Mastercycler® 384

Bedienungsanleitung
Operating Manual
Mode d'emploi
Istruzioni d'impiego
Manual de Instrucciones

eppendorf

Zertifikat / Certificate / Certificat

Autorisierter Thermocycler
Authorized Thermal Cycler
Thermocycleur autorisé

Dieses Gerät, Werk-Nummer ***5334*** , ist ein autorisierter Thermocycler.
This instrument, Serial No. , is an Authorized Thermal Cycler.
Le présent appareil portant le numéro de série est un thermocycleur autorisé.

Sein Verkaufspreis enthält die Lizenzkomponente (up-front fee component) einer Lizenz unter den Patenten des Polymerase-Ketten-Reaktion (PCR) Prozesses, die von Hoffmann-La Roche Inc. und F. Hoffmann-La Roche Ltd. gehalten werden und die somit die Anwendung des PCR-Prozesses für interne Forschung und Entwicklung auf diesem Gerät erlaubt. Eine weitere Lizenzkomponente (running royalty component) dieser Lizenz kann von Perkin-Elmer erworben oder über den Kauf autorisierter Reagenzien erhalten werden. Dieses Gerät ist auch ein autorisierter Thermocycler für den Gebrauch mit Anwendungslizenzen, die von Perkin-Elmer erworben werden können. Seine Nutzung mit autorisierten Reagenzien beinhaltet ebenso eine limitierte PCR-Lizenz in Übereinstimmung mit in den Packungen der Reagenzien ausgewiesenen Rechte. Der Kauf dieses Produktes allein überträgt dem Käufer weder die komplette Lizenz noch das Recht, den PCR-Prozess durchzuführen. Weitere Informationen über den Kauf von Lizenzen, um den PCR-Prozess durchzuführen, können über folgende Kontaktadresse angefordert werden: Direktion der Lizenzabteilung bei der Perkin-Elmer Corporation, 850 Lincoln Centre Drive, Foster City, California 94404.

Der Thermocycler verliert den Status als autorisierter Thermocycler, wenn bei einer Weitergabe des Geräts die Werk-Nummer und/oder dieses Zertifikat abhanden kommen.

Perkin-Elmer übernimmt keine Garantie für dieses Gerät.

Its purchase price includes the up-front fee component of a license under the patents on the Polymerase Chain Reaction (PCR) process, which are owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd., to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Perkin-Elmer or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Perkin-Elmer. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at The Perkin-Elmer Corporation, 850 Lincoln Centre Drive, Foster City, California 94404.

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Perkin-Elmer does not guarantee the performance of this instrument.

Zertifikat / Certificate / Certificat

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Aucune garantie concernant le présent matériel n'est prise en charge par la société Perkin-Elmer.

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1 Introduction

The Eppendorf Mastercycler® 384 is a thermocycler for carrying out the "Polymerase Chain Reaction" (PCR) and related methods in the research laboratory.

Temperature control of the Mastercycler® 384 is carried out with Peltier elements, which allow very rapid temperature changes. When combined with optimum block geometry, this enable rapid heat transfer into the sample.

Two possibilities for temperature control are provided: the "Block Control" mode and the specially developed "Tube Control" mode, with which the software matches the temperature to the sample volume.

The heated "Specialized lid 384" prevents condensation in the upper vascular areas and its compression mechanism ensures safe sealing of the 384-PCR plates during the PCR.

The device is 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.

To optimize PCR reactions, the Mastercycler® 384 has a gradient function, which allows the temperature distribution to be varied via the thermoblock at each temperature step.

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

2 Safety precautions

Before using the Mastercycler® 384 for the first time, please read the complete operating manual. The Mastercycler® 384 may be used only for performing PCR and related methods in a research laboratory.

It is essential to observe the following safety precautions:

Voltage:
Before plugging in, compare your power supply with the electrical requirements listed on the identification plate of the device.
The device must be plugged into a grounded socket.
Before carrying out maintenance or cleaning work or replacing the fuses, switch off the device and disconnect it from the mains supply.

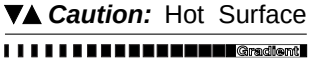
Dangerous materials:
The Mastercycler® 384 must not be used to process explosive, flammable and highly reactive substances and must not be operated in a hazardous or potentially explosive environment.
You must observe the relevant safety regulations when handling pathogenic material, radioactive substances or other substances hazardous to health.

Liquids:
Please ensure that no liquid enters the device.

Operating environment:
Please ensure that the ventilation for the device remains unblocked at all times.

Risk of injury:
The generally applicable precautionary measures should be followed when handling the device. In particular, watch out for your hands when closing the lid.

Danger of burning:
The thermoblock, the inside of the heated lid and the microtiter plates can rapidly reach a temperature in excess of 50 °C. Care must therefore be taken to avoid burns!
Do not open the heated lid until the temperatures have decreased to 30 °C.
Do not use any materials (PCR-Plate 384, sealings, foils, mats) which are not sufficiently temperature-stable (up to 120 °C).



Symbol on the thermoblock:
This symbol warns the user that the thermoblock (▼) or the inside of the lid (▲) may be hot .
▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ shows that when the Gradient mode is in operation, the temperature is higher on the right-hand side than on the left-hand side.

Repairs may only be carried out by an authorized specialist. Only use spare parts recommended by Eppendorf. Disconnect the device from the mains supply before opening.

3 Installation

3.1 Delivery package

The delivery package contains the following items:

1 Mastercycler® 384
1 Main power cable
1 Operating manual
1 Personal card
PCR plate 384 (2 pcs.)
PCR Foil, adhesive (5 pcs.)

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.

Dimensions:	Width:	26 cm
	Depth:	41 cm
	Height:	27 cm
Mains connection:	1 safety plug socket for the Mastercycler® 384. 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® 384 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

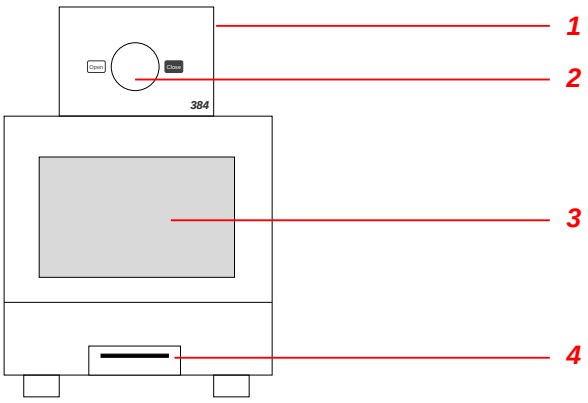


Fig. 1: Front view

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

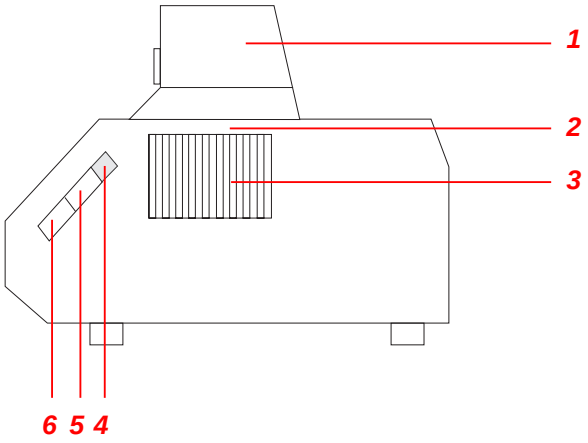


Fig. 2: Side view

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

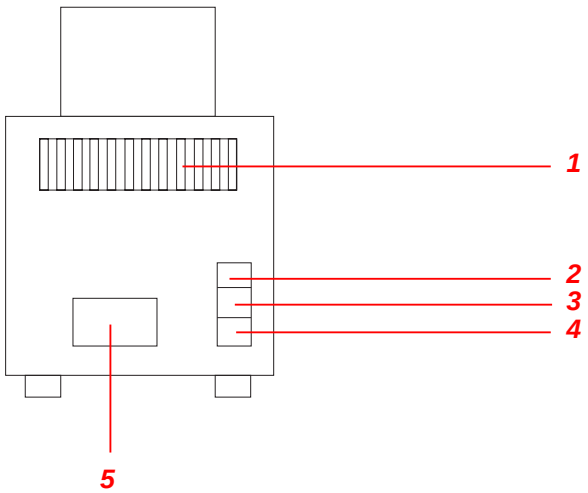


Fig. 3: Rear view

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

4 Technical description

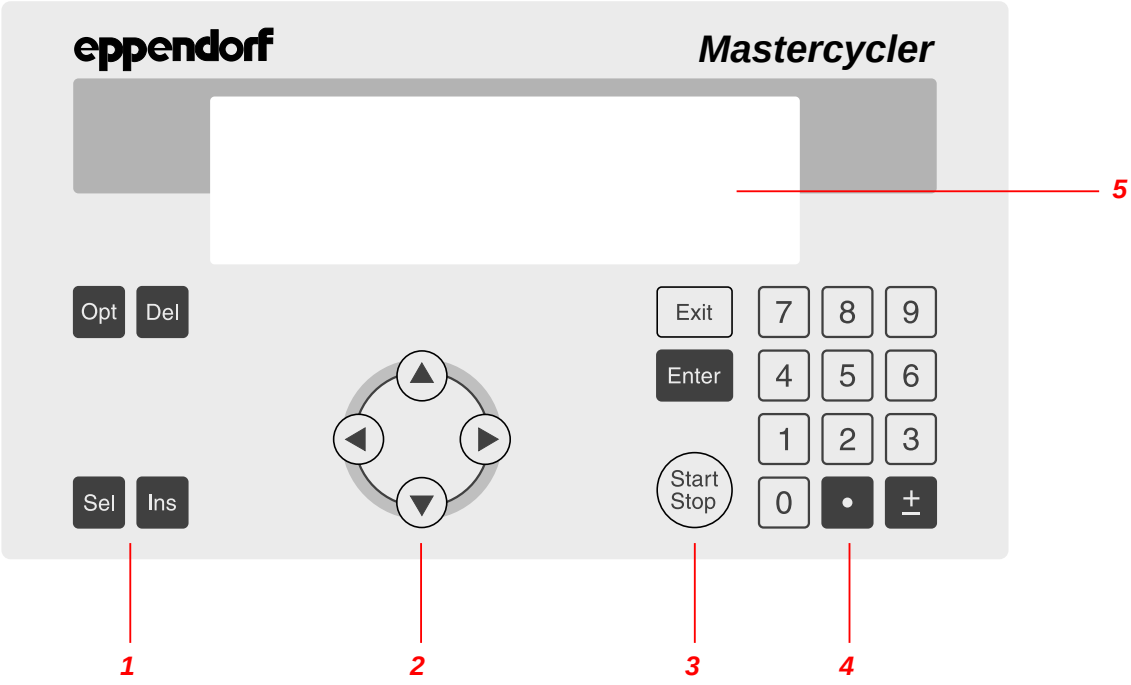


Fig. 4: Display and control panel

1 Programming keys
2 Cursor keys
3 Control keys
4 Numeric keypad
5 Display

4.2 Keys

Programming keys

Opt

– To display the calculated run time of a program and the time of the expected end of the running program.
– To select programming options for "Temperature" and "Time" commands (ramp, gradient, increment).

Del

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

Sel

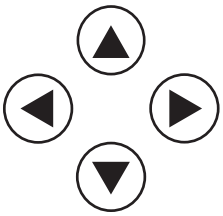
– To select a program command.
– To select a menu item from a list instead of the ▲ / ▼ keys.
– To enter letters when naming programs and command texts.
The direction of selection of the letters can be changed by pressing the ± key.

Ins

– To insert program lines during the creation of a program.

4 Technical description

Cursor keys



The cursor appears in the display as a dark field and is moved with the cursor keys.

– The cursor keys are used to move into, or to change between, input fields.

– If a menu should be selected, the cursor must be moved to that menu and the selection should then be confirmed by pressing Enter.

Control keys

Exit

– To exit a menu or to return to the next-highest menu level.

– To exit the program view during a running program, e.g. in order to work in the programming level.

Enter

– To confirm an entry.

– To call up a menu which has been selected using the cursor.

Start
Stop

– To start the program directly in the processing level ("FILES/Edit" menu). To start all other programs, the "Start" menu must be used.

– To cancel or to interrupt a program.

Numeric keys

0 to 9

– To enter numbers.

– For direct selection of commands during programming.

Decimal point key

.

– To enter a decimal point.

+/- Prefix key

±

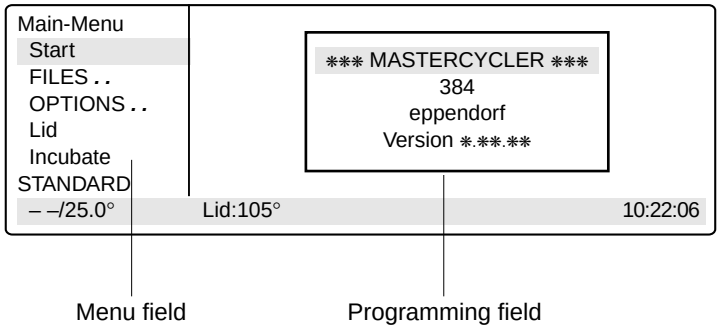
– To select the plus/minus prefix for a number.

– To reverse the alphabetical order when entering letters.

4.3 Display

Press the main power switch on the rear of the device (Sec. 4.1, Fig. 3). The device name and the software version installed appear briefly in the display.

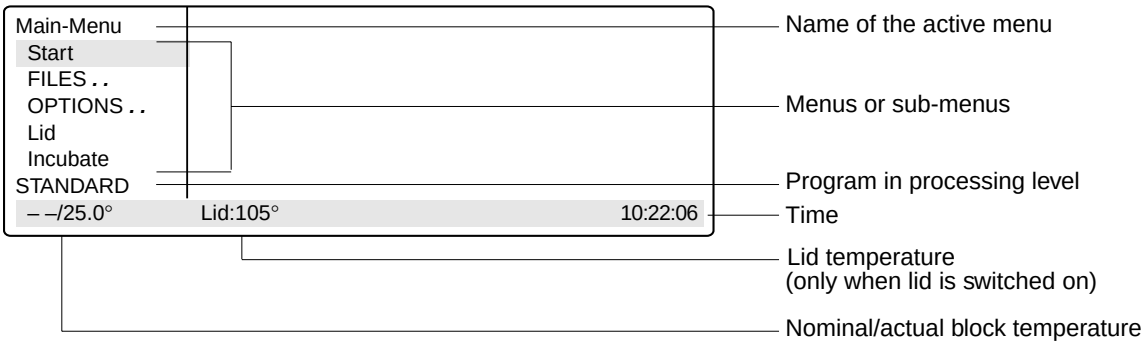
The main menu then appears in the display:



The eight-line display is divided into two areas. The menus available for selection are displayed and selected in the menu field on the left. The programming field is used for programming and for setting the device parameters.

5 Description of menus

5.1 Main menu



All menus available for selection are listed in the main menu, in which the menus containing submenus are shown in upper case letters and are followed by dots. A menu or a sub-menu can be called up in three different ways:

1. Select using the ▲ / ▼ keys and confirm by pressing Enter.
2. Select using the Sel key and confirm by pressing Enter.
3. Select the menu using the internal position number (shown in brackets in the text) and confirm by pressing Enter.

Menus are exited by pressing the Exit key. To access the main menu again, it may be necessary to press the Exit key several times.

Overview of the entire menu structure:

Main-Menu				
Start	(1)			To start the program (Sec. 5.2)
FILES . .	(2)			To program (Sec. 5.3)
	Edit	(1)		To change a program
	Load	(2)		To load any program
	Standard	(3)		Example program installed in the factory
	New	(4)		To create a new program
	Delete	(5)		To delete the program
OPTIONS . .	(3)			System settings (Sec. 5.4)
	Editor	(1)		To display additional functions
	Printer	(2)		To connect a printer
	Gradient	(3)		To display the temperature distribution in the block
	GENERAL . .	(4)		
		Clock	(1)	To set the clock
		Remote	(2)	To connect a PC
		Sound	(3)	Tone
		Etc	(4)	Menu display selection Auto-Restart selection
Lid	(4)			To preheat the lid (Sec. 5.5)
Incubate	(5)			To heat the thermoblock and the lid (Sec.5.6)

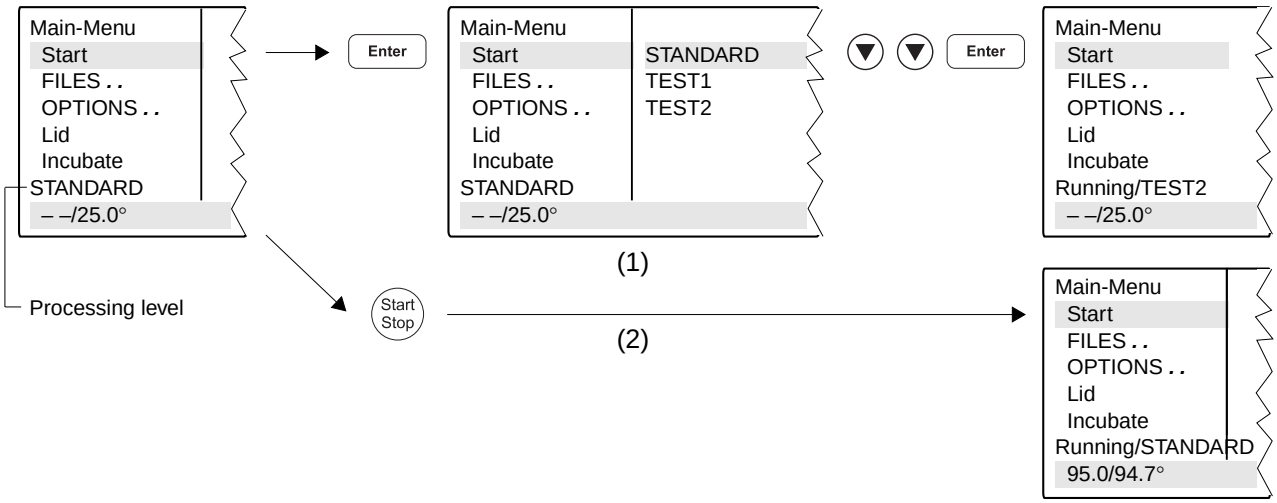
5 Description of menus

5.2 Start

The programs are started from the "Start" menu.

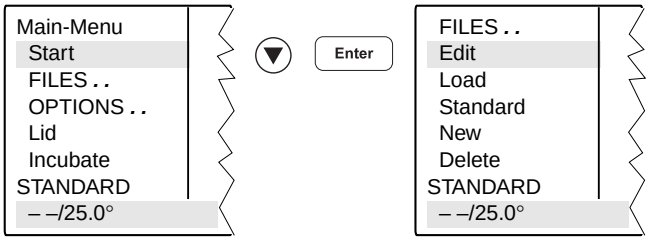
If a program which has been stored has to be started, it is selected with the cursor keys from the displayed list after Enter has been pressed and started by pressing Enter (1).

Note: The program displayed in the processing level can be started directly by pressing the Start Stop key (2).



5.3 FILES

The "FILES" menu contains several submenus with which programs can be changed, loaded, created or deleted.



5.3.1 Edit

The "Edit" menu is used to change programs.

The program which is to be changed must be in the "processing level" and if necessary loaded into the processing level via the "Load" menu (see Sec. 5.3.2).

After a change, the program concerned can either be saved under a new name or the name is retained. In the later case, the original program is overwritten.

Note: If the modified program is not saved, but started directly from the processing level with the Start Stop key, two different programs with the same name (one in the internal memory and one in the processing level) will exist.

5.3.2 Load

The "Load" menu is used to load a saved program into the processing level.

A list of the existing programs appears from which programs can be loaded from the internal memory or from the personal card. If there is a program in the processing level which has not yet been saved, a safety check is made allowing this program to be saved.

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

5.3.3 Standard

It is not necessary to rewrite a program completely; an example program can be supplemented or changed and then saved under a new name. In order to facilitate the creation of new programs, a "Standard" example program is installed in the factory.

The example program is loaded and changed into the processing level. The exact procedure is described in Sec. 7.4.

The "Standard" example program cannot be deleted but can be individually varied. After the changes have been made, the example program is saved under the name "Standard".

To restore the example program set in the factory example program, the "Standard" program must be deleted in the"Delete" submenu. When the "Standard" program is loaded, the original example then appears in the processing level.

Note: All programs which are in the internal memory can be used as an example program.

5.3.4 New

The "New" menu is used to create new programs without using an example. The program head parameters, for which entries are essential, are shown in the processing level and the program is given the provisional title UNNAMED. The exact programming is described in Sec. 7.3.

5.3.5 Delete

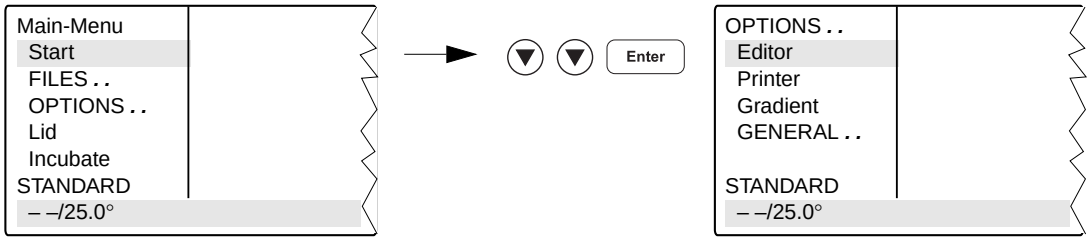
Programs from the internal memory or from the personal card are deleted in the "Delete" menu.

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

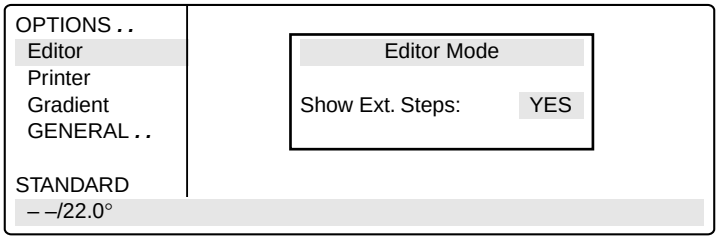
The "OPTIONS" menu can be used to define general system settings for the program editor, printer, time/date, etc.



Selection of YES/NO, ON/OFF and other settings in the relevant submenu are carried out using the Sel key.

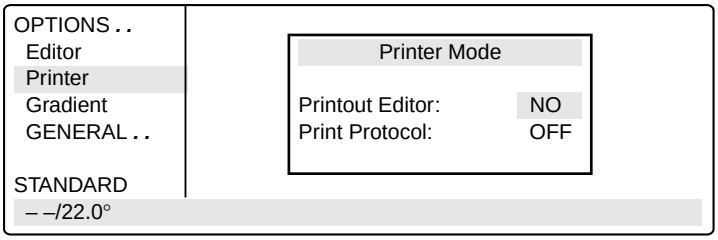
These menus are exited by pressing the Exit key.

5.4.1 Editor



Show Ext. Steps	Display of the temperature options (temperature increment, time increment, ramp, ramp increment, gradient).
YES	Selected options 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 selected, the command line concerned is marked with an "*". The options can be displayed and entered or changed by pressing the Opt key.

5.4.2 Printer



Entries are necessary only when a printer is connected.

Printout Editor

YES	The program in the 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	No printing takes place. "NO" is automatically reset after a program has been printed out.

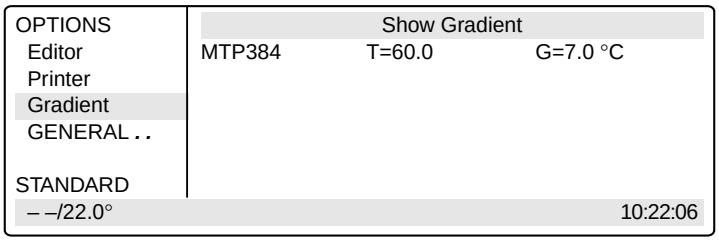
Print Protocol

ON	First the program overview is printed out and during the run a protocol with the time of the executed commands.
OFF	No printout is made.

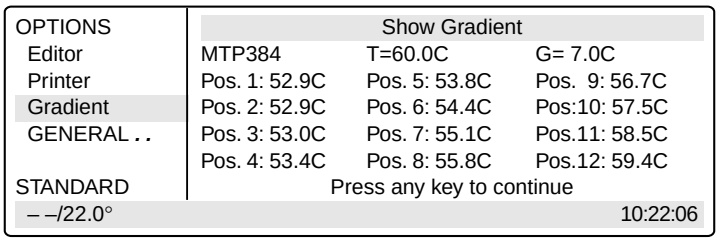
5.4.3 Gradient

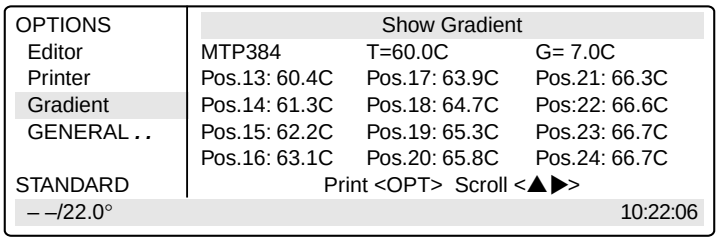
The "Gradient" menu can be used to indicate how the temperature distribution varies over the thermoblock during programming of a gradient.

A gradient can be entered with every temperature command so that the columns of the thermoblock are subject to different temperature controls. To program a gradient see Sec. 7.2.3 and 7.3.1. The temperature in the cavities rises from the left side of the thermoblock to the right side.



After a temperature and a gradient have been entered, the temperature distribution is indicated over the left half of the block. To display the right-hand side of the block, press any key:



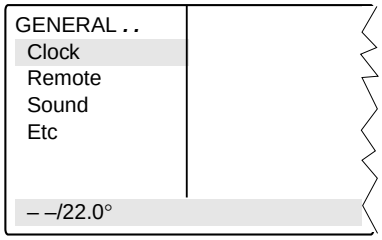


The gradient may be a maximum of ± 10 °C with a resolution of 0.1 °C. This means that the overall temperature span is 20 °C. The selected temperature is in the middle of the block.

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

The "General" menu is used to enter general settings and to test the device.

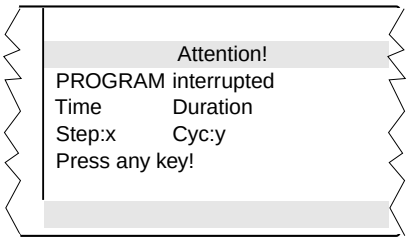


Clock	Format:		The time in the 24-(military) or 12-hour-clock format (choice of PM or AM) can be selected using the Sel key.
	Time:		To enter the current time in hours:minutes:seconds.
	Date:		To enter the current date as day/month/year. The month can be selected using the Sel key.
Remote	Baud rate:		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.

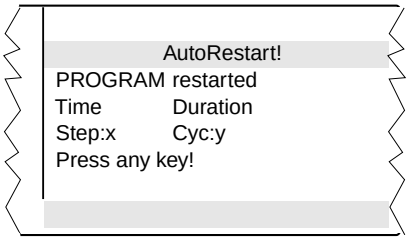
LAST: After the device has been switched on, the most-recently used menu appears in the display.

Auto Restart: NO: A program interrupted by a power failure is not continued.



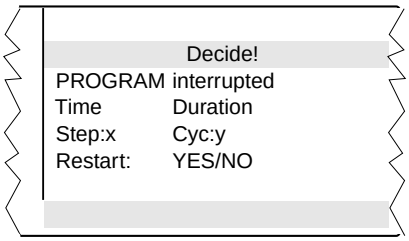
YES: Depending on the duration of a power failure, an interrupted program will automatically continue or can be manually continued or stopped.

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 Start Stop key and confirm by Enter.

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.



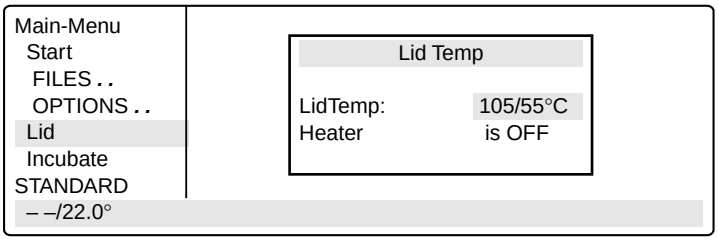
If the interrupted program is to be continued, select "YES" with Sel and confirm with Enter. Press any key to return to the program view.

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

5 Description of menus

5.5 Lid

The heated lid can be independently controlled with the "Lid" menu (e.g. to preheat the lid).



LidTemp: Display of the nominal/actual temperature. The temperature selected for the heated lid is entered as the nominal temperature.
The lid heating switches off when a temperature value of 0 °C is entered.

Heater is ON: Switch on the lid heating.

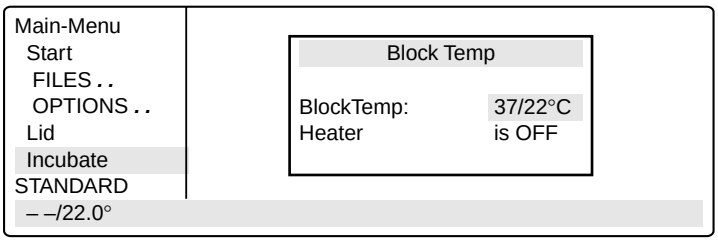
Heater is OFF: Switch off the lid heating.

Note: 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.

Caution: Depending on the temperature (up to 110 °C) selected, the lid can get very hot. Risk of injury from burns!

5.6 Incubate

The "Incubate" menu is used to maintain the thermoblock at any temperature (e.g. for incubation experiments or to preheat the block). The heated lid is thereby automatically brought to the block temperature.



BlockTemp: Nominal/actual temperature.
The temperature for the thermoblock is entered as the nominal temperature.
It is possible to enter values from +4 °C to 99 °C. It is not possible to select a gradient.

Heater is ON: Switch on the block temperature control.

Heater is OFF: Switch off the block temperature control.

Note: 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.

Caution: If the "Incubate" menu has been selected, then depending on the temperature selected, the thermoblock and the heated lid can get very hot, which means that there is a high burns risk for the user!

6 Operation

6.1 General

6.1.1 Loading the samples

The thermoblock can be loaded with PCR plates 384.

To achieve optimal temperature transition, only PCR plates 384 with a V-shaped base should be used (see "Ordering information"). PCR plates 384 must fit tightly into the thermoblock and should not move in any way. Microtiter plates which are not sufficiently temperature-stable (up to approx. 120 °C) should not be used.

In general, this also applies to foils used to seal microtiter plates. However, due to the temperature-controlled lid heating, it is also possible to use foils which would not be resistant above 100 °C, because in this case, the lid heating can then be set, for example, to 95 °C.

6.1.2 Heated lid "Specialized lid 384"

After the samples have been loaded, the heated lid is closed over the thermoblock and locked, the locking lever is moved to the right.

The lid temperature (up to 110 °C) is controlled automatically by the program (see Sec. 7.2.2). The program start can be speeded up by preheating the heated lid by means of the "Lid" menu (see Sec. 5.5).

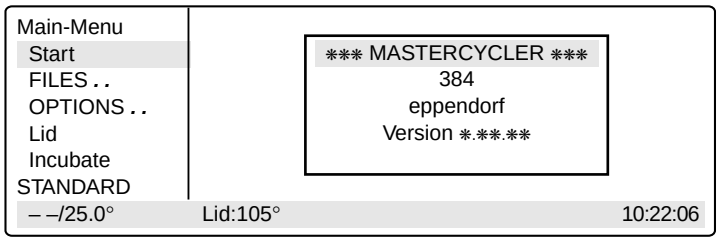
Caution: Depending on the temperature selected (up to 110 °C), the thermoblock and the PCR plates 384 can get very hot. Risk of injury from burns!

Since the heated lid prevents the liquid from condensing in the upper part of the tube, it is not necessary to use an oil layer.

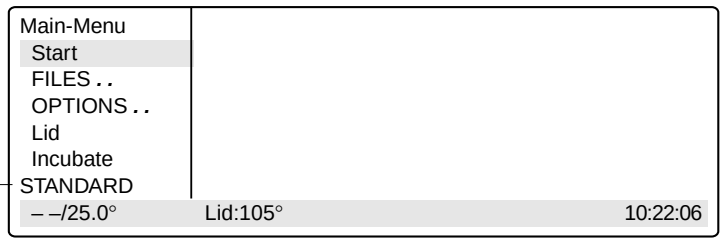
6.2 Switching on the device

– 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

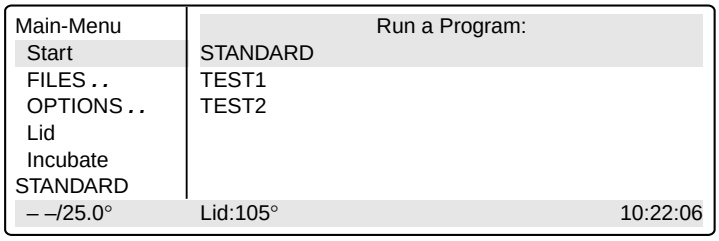
– The program in the processing level can be started immediately by pressing the Start Stop key (see also Sec. 5.2).

– To start another program, press Enter.

  A list of the programs appears in the display.

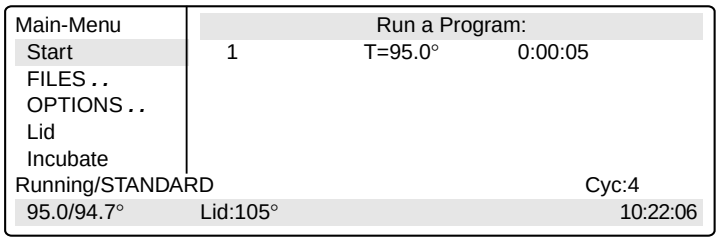
– Select the program name using the ▼ or ▲ key (the program name appears with a dark background).

  The procedure for starting programs which are stored on a personal card is described in Sec. 9.3.



– Press Enter.

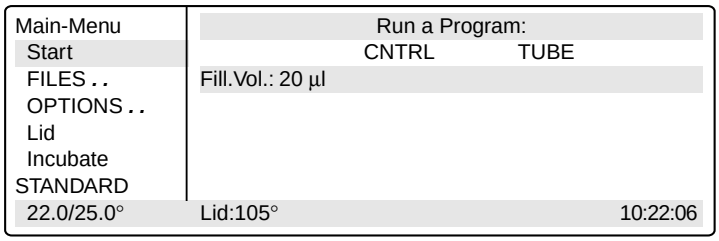
  The program starts immediately after a "Testing Program".



  If the program contains the "CNTRL/TUBE" command, it is necessary to enter in the filling volume after the program has started (see Sec. 7.2.1).

– Enter the filling volume and confirm by pressing Enter.

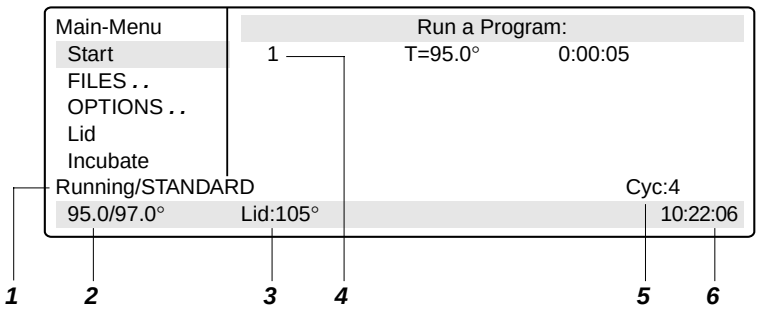
  If no entry is made for "Fill.Vol.", the program will not run.
  "Enter Volume" appears in the main menu instead of the program name and a long acoustic signal is emitted until the entry is made.



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 Exit.



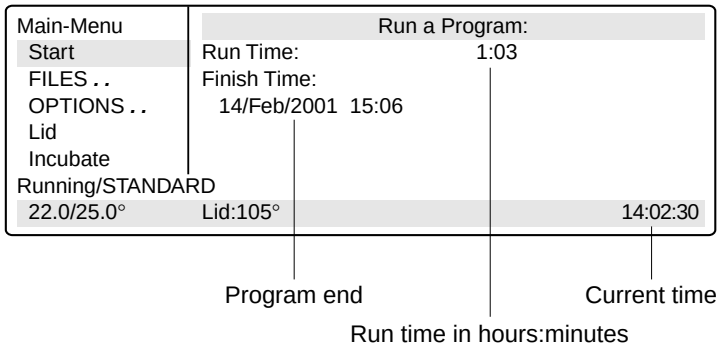
1 Program flashes in alternation with "Running"
2 Nominal/actual block temperature
3 Lid temperature
(only when lid is switched on)
4 Program line number, program command
Example: Temperature command (T)
with nominal temperature and cycle length
5 Number of completed cycles
6 Time

6.4 Displaying the running time of a program

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

– This can be done by pressing Opt.

Note: The key Opt has this function only when the program is running (see Sec. 4.2).
After a few seconds the display jumps back to the program sequence.



6.5 Interrupting a program

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

– Press Start Stop.

– Using the Sel key, select between:

STOP To abort the program
PAUSE To interrupt the program
RUN Back to program sequence

Main-Menu	Run a Program:	
Start	STANDARD running...	
FILES . .	Program: STOP	
OPTIONS . .		
Lid		
Incubate		
Running/STANDARD		
95.0/94.5°	Lid:105°	10:22:06

– Using the Sel key, move to "Pause".

– Confirm "Program:PAUSE" by pressing Enter.

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".

Main-Menu	Run a Program:	
STOP !	STANDARD running...	
FILES . .	Program: PAUSE	
OPTIONS . .		
Lid		
Incubate		
Paused/STANDARD		
95.0/94.5°	Lid:105°	10:22:06

6.6 Continuing an interrupted program

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

– Press Start Stop.

Using the Sel key, select between:
STOP To abort the program
PAUSE To interrupt the program
RESUME To continue the program

– Using the Sel key, move to "RESUME".

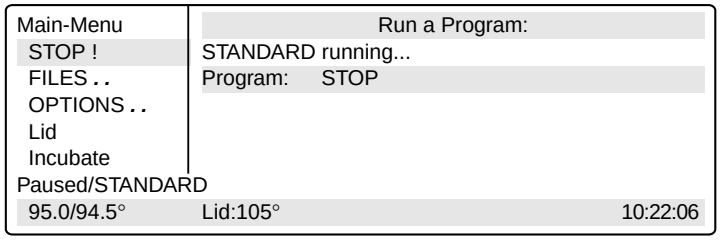
Main-Menu	Run a Program:	
STOP !	STANDARD PAUSED	
FILES . .	Program: PAUSE	
OPTIONS . .		
Lid		
Incubate		
Paused/STANDARD		
95.0/94.5°	Lid:105°	10:22:06

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

Main-Menu	Run a Program:	
STOP !	STANDARD PAUSED	
FILES . .	Program: RESUME	
OPTIONS . .		
Lid		
Incubate		
Paused/STANDARD		
95.0/94.5°	Lid:105°	10:22:06

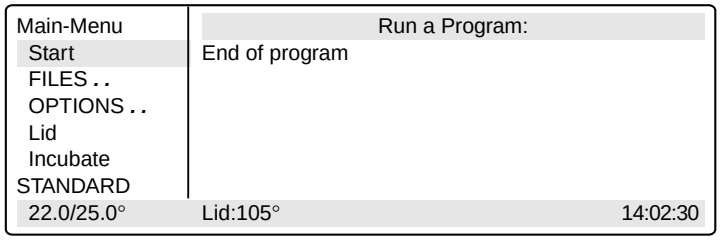
6.7 Aborting a program

– Press Start Stop.
– Confirm "Program:STOP" by pressing Enter.



6.8 Program end / switch off the equipment

At the end of a program,
the following appears in the display:



– Exit the program by pressing Exit.

If no new program is to be started, set the power switch at the back of the equipment to 0 to switch it off.

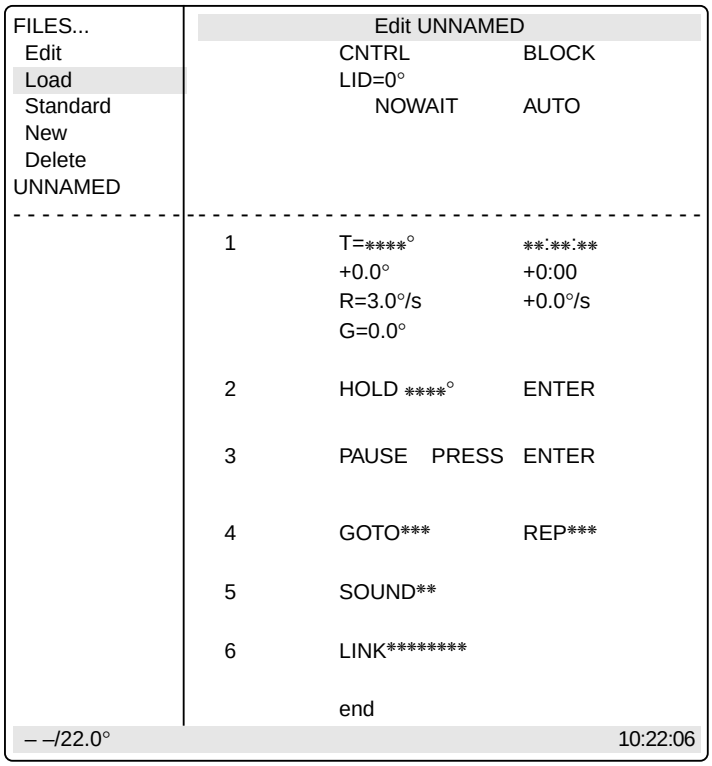
7 Programming

7.1 Program structure

Each program is divided into the program head and the program sequence.

The settings for the thermoblock and the heated lid which apply to the entire program are entered in the program head.

The program sequence can contain up to 40 program lines in which the individual commands are programmed. There are 6 different commands available here which can be used any number of times.



Programming always takes place in the processing level. This is used to create a new program with the "New" menu or an example program loaded into the processing level and modified with the "Load" menu (see Sec. 5.3).

The entries are made with the cursor and programming keys as well as the numeric keypad (see Sec. 4.2).

If values are entered outside the permitted range, the message "Value out of range" and the limit value still possible are entered automatically.

7.2 Description of the commands

7.2.1 CNTRL

This command is used to select the type of temperature control, which can be based either on the thermoblock or on the sample.

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

TUBE The software adapts the temperature of the thermoblock to the temperature in the sample. For this reason, the filling volume must be entered immediately after the start of the program.

Possible entry values	5 to 25 µl filling volume
Entry increments	1 µl

7.2.2 LID

To determine the settings of the heated lid. At LID, the lid temperature wanted is entered. 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 temperature of the heated lid, coupling of the lid temperature to the block temperature can be established at the beginning and 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 (see Sec. 5.5).

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 is switched off in relation to the block temperature. This takes place at temperatures < 22 °C and times > 5 minutes and also applies to Hold or Pause commands with temperatures < 22 °C.

7 Programming

The program commands described in Sections 7.2.3 to 7.2.9 can be selected with Sel or directly with the stated number.

7.2.3 T Selection also possible using 1

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

The following options may be entered for every temperature command by positioning the cursor on the program line number and pressing the Opt key.

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

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 (± °C): For each cycle, the temperature is increased or decreased by the value entered here.

Please note that the temperature cannot exceed 99 °C. For example, with a temperature increment of +0.1 °C and 25 cycles, the starting temperature cannot be greater than 96.5 °C. First enter the numerical value and then the plus or minus sign.

Permitted values	0.0 to 10.0 °C
Entry increments	0.1 °C
Entry for temperature increase	"+"
Entry for temperature decrease	"–"

± 0:00 Time increment (± s): For each cycle, the time is extended or reduced by the value (max. 1 minute) entered here.

Permitted values	0:00 to 1:00
Entry increments	1 s
Entry for time extension	"+"
Entry for time reduction	"–"

R = 3 °/s Ramp (K/s): The ramp states the speed of the heating or cooling curve of a cycler. The higher the value entered, the more rapidly the heating or cooling takes place. Most reactions require rapid heating or cooling rates, i.e. a high value. For processes in which heating or cooling should take place slowly, different values may be entered for the ramp.

Permitted values	0.3 to 3 K/s
Entry increments	0.1 K/s

± 0.0 °/s Ramp increment (± s): For each cycle, the ramp is raised or lowered by the value entered here.

Please note that the ramp cannot exceed 3 K/s, i.e. with an initial ramp entry of 0.3 and a ramp increment of 0.1, a maximum of 27 cycles is possible. First enter the numerical value and then the plus or minus sign.

Permitted values	0.0 to 1.0 K/s
Entry increments	0.1 K/s
Entry for increasing the ramp speed	"+"
Entry for decreasing the ramp speed	"–"

G = 0.0° Gradient: When an entry is made here, the columns of the block are set to different temperatures. The nominal temperature set at "T" is in the center of the block. The lowest temperature is on the left-hand side and increases gradually up to the highest temperature on the right-hand side. This means that a temperature difference of up to 20 °C across the block can be generated. The temperature distribution can be viewed in the OPTIONS/Gradient menu (see Sec. 5.4.3).

Please note that the temperature cannot exceed 99 °C, i.e. if a gradient of ±10 °C is built up, the nominal temperature may not be greater than 89 °C.

Permitted values	± 0.0 to ± 10.0 °C
Entry increments	0.1 °C

7.2.4 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 Enter.

Note: A program should not begin with a HOLD command and a temperature < 22 °C because the temperature-control for the lid will not start. If, for example, 4 °C is required at the beginning of a program, HOLD should be connected to the main program via a LINK command (see Sec. 7.7, "Examples of programming").

HOLD ** ENTER

Permitted values	4 to 99 °C
Entry increments	0.1 °C

7.2.5 PAUSE Selection also possible using 3

The program is interrupted and the temperature remains at the value for the last temperature command. The program is continued by pressing Enter.

A user-defined text may be entered by pressing Sel instead of the preset text "PRESS ENTER".

PAUSE PRESS ENTER

Text length	0 to 11 characters

7.2.6 GOTO Selection also possible using 4

To enter data for cycle repeats. With GOTO, the program line number is entered from which commands are to be repeated. REP specifies how often the commands are to be repeated.

GOTO *** REP *

Permitted values	
Program line number	1 to 40
Repeats	1 to 99

7.2.7 SOUND Selection also possible using 5

To emit an acoustic signal (e.g. for displaying a programmed pause or when a program is ended).

SOUND **

Permitted repeats	1 to 10 tones

7.2.8 LINK Selection also possible using 6

To link up with another program in the internal memory. Via the LINK command, the current program is ended and the selected program is started. With the aid of LINK commands, a maximum of five programs may be run in succession. The CNTRL, LID, NOWAIT/WAIT, AUTO/FIX commands in the program head are not taken into consideration during the subsequent programs.
It is not possible to branch to programs in the processing level ("Edit") or on the personal card using LINK commands (it is possible from the personal card to programs in the internal memory).

LINK ********

Permitted number of programs	5

The name of the program to be called up must be entered (select letters using the Sel key). If the name of the program itself is entered, the program goes into an endless loop and can only be cancelled with "Stop".

7.2.9 end

"end" appears automatically as the last line of a program. It switches off the temperature control for the block and for the lid.

7.3 Creating a new program

The creation of a new program without an example program is described by means of the following example:

Program example:

Program head

Temperature control based on thermoblock: CNTRL "BLOCK"

Lid temperature	LID=105 °C
Lid setting	Program start independent of lid temperature: "NOWAIT"
	At the end of the program, lid heating switched off in relation to the block temperature: "AUTO"

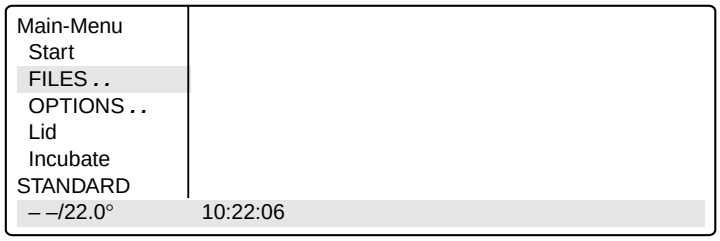
Program sequence

Initial denaturation	95 °C for 2 minutes
20 cycles:	
Denaturation	95 °C for 45 seconds
Annealing	61 °C for 45 seconds with a gradient of ± 10 °C
Elongation	72 °C for 45 seconds
Final elongation	72 °C for 2 minutes

Cooling of the samples and holding the temperature HOLD=22 °C

7.3.1 Programming

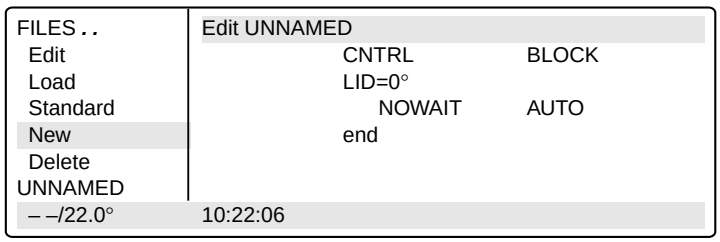
– Select the "FILES" menu using the ▼ or ▲ key and call up by pressing Enter.



– Select the "New" menu using the ▼ or ▲ key and call up by pressing Enter.

Note: If a program which has not yet been saved is in the processing level, a safety check appears automatically which then allows the program to be saved.

Select the relevant entry field with the ▲, ▼, ◀, ▶ keys or Enter.

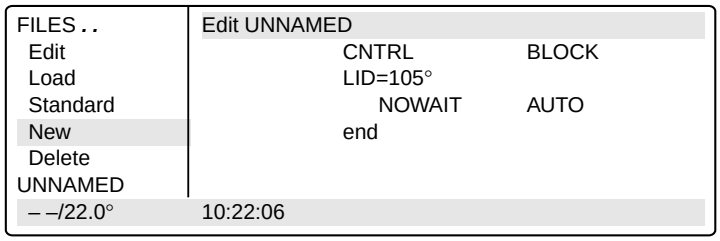


CNTRL BLOCK

– Using the Sel key or confirm by pressing Enter.

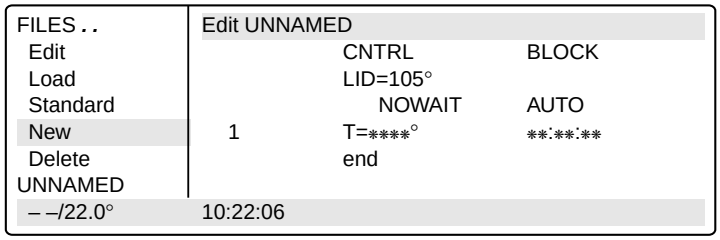
LID=105° NOWAIT AUTO

– Enter values by pressing 1 0 5 keys.
Change "NOWAIT" and "AUTO" (Confirm with Sel) or with Enter.

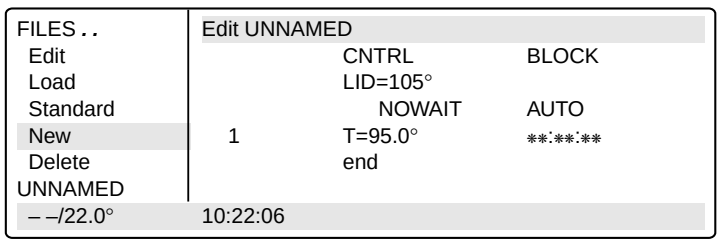


Initial denaturation
T=95.0° 0:02:00

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

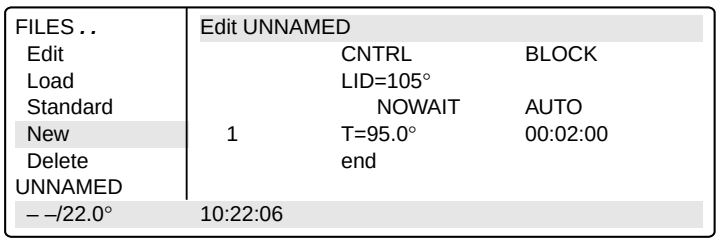


– Enter the temperature, by pressing 9 5 keys and confirm by pressing Enter.



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

Press the [0] [0] [2] keys and confirm by pressing [Enter].



Denaturation

T=95.0° 0:00:45

Program as in the previous step. Press [4] [5] to enter the time and confirm with [Enter].

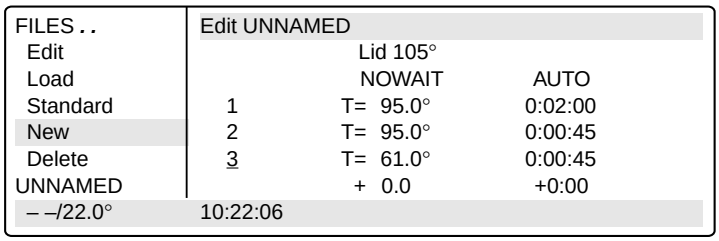
Annealing

T=61.0° 0:00:45

– Select Temperature (T) program line with [Sel] and confirm with [Enter].

– Load temperature options with [Opt] and confirm with [Enter].

Note: The cursor must be on the program line number in front of the temperature command.



– Enter temperature [6] [1] and confirm by pressing [Enter].

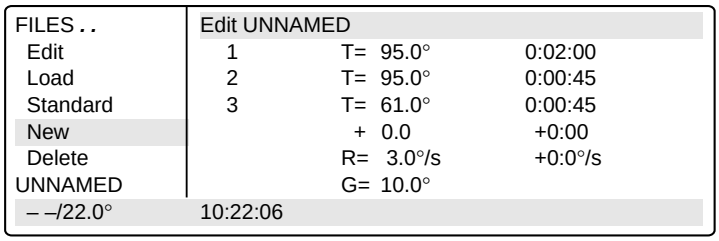
– Enter time [4] [5] and confirm by pressing [Enter].

– Press [Enter] to proceed to Gradient (G).

Gradient

G=10.0°

– Enter the gradient with [1] [0] and confirm by pressing [Enter].



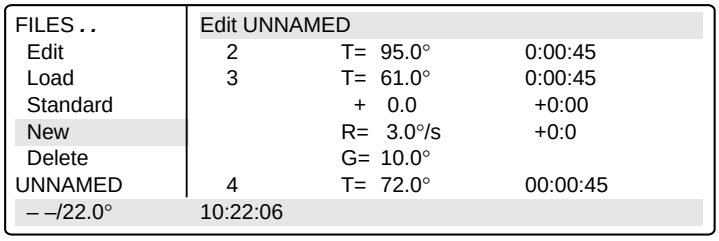
Elongation

T=72.0° 0:00:45

– Select the command T with [Sel] and confirm by pressing [Enter].

– Press the [7] [2] keys and confirm by pressing [Enter].

– Press the [4] [5] keys and confirm by pressing [Enter].



Cycle

GOTO 2 REP 19

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

– Line number from which the program section is to be repeated. Press 2, confirm by pressing Enter. Enter the number of repeats (REP). Confirm 1 9 by pressing Enter.

Note: The total number of cycles is REP + 1 (in the example 19 + 1 = 20).

FILES . .	Edit UNNAMED		
Edit	3	T= 61.0°	0:00:45
Load		+ 0.0	+0:00
Standard		R= 3.0°/s	+0:0
New		G= 10.0°	
Delete	4	T= 72.0°	00:00:45
UNNAMED	5	GOTO***	REP***
– –/22.0°	10:22:06		

Final elongation

T=72.0° 0:02:00

– Select the command T with Sel and confirm by pressing Enter.

– Press the 7 2 keys and confirm by pressing Enter.

– Press the 0 0 2 keys and confirm by pressing Enter.

FILES . .	Edit UNNAMED		
Edit		+ 0.0	+0:00
Load		R= 3.0°/s	+0:0
Standard		G= 10.0°	
New	4	T= 72.0°	00:00:45
Delete	5	GOTO 2	REP 19
UNNAMED	6	T= 72.0°	00:02:00
– –/22.0°	10:22:06		

Cooling of samples

HOLD 22.0° ENTER

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

– Enter the temperature. press 2 2, and confirm by pressing Enter.

FILES . .	Edit UNNAMED		
Edit		R= 3.0°/s	+0:0
Load		G= 10.0°	
Standard	4	T= 72.0°	00:00:45
New	5	GOTO 2	REP 19
Delete	6	T= 72.0°	00:02:00
UNNAMED	7	Hold 22.0°	ENTER
– –/22.0°	10:22:06		

Saving a programm see Sec. 7.3.2.

7.3.2 Saving a program

– End command entry with Exit.

– To save "YES" select with Sel and confirm with Enter.

FILES . .	Edit UNNAMED
Edit	UNNAMED not saved
Load	SAVE: YES
Standard	ProgName: UNNAMED
New	
Delete	
UNNAMED	
– –/22.0°	10:22:06

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

– Confirm the name by pressing Enter or – if an other name is desired – delete by pressing Del (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. To do this, select letters using the Sel and possibly the ± key. Press the ▶ key to move to the next position etc., entering numbers if necessary via the numeric keypad. Confirm name by pressing Enter.

FILES . .	Edit UNNAMED
Edit	UNNAMED not saved
Load	SAVE: YES
Standard	ProgName: Gradient
New	
Delete	
UNNAMED	
– –/22.0°	10:22:06

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 Sel key, press Enter and enter a new name. Confirm the new name by pressing Enter.

FILES . .	Edit UNNAMED
Edit	Testing...
Load	Gradient exists
Standard	Overwrite: YES
New	
Delete	
UNNAMED	
– –/22.0°	10:22:06

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.

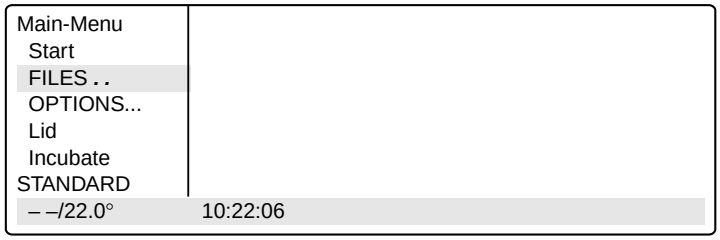
The program in the processing level can be started by pressing the Start Stop key.

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

7.4 Modifying a program

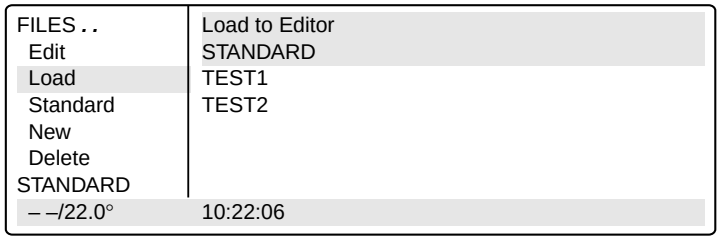
If an existing program is to be modified, it is loaded for this purpose into the processing level from the internal memory or from the personal card (see Sec. 9.3). If a program which has not yet been saved is in the processing level, a safety check appears automatically which then allows the program to be saved.

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

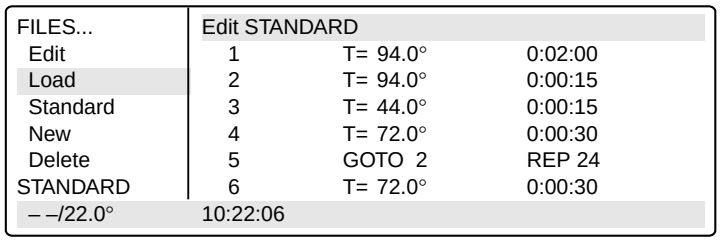


– Select the "Load" menu using the ▼ or ▲ key and call up by pressing Enter.

  A list of existing programs appears in the display.



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



– Using the ▲, ▼, ◀, ▶ keys or Enter, move the flashing cursor onto the parameter which is to be changed.

– Enter data using the numeric keypad or select between settings using the Sel key or enter letters using the Sel 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 Ins key. A new line is inserted above the cursor.

– Select the wanted command using the Sel key.

Note: Press the Opt 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 Del key. The line is deleted.

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

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

– The modified program can be saved using the same name, in which the old program is overwritten. Confirm the check "Overwrite:YES" by pressing Enter.

If the program should not be overwritten, and a new program is to be created, answer "Overwrite:" with "NO" (Sel and Enter keys) and enter a new program name with Sel or the numeric keypad.

Caution: If the changed program is not stored, there will be two different programs with the same name. The program which has been changed is in the processing level and the program which has not been changed is in the device memory.

The modified program can be started in the processing level Start Stop.

– After the end of the program "End of Program" appears in the display. Press the Exit key.

– If you now want to save the program which has been changed, select "Load" menu with ▼ and press Enter. "Program not saved – Save YES" appears in the display.

– Press Enter to save the program .

7 Programming

7.5 Creating programs using an example program

For fast programming, the "Standard" example program can be used. A program does not need to be completely rewritten; the sample can be supplemented, modified and then saved under a new name (see Sec. 7.4).

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

– Select "Standard" and call up by pressing Enter.

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

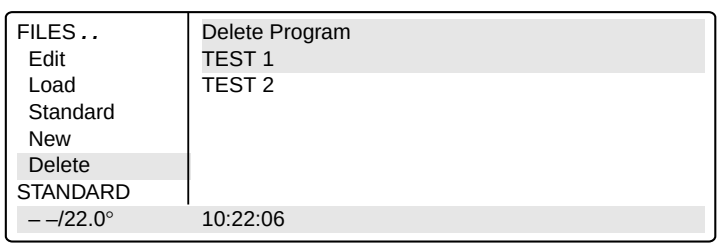
	CNTRL		BLOCK	Temperature control on the block.
	LID=105°			Sets heated lid to 105 °C.
	NOWAIT		AUTO	NOWAIT = Program is started immediately. AUTO = Lid heating is switched off automatically when a temperature <22 °C is maintained for more than 5 minutes
1	T=94.0°		0:02:00	Maintains 94 °C for 2 min. (initial 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 °C and maintains temperature.
	end			Program end

The example program "Standard" cannot be deleted, but 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.

7.6 Deleting a program

– Select the "FILES" menu with the ▼ or ▲ key and call up by pressing Enter.

– Select "Delete" with the ▼ key and call up by pressing Enter.
A list of the existing programs appears in the display.



– Select the program name by pressing the ▼ or ▲ key.

– The program is deleted by pressing Enter.

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

7.7 Examples of programming

The following passage contains several examples of the versatility of the commands and functions of the Mastercycler® 384.

7.7.1 Gradient PCR

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.

Example	***Gradient PCR***		
	Temperature control of samples	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 cycle repetition	GO TO 2	REP 29
6	Final elongation	72°	2 min
7	Cooling and storage	HOLD 20°	ENTER
	End	end	

7.7.2 Using the "PAUSE" and "HOLD" commands

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 the program is stopped after the block preheating, in order to place the samples in the hot block

Caution: There is a danger of injury from burning when inserting the samples!

After the final elongation, the block is cooled down to room temperature.

Example	***PCR with program pause and maintaining the final temperature***		
	Temperature control of samples	CONTROL	TUBE
	Temperature control of lid	LID	105°
		NOWAIT	AUTO
1	Preheating	94 °	5 min
2	***Pause for adding substance***	***PAUSE***	
3	Initial denaturation	94°	2 min
4	Denaturation	94°	15 sec
5	Annealing	55°	15 sec
6	Elongation	72°	30 sec
7	Number of cycle repetition	GO TO 3	REP 29
8	Final elongation	72°	2 min
9	***Cooling and storage***	***HOLD 20°***	***ENTER***
	End	end	

7.7.3 The temperature increment

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 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.

Example	***Touch Down PCR***		
	Temperature control of samples	CONTROL	TUBE
	Temperature control of lid	LID	105°
		NOWAIT	AUTO
1	Initial denaturation	94°	2 min
2	Denaturation	94°	15 sec
3	Annealing	65°	15 sec
	Temperature increment	***–1***°	
4	Elongation	72°	30 sec
5	Number of cycles	GO TO 2	REP 15
6	Denaturation	94°	15 sec
7	Annealing	50°	15 sec
8	Elongation	72°	30 sec
9	Number of cycle repetition	GO TO 6	REP 13
10	Final elongation	72°	2 min
11	Cooling and storage	HOLD 20°	ENTER
	End	end	

7.7.4 The time increment

The time increment, which can be programmed as desired with every temperature command, enables a defined extension of the lag time at a specific temperature for each cycle.

With Long PCR, this function can be used to amplify long fragments for a successive extension of the elongation.

In the example given, the elongation time is extended by 10 seconds for each cycle, which means that, for the 30th cycle, the elongation time is 290 seconds longer than for the first cycle.

Example	***PCR with successive extension of the elongation time***		
	Temperature control of samples	CONTROL	TUBE
	Temperature control of lid	LID	105°
		NOWAIT	AUTO
1	Initial denaturation	94°	2 min
2	Denaturation	94°	15 sec
3	Annealing and Elongation	68°	10 min
	Time increment		***+0:10 sec***
4	Number of cycle repetition	GO TO 2	REP 29
5	Final elongation	72°	15 min
6	Cooling and storage	HOLD 20°	ENTER
	End	end	

7.7.5 Regullating the temperature-control speed

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.

Example	***PCR with reduced heating rate***		
	Temperature control of samples	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 cycle repetition	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.

Example	***PCR with program loop***		
	Temperature control of samples	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
	Temperature increment	***+1°***	
	Heating rate	***R = 0.3 °/s***	
5	Number of cycle repetition	GO TO 4	REP 16
6	Number of cycle repetition	GO TO 2	REP 29
7	Final elongation	72°	2 min
8	Cooling and storage	HOLD 20°	ENTER
	End	end	

7.7.6 Sample cooling with/without subsequent PCR

There are various possibilities for cooling the thermoblock:

1. Set the "Incu" menu to 4 °C and "Heater is ON" (see Sec. 5.6).
2. At the end of a program with the command "HOLD" (see Sec. 7.2.4).
3. If the thermoblock is to be cooled immediately before the start of a PCR program, this is done by using a series-connected program linked to the PCR program with the "LINK" command. In so doing, ensure that
   – the entries in the program head of the series-connected program also apply to the following PCR program,
   – the "FIX" lid setting is selected so that the heated lid operates after cooling.

Example:
Program "KUEHLEN" (cooling):

CNTRL TUBE
LID = 105°
NOWAIT | FIX

1 HOLD 4 °C ENTER

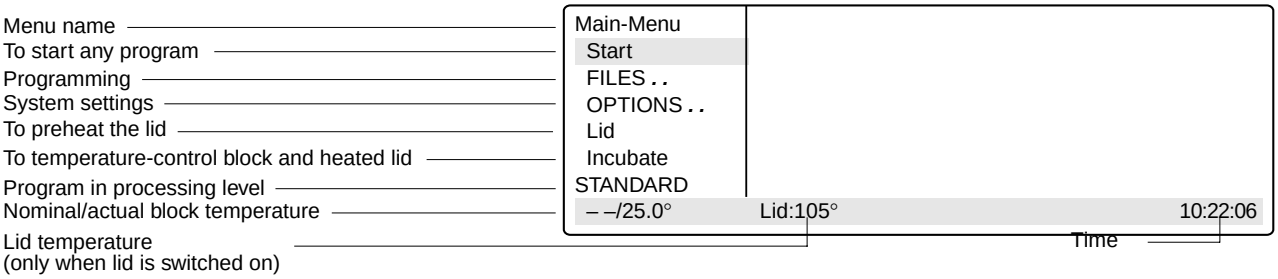
2 LINK PCRPROT
end

After entry of the filling volume, the thermoblock is cooled to 4 °C. After pressing Enter, a PCR program "PCRPROG" runs. Since the program head parameters of the first program always have priority, those of the "KUEHLEN" (cooling) program are automatically taken over (lid temperature 105 °C etc.).

8 Short instructions

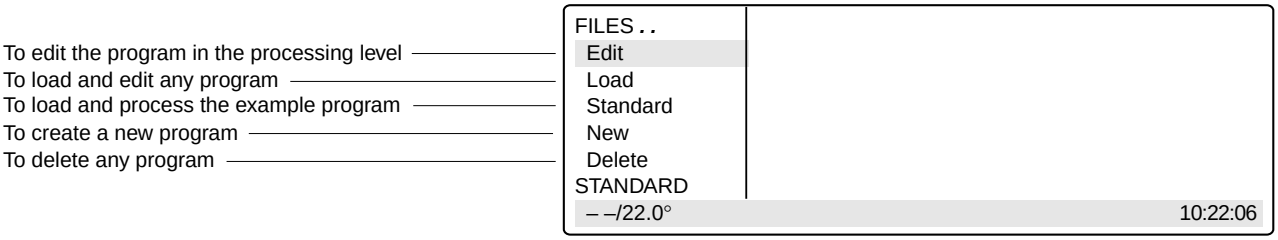
Please read the operating manual completely before working with the short instructions!

Switching on the device



Programming

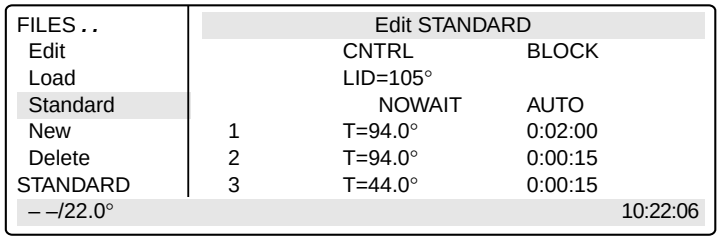
– Select the FILES menu using the ▲ / ▼ keys and press Enter.



Changing the "Standard" example program

– Select "Standard" using the ▼ key and press Enter.
– Using the ▲, ▼, ◀, ▶ keys, move the cursor to or between the entry positions.
– Select the program commands using the Sel key, enter the settings or the texts and confirm by pressing Enter.

CNTRL	To define temperature regulation
BLOCK	Based on thermoblock
TUBE	Based on sample volume
LID	To determine the lid temperature
NOWAIT	To start a program immediately
WAIT	Program waits for lid temperature
AUTO	Lid switches off at <22 °C and time >5 min.
FIX	Lid switches off at end of program

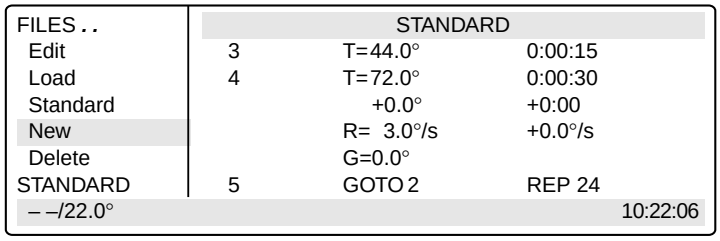


Program commands

T	Temperatures, times, increments, ramp, gradient
HOLD	To maintain temperature entered until program is continued with ***Enter*** (4–99 °C)
PAUSE	To interrupt program until program is continued with ***Enter*** (0–11 characters)
GOTO/REP	To repeat program parts and number of cycles (1–40 lines, 1–99 repeats)
SOUND	Acoustic signal (1–10 tones)
LINK	To branch to other programs (1–5 programs)

– Enter the options for the temperature command using the Opt key. The cursor must be positioned on the program line number.

T	Temperature (0–99°)
	Time (0:00:01–9.59:59)
	Temperature increment (0.0 –10.0°, + or –)
	Time increment (0:00–1:00, + or –)
R	Ramp (0.3–3 K/s)
	Ramp increment (0.0–1.0 °/s, + or –)
G	Gradient (±0,0 – ±10 °C)

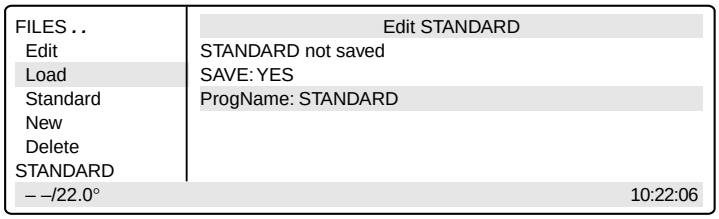


– Confirm entries by pressing Enter.
– Complete programming by pressing Exit.
– Save the program.

8 Short instructions

Saving a program

– Exit the program using the Exit key.
– Answer the question "Save: YES" by pressing Enter.
– Define the program name using the Sel key.
– Confirm the name by pressing Enter.



Adding a program line

– Using the ▲ / ▼ keys, move the cursor to the front of the line.
– Press the Ins key; a new line is then added above the cursor. Select command required with Sel.

Deleting a program line

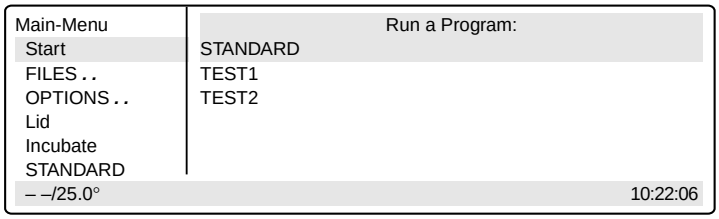
– Using the ▲ / ▼ keys, move the cursor onto the program line number.
– Press the Del key; the line is then deleted and the cursor moves to the next line.

System settings

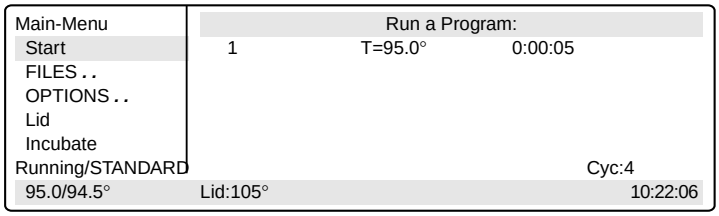
To temperature-control lid and block	Incubate menu	Block temperature 37 °C, Heater is ON
To preheat the lid	Lid menu	LidTemp 105 °C, Heater is ON
To print programming	OPTION menu	Printout Editor: YES
To print program sequence	OPTION menu	PrintProtocol: ON

Starting a program

– Switch on the device. If necessary, preheat the lid and the block.
– Select the "Start" menu using the ▲ / ▼ keys and press Enter.
– Select the program name using the ▲ / ▼ keys and press Enter.

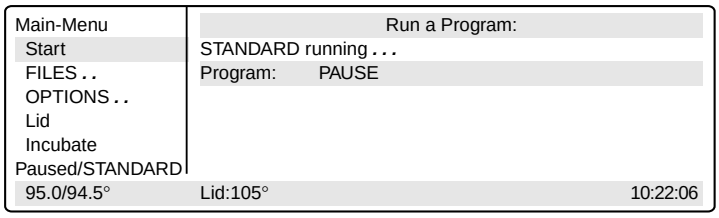


– With CNTRL TUBE Enter sample volume "Fill.Vol.". Values: 5 to 25 µl
– Confirm by pressing Enter. The program starts immediately.
– To see the program run time, press the Opt key.



Interrupting a program

– Press the Start Stop key.
– Using the Sel key, select the "PAUSE" program.
– Confirm by pressing Enter.

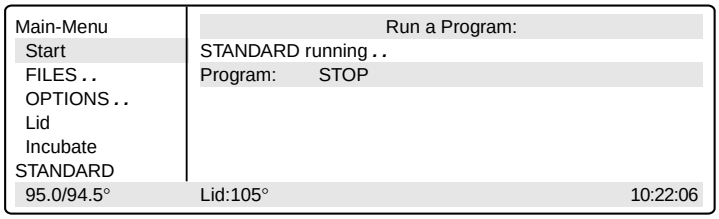


Continuing an interrupted program

– Press the Start Stop key.
– Using the Sel key, select "RESUME".
– Confirm by pressing Enter.

Stopping a program

– Press the Start Stop key.
– Confirm "STOP" by pressing Enter.
– Exit the program by pressing Exit.



9 Personal card

Programs can be saved externally and be transferred to other devices of the Mastercycler® 384, Mastercycler®, Mastercycler® gradient and Mastercycler® personal using the personal card.

Depending on the program length, a maximum of 10 programs can be saved on a personal card.

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.

During formatting, the nominal/actual steps appear in the bottom line of the display.

9.3 Processing programs

When the personal card is inserted in the device, a differentiation is automatically made between the personal card memory ⟨MCARD⟩ and the internal memory of the Mastercycler® 384 ⟨INTERN⟩.

Only programs of a memory are listed in the "Start", "FILES/Load" and "FILES/Delete" menus. The memory which is currently closed appears in the display at the head of a program list as ⟨INTERN⟩ or ⟨MCARD⟩. On selecting the closed memory and confirming with Enter, this memory is opened and the other memory closed.

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

9 Personal card

Starting a program from a card

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

– Select ⟨MCARD⟩ and call up by pressing Enter.
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 start by pressing Enter.

Note: 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 sequence. 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

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

– Select ⟨MCARD⟩ and open by pressing Enter.
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 delete by pressing Enter.

Loading a program from a card into the processing level

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

– Select ⟨MCARD⟩ and open by pressing Enter.
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 load by pressing Enter.

Saving a program on a card

– After a program has been compiled or modified, exit the processing level by pressing Exit.

– Confirm the inquiry "Save: YES" by pressing Enter.

– For the inquiry "Target:INTERN", select ⟨MCARD⟩ using the Sel key and confirm by pressing Enter.

– Enter the program name (max. 8 characters) and confirm by pressing Enter.

Note: The program can be saved under the same name as in the memory of the Mastercycler.

Programs with LINK commands

A program cannot be started from a personal card if it contains program branching – LINK commands – to other programs on the personal card. It is possible to start the program if branching to another program in the internal memory of the device is available.

A program from the internal memory cannot call up a program from the personal card using a LINK command.

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).

Connect the Mastercycler® 384 and printer via a commercially available PC printer cable. The cyclers and printer must be switched off during installation. If the printer is not used frequently, the printer cable should be disconnected from the cycler port and only be installed when needed.

The parameters for the printer are set in the "OPTIONS/Printer" menu (see Sec. 5.4.2).

Example of a program printout:
Setting "Printout Editor: YES":

```
******MASTERCYCLER 384******
          eppendorf
          Version x.xx.xx
          Time: 13:41:02
          Date: 27/Feb/2001
**************************

LISTING
Program:          STANDARD          <-- Program name
Created:          23/Feb/2001 11:46 <-- Date last stored
Step:
      CNTRL         BLOCK

      LID=105C
        NOWAIT      AUTO

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

2     T = 44.0C     0:00:15
          +0.0C      + 0:00
      R = 3.0C/s    +0.0C/s
      G = 0.0C

.     .
.     .
.     .

7     HOLD  22.0C  ENTER

END OF LISTING
```

Example of a program protocol:
Setting "Print Protocol: ON":

First a program is printed out 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: 27/Feb/2001 7:56:12
Initial Blocktemp:                 24.7 C
Initial Lidtemp:                     23 C

                                   Tube:plate 384      Fill.Vol.:10 µl

Run Time:                            1:26
Finish Time:                       27/Feb/2001       9:22

Count     Cycle     Step     Command                        Time

                             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:             27/Febr/2001       9:22:51

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® 384, Mastercycler®, Mastercycler® gradient and Mastercycler® personal devices.

If a program contains temperature commands with a gradient function, this function is omitted on transfer of the program to the Mastercycler® and Mastercycler® personal. In the transfer between the Mastercycler®. gradient and Mastercycler® 384, the temperature distribution of a programmed gradient takes place according to the block concerned.

11 Maintenance

The Mastercycler® 384 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 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.

Error message	*Cause*	*Solution*
Memory Error	The internal memory for programs is defective.	Evaluate further messages.
Memory full!	Memory is full.	Before storing, delete programs which are not required.
MemoryCard (Blank)	Card has incorrect logical identification and/or is blank.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard Defective!	Card is defective.	Use a new card.
MemoryCard HdChksumErr	Error in check sum.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard ManIDErr	Incorrect identification.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard MemOverErr!	Incorrect memory address.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard MemRangeErr	Error in memory range.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard MemSizeErr	Incorrect memory size.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard MemUnderErr!	Incorrect memory address.	Card must be formatted. Automatic procedure when program is saved.
MemoryCard Missing!	No card has been inserted.	Insert card.
MemoryCard Not Valid	Card is invalid.	Insert card correctly (check that card is facing in the correct direction). Card should be formatted before saving begins.

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.

12 Troubleshooting

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® 384

Sample capacity:	1 PCR plate 384
Temperature range:	4 to 99 °C
Temperature uniformity across the block:	20 °C to 72 °C ± 0.5 K 95 °C ± 0.9 K
Block homogeneity ($\bar{S}_{95}$):	20 °C to 72 °C ± 0.3 K 95 °C ± 0.4 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. 100 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
Power requirement:	500 W 500 W
Operating current:	2.6 A 5 A
Fuses:	2 x T 5 A, 250 V 2 x T 6.3 A, 250 V
Overvoltage category:	II
Pollution degree:	2
Contamination level:	I
Operating conditions:	15 to 35 °C, up to 70 % rel. humidity
Storage conditions:	–20 to 70 °C, up to 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® 384
5334 000.003	230 V, 50–60 Hz
5334 000.011	115 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
5334 900.101	Operating manual for Mastercycler® 384
5332 300.018	Personal card
	Consumables
0030 127.633	Heat Sealing Foil (100 sheets)
0030 127.480	PCR Film, adhesive (100 sheets)
0030 127.471	PCR Foil, adhesive (100 sheets)
0030 127.447	PCR plate 384, colorless (50 pcs.)
	Recommended auxiliary equipment
5390 100.029	Base Plate for 384 well plate for Heat Sealer
	Eppendorf Research® pro Multi-channel pipette with charging adapter
4860 000.518	0.5– 10 µl, 8-channel
4860 000.534	5–100 µl, 8-channel
0030 127.641	Foil Stripper
5390 000.016	Heat Sealer 115 V / 60 Hz
5390 000.024	Heat Sealer 230 V / 50 Hz
	PCR Reagents
	see Main Catalog

14 a Ordering information for USA and Canada

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® 384
5334 000.003	230 V, 50–60 Hz
5334 000.011	115 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
5334 900.101	Operating manual for Mastercycler® 384
5332 300.018	Personal card
	Consumables
0030 127.633	Heat Sealing Foil (100 sheets)
0030 127.480	PCR Film, adhesive (100 sheets)
0030 127.471	PCR Foil, adhesive (100 sheets)
0030 127.447	PCR plate 384, colorless (50 pcs.)
	Recommended auxiliary equipment
5390 100.029	Base Plate for 384 well plate for Heat Sealer
	Eppendorf Research® pro Multi-channel pipette with charging adapter
2246 140-1	0.5– 10 µl, 8-channel
2246 141-9	5–100 µl, 8-channel
0030 127.641	Foil Stripper
5390 000.016	Heat Sealer 115 V / 60 Hz
5390 000.024	Heat Sealer 230 V / 50 Hz
	PCR Reagents
	see Main Catalog

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Eppendorf AG
22331 Hamburg · Germany
Phone: +49 40-5 38 01-0
Fax: +49 40-5 38 01-556
e-mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com
Application Hotline:
Phone: +49 180-3 66 67 89
e-mail:
application-hotline@eppendorf.com

Brinkmann Instruments, Inc.
One Cantiague Road,
P.O. Box 1019
Westbury, New York 11590-0207
(USA)
Phone: 800-645-3050
Fax: 516-334-7506
e-mail: info@brinkmann.com
Internet: www.brinkmann.com

Printed in Germany

Support and Services Directory

Contact Information

	United States	Canada
Business Hours:	8:30 a.m. to 6:00 p.m. EST	8:30 a.m. to 6:00 p.m. EST
Phone:	800-645-3050 516-334-7500	800-263-8715 905-826-5525
Fax:	516-334-7506	905-826-5424
Address:	Eppendorