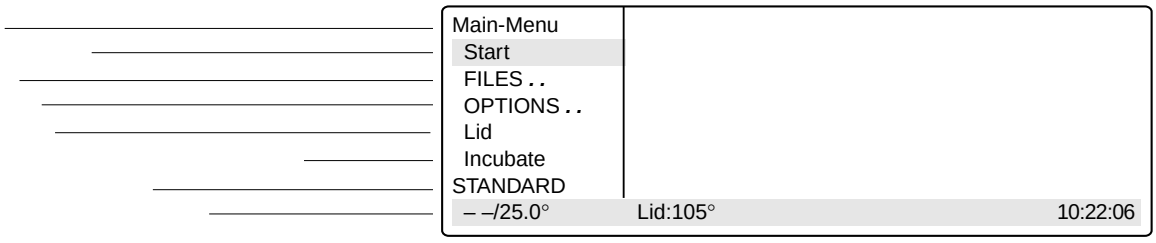
If sensitive samples should remain cooled until the PCR takes place, the thermoblock must be preheated. This can be carried out in two ways:

1. Set the "Incu" menu to 4

8 Short instructions

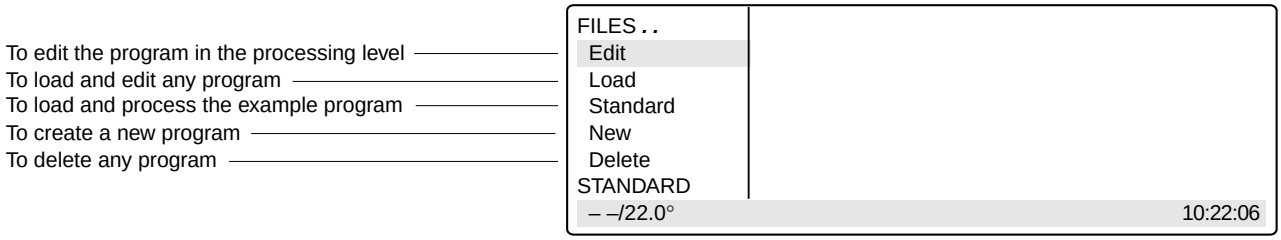
It is essential to read the operating manual completely before working with the short instructions.

Switching on the device



Programming

– Select the FILES menu using the ▲ / ▼ keys and press Enter.



Changing the "Standard" example program

– Select "Standard" using the ▼ key and press Enter.

– Using the ▲, ▼, ◯, keys, move the cursor to or betweeging t37.7/O0dL5555c0n1working with the .nsTo start any program

t

Programming
System settings
To preheat the lid
To temperature-control block and heated lid
Program in processing level
Nominal/actual block temperature
Lid temperature
(only when lid is switched on)

8 Short instructions

9 Personal card

Depending on the length of the program, a maximum of ten programs can be stored on a personal card. When a program is started from a personal card, the program is loaded into a temporary memory (but not into the internal memory or into the processing level) for the duration of the program processing.

Programs can be transferred to other devices of the Mastercycler®, Mastercycler® gradient and Mastercycler® personal using the personal card.

For the "Start", "FILES/Load", "FILES/Edit" and "FILES/Delete" menus, a difference will be made between the memory of the personal card ⟨MCARD⟩ and the internal memory of the Mastercycler® ⟨INTERN⟩ only when the personal card has been inserted.

Menus "Start", "FILES/Load" and "FILES/Delete"
Only the programs of a memory appear in the display. The memory which is currently closed appears in the display at the head of a program list as ⟨INTERN⟩ or ⟨MCARD⟩. The closed memory can be opened by selecting and then confirmed by pressing En. This also closes the previous memory.

Menu "FILES/Edit"
The memory target (Target) must be selected using the Sel key before the program name is determined.

9.1 Safety precautions

The gold-colored contact area on the personal card must not be damaged, scratched or contaminated in any way. Avoid touching the contact area with your fingers.

Electrostatic charges can destroy stored programs.

9.2 Operation

Inserting the card

– With the arrow facing forwards and the gold-colored contact area facing upwards, insert the card into the slot under the control panel.

– Insert the card up to the stop. It clicks into place automatically.

Removing the card

– Insert the card up to the stop.

– The card is pushed out by spring pressure and can then be removed.

Formatting the card

A new card must be formatted. The request for formatting appears automatically in the display only when it is necessary. The request for formatting is also made if the card has been erroneously inscribed.

– "Memory card not valid"
Confirm "Format: Yes" by pressing Enter.

The nominal/actual steps appear in the bottom line of the display.

9.3 Processing programs

Starting a program from a card

– Select the "Start" menu item and call up by pressing .

– Select ⟨MCARD⟩ and call up by pressing .
If the memory name ⟨INTERN⟩ appears in the display instead of ⟨MCARD⟩, this means that the program memory of the personal card has already been opened.

– Select a program and call up by pressing .

Note: For starting purposes, programs are not loaded into the internal memory or into the "Edit" processing level. After the program has ended, the program which has been started by the card is no longer available on the device.

Deleting a program from a card

10 Interface description

10.1 Printer / PC connection

Printer connection

The printer connection socket (25 pins, Centronics parallel interface, PC compatible) is located below the PC connection socket on the right-hand side of the device (see Sec. 4.1, Fig. 2.6).

Connect the Mastercycler® or Mastercycler® gradient and printer via a commercially available PC printer
Print Protocol: ON

Sel

Enter

The program sequence is printed out.

10 Interface description

Example of a program printout:

✲✲ M A S

Example of a program protocol:

Prior to the start, the program is printed (see page 46) and during the program sequence, all temperatures and times are printed in a protocol.

Program ... started at	–	Date and time of start of program
Initial Blocktemp	–	Block temperature at start
Initial Lidtemp	–	Lid temperature at start
Run Time	–	Estimated run time
Finish Time	–	Estimated time for end of program
Count	–	Count of program steps
Cycle	–	Count of cycles
Step	–	Program step no. from programming
Command	–	Command type and programmed values
Time	–	Time at end of a command
Completion Time	_	Time at end of program

```
Program STANDARD started at Date:        15/Apr/1999      7:56:12
Initial Blocktemp:                       24.7 C
Initial Lidtemp:                         25 C
Run Time:                                  1:26
Finish Time:                             15/Apr/1999      9:22


Count     Cycle     Step     Command                          Time

                             Tube:0.2 ml          Fill.Vol.:20 µl

                             CNTRL                TUBE        7:56:21

                             LID=105C
                               NOWAIT   AUTO                   7:56:22

1         1         1        T=94.0C    0:02:00
                              +0.0C      + 0:00
                             R=3.0C/s    +0.0C/s
                             G=0.0C                           7:59:05

2         1         2        T=94.0C    0:00:15
                              +0.0C      + 0:00
                             R=3.0C/s    +0.0C/s
                             G=0.0C                           7:59:21

.
.

Completion Time:             15/Apr/1999      9:24:55

E N D   O F   P R O T O C O L
```

10 Interface description

PC connection

The 9 pin PC connection socket (serial interface RS 232) is located above the printer port on the right-hand side of the device (see Sec. 4.1, Fig. 2.5).

The parameters for the computer are set in the "OPTIONS/GENERAL/Remote" menu.

Selection of transfer rate: Baud rate 19,200, 9,600, 4,800, 2,400 or 1,200

Detailed information about PC connection and the connecting cable required is available on request.

The computer to be connected must correspond to the EN 60950 or UL 1950 regulations.

A special operating manual is available for serial communication. Further information is available on request.

10.2 Program transfer

If programs are stored on a personal card, they can be transferred via the card to other Mastercycler®, Mastercycler® gradient and Mastercycler® personal devices. Programs created on a Mastercycler® personal can also be transferred to a Mastercycler® gradient. The Mastercycler® gradient has an additional function, whereby a Gradient function is added to the temperature command, i.e. a temperature gradient can be built up in the thermoblock. This function cannot be used when programs are transferred to the Mastercycler® and Mastercycler® personal.

11 Maintenance

The Mastercycler® / Mastercycler® gradient can be cleaned using water or a mild laboratory cleaning agent. The device should not come into contact with organic solvents or aggressive solutions. Ensure that no liquid enters the device. For safety reasons, the device must be switched off and disconnected from the power supply before cleaning begins.

The electrical safety fuses are located between the main power switch and the main power plug at the rear of the device. They can be removed by sliding them up one catch. Before the fuses are replaced, the device must be switched off and disconnected from the mains supply. Only fuses with the correct voltage values may be used (information on the fuse type can be found at the rear of the device).

The device may only be opened by qualified service personnel. The warranty will not be honored in the event of damage caused by unauthorized servicing.

12 Troubleshooting

Programming errors or errors regarding the handling of the personal card can be eliminated after the cause of the error has been established.

Technical errors can be caused by interference (e.g. power failure or power fluctuations). In some cases, it is possible to eliminate the error by switching off the device for a short period. Wait ten seconds before switching on the device again. If the error recurs, contact a service technician.

12.1 Error messages

Error message	*Cause*	*Solution*
BLOCK ChkSumErr	Technical defect.	Contact SERVICE.
BLOCK TOO HOT!	Defective regulation or defective electronics.	Contact SERVICE.
Card changed	Card removed immediately before being inscribed.	Insert card correctly and repeat procedure.
Card content not o.k.	Content of card is defective.	Card must be formatted. Automatic procedure when program is saved.
Card not in slot	Card removed during saving process.	Insert card correctly and repeat procedure.
Enter Tube	Not all entries have been made for a program which has been started.	Enter tube type and sample volume.
Err GOTO (1)	Branching to a program line number is impossible because this program line number is in a program area which has already been selected for repeating using another GOTO command.	Check GOTO command and modify or delete if necessary. Program line number for additional GOTO command must not be in a section which is already addressed by a GOTO command.
Err GOTO (2)	More than three GOTO commands used for one program range.	Three GOTO commands can be used in one program range when the areas which have been addressed overlay each other completely, i.e. the program area of the second command is greater than that of the first command. The third GOTO command must cover an even greater area than that of the second command.
Err Invalid	Invalid command. Parameter has not been entered.	Enter command completely or delete command.

12 Troubleshooting

Error message	*Cause*	*Solution*
***E**rr Link (1) ... Line ..	LINK command cannot be executed. Program not found. Program name and program line of defective LINK command appear in the display.	Only programs from the internal memory can be called up by the LINK command. Programs on a personal card and the program in the processing level ("Edit") cannot be called up by the LINK command.
***E**rr Link (2) ... Line ..	Too many programs are connected by LINK commands. Program name and program line of defective LINK command appear in the display.	A maximum of five programs, i.e. four LINK commands, can be processed in succession.
***E**rr No Program	No executable program available in memory.	Load program from memory or personal card, or define a program in "Edit" menu and restart.
***F**AN ERROR!	Defective internal fan.	Switch off device. Contact SERVICE.
***F**AT Full	Number of programs which can be administrated has been exceeded. Internal memory: 99 programs personal card: 10 programs	Delete programs which are not required.
***I**nternal EE-Err	Technical defect.	Contact SERVICE.
***L**EFT BLOCK DEFEKTIV!	Defective regulator or electronics on left-hand block.	Switch off device. Contact SERVICE.
***L**ID TOO HOT!	Defective electronics.	Contact SERVICE.
***L**ink not found	Program branching is not possible because the program which was supposed to be called up has been deleted.	Check the LINK command. Enter the missing program.
***L**ink Error	Program branching is not possible because the program which was supposed to be called up has been deleted.	Check the LINK command. Enter the missing program.
***M**Card Error	The memory of the personal card is defective.	Evaluate further messages.
***M**emory corrupted	The internal memory is defective.	The internal memory must be deleted. This deletes all programs.

Memory Error	The internal memory for programs

12 Troubleshooting

Error message	*Cause*	*Solution*
MemoryCard Read Err!	Card cannot be read.	Insert card correctly (check that card is facing in the correct direction).
MemoryCard Read-Only!	Card is marked as write-protected.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard Write Err!	Writing error on card.	– Insert card correctly (check that card is facing in correct direction). – Do not touch/move card during saving.
MID BLOCK DEFEKTIV!	Defective regulator or electronics on middle block.	Switch off device. Contact SERVICE.
Name Invalid!	Program name contains an invalid character.	Enter program name again.
POWER ERROR!	Voltage error or defective fuse.	Contact SERVICE.
Printer: OFF LINE	Printer is not ready to receive.	Select the "ON LINE" printer setting.
Printer: no response	No printer is available or the printer is not switched on.	– Select "OFF" in the menu OPTION/Printer/Print Protocol. – Switch on the printer.
Program is running!	A program is being processed.	Lid temperature cannot be modified at the moment.
Program too large!	Program is too large.	Max. 40 program lines possible.
Restarting Program!	A power failure occurs or the device is switched off briefly when a program is running.	The program continues automatically.
RIGHT BLOCK DEFEKTIV!	Defective regulator or electronics on right-hand block.	Switch off device. Contact SERVICE.
SLOWING DOWN!	The heating rate or cooling rate has slowed down.	If error recurs, contact SERVICE.
Steps free	Display of free memory spaces is < 100 steps. 1 step = 1 program line.	Delete programs which are not required or store them on the personal card.

Error message	*Cause*	*Solution*
TSensor_Err	– Permitted operating conditions have not been maintained. – Technical defect.	– Operate device at permitted temperature and humidity only – Contact SERVICE.
Value out of range!	Value is not within the permitted value range.	The closest permitted value is entered automatically.
Warn Ramp Max ... Line ...	The ramp is limited to 3 K/s.	Check entries for ramp and ramp increment, and change entries if necessary.
Warn Ramp Min ... Line ...	The ramp is limited to 0.3 K/s.	Check entries for ramp and ramp increment, and change entries if necessary.
Warn Temp Max	Temperature limits of 99 °C exceeded. If program is started, temperature is limited to 99 °C.	Check temperature commands and reduce value of temperature increment so that only values below 99 °C can be reached.
Warn Temp Min	Temperature limit of 4 °C not reached. If program is started, temperature is limited to 4 °C.	Check temperature commands and modify values of temperature increment so that only values above 4 °C can be reached.
Warn Time Max	Cycle time of 9:59:59 exceeded. If program is started, cycle time is limited to 9:59:59.	Check temperature commands and modify values of time increment so that only values below 9:59:59 can be reached.
Warn Time Min	Cycle time of 0:00:01 not reached. If program is started, cycle time is limited to 0:00:01.	Check temperature commands and modify values of time increment so that only values above 0:00:01 can be reached.

13 Technical data

Mastercycler® / Mastercycler® gradient

Sample capacity:	1 PCR plate 96 or 96 0.2 ml PCR tubes or 77 0.5 ml PCR tubes, thin-walled
Temperature range:	4 to 99 °C
Temperature uniformity across the block:	20 °C to 72 °C ± 0.6 K 95 °C ± 1.0 K
Block homogeneity ($\bar{S}_{95}$):	20 °C to 72 °C ≤ ± 0.4 K 95 °C ≤ ± 0.5 K
Control accuracy:	± 0.2 K
Temperature-control speeds:	Heating rate: approx. 3 K/s, measured on the block Cooling rate: approx. 2 K/s, measured on the block
Number of programs:	max. 99 max. 10 on a personal card
Maximum number of cycles:	99
Dimensions:	Width: 26 cm Depth: 41 cm Height: 27 cm
Weight:	approx. 12.4 kg

Voltage/frequency:	230 V, 50–60 Hz	115 V, 50–60 Hz	100 V, 50–60 Hz
Power requirement:	500 W	500 W	500 W
Operating current:	2.6 A	5 A	6 A
Fuses:	2 x T 5 A, 250 V	2 x T 6.3 A, 250 V	2 x MT 8 A, 250 V

Overvoltage category:	II
Pollution degree:	2
Contamination level:	I
Operating conditions:	15 to 35 °C, 70 % rel. humidity
Storage conditions:	–20 to 70 °C, 85 % rel. humidity

The device is CE-approved, fulfills UL 3101-1 and CSA C 22.2 No. 1010.1.

Technical specifications subject to change!

14 Ordering information

Please use only the accessories recommended by Eppendorf. Using disposables which we have not recommended can reduce the precision, accuracy and life of the device. We do not honor any warranty or accept any responsibility for damage resulting from such action.

Order no.	*Description*
	Mastercycler®
5333 000.018	230 V, 50–60 Hz
5333 000.026	115 V, 50–60 Hz
5333 000.034	100 V, 50–60 Hz
	Mastercycler® gradient
5331 000.010	230 V, 50–60 Hz
5331 000.045	115 V, 50–60 Hz
5331 000.037	100 V, 50–60 Hz
	Accessories
0013 609.349	Fuse, time-lag, 5 A, 250 V (1 pc.) for 230 V
0013 565.333	Fuse, time-lag, 6.3 A, 250 V (1 pc.) for 115 V
0013 611.432	Fuse, semi time-lag, 8 A, 250 V (1 pc.) for 100 V
5331 220.002	*in situ* Adapter
5331 900.062	Operating manual for Mastercycler® and Mastercycler® gradient
5332 300.018	Personal card
5331 226.027	Personal card for temperature-validation system
5331 222.005	Temperature-validation system
5331 224.008	Temperature sensor for 0.2 ml tube positions
	Consumables
0030 124.332	PCR-tubes 0.2 ml, colorless (1000 pcs.)
0030 124.502	PCR-tubes 0.5 ml, colorless, thin-walled (500 pcs.)
0030 124.359	8-strip 0.2 ml PCR tubes, colorless, per 120 pcs. (= 960 tubes)
0030 121.023	Safe-Lock microcentrifuge tubes 0.5 ml, colorless (500 pcs.)
0030 128.648	Eppendorf twin.tec PCR Plate 96, skirted, colorless (25 pcs.)
0030 128.656	Eppendorf twin.tec PCR Plate 96, skirted, yellow (25 pcs.)
0030 128.680	Eppendorf twin.tec PCR Plate 96, skirted, red (25 pcs.)
0030 128.664	Eppendorf twin.tec PCR Plate 96, skirted, green (25 pcs.)
0030 128.672	Eppendorf twin.tec PCR Plate 96, skirted, blue (25 pcs.)
0030 128.575	Eppendorf twin.tec PCR Plate 96, semi-skirted, colorless (25 pcs.)
0030 128.583	Eppendorf twin.tec PCR Plate 96, semi-skirted, yellow (25 pcs.)
0030 128.613	Eppendorf twin.tec PCR Plate 96, semi-skirted, red (25 pcs.)
0030 128.591	Eppendorf twin.tec PCR Plate 96, semi-skirted, green (25 pcs.)
0030 128.605	Eppendorf twin.tec PCR Plate 96, semi-skirted, blue (25 pcs.)

14 Ordering information

	Consumables
0030 127.498	Cap Strips, (8-strips) (25 x 12 pcs.)
0030 127.650	Heat Sealing Film (100 pcs.)
0030 127.668	Peel-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.676	Pierce-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.480	PCR Film, adhesive (100 sheets)
0030 127.471	PCR Foil, adhesive (100 sheets)
0030 127.501	*in situ* Frames, 25 µl (100 pcs.)
0030 127.510	*in situ* Frames, 65 µl (100 pcs.)
0030 127.528	*in situ* Frames, 125 µl (100 pcs.)
0030 127.536	*in situ* Frames, 300 µl (100 pcs.)
	Recommended auxiliary equipment
5425 715.005	Adapter for 0.2 ml PCR tubes for 1.5/2.0 ml rotor (6 pcs.)*
5425 716.001	Adapter for 0.5/0.6 ml tubes for 1.5/2.0 ml rotor (6 pcs.)*

14 a Ordering information for North America

Please use only the accessories recommended by Eppendorf. Using disposables which we have not recommended can reduce the precision, accuracy and life of the device. We do not honor any warranty or accept any responsibility for damage resulting from such action.

Order no.	*Description*
	Mastercycler®
5333 000.018	230 V, 50–60 Hz
5333 000.026	115 V, 50–60 Hz
5333 000.034	100 V, 50–60 Hz
	Mastercycler® gradient
5331 000.010	230 V, 50–60 Hz
5331 000.045	115 V, 50–60 Hz
5331 000.037	100 V, 50–60 Hz
	Accessories
0013 609.349	Fuse, time-lag, 5 A, 250 V (1 pc.) for 230 V
0013 565.333	Fuse, time-lag, 6.3 A, 250 V (1 pc.) for 115 V
0013 611.432	Fuse, semi time-lag, 8 A, 250 V (1 pc.) for 100 V
5331 220.002	*in situ* Adapter
5331 900.062	Operating manual for Mastercycler® and Mastercycler® gradient
5332 300.018	Personal card
5331 226.027	Personal card for temperature-validation system
5331 222.005	Temperature-validation system
5331 224.008	Temperature sensor for 0.2 ml tube positions
	Consumables
0030 124.367	PCR tubes, 0.2 ml, colorless (1000 pcs.)
0030 124.600	PCR tubes, 0.5 ml, colorless, thin-walled (500 pcs.)
0030 124.383	8-strip 0.2 ml PCR tubes, colorless, per 120 pcs. (= 960 tubes)
22363611	Safe-Lock microcentrifuge tubes, 0.5 ml, colorless (500 pcs.)
0030 128.648	Eppendorf twin.tec PCR Plate 96, skirted, colorless (25 pcs.)
0030 128.656	Eppendorf twin.tec PCR Plate 96, skirted, yellow (25 pcs.)
0030 128.680	Eppendorf twin.tec PCR Plate 96, skirted, red (25 pcs.)
0030 128.664	Eppendorf twin.tec PCR Plate 96, skirted, green (25 pcs.)

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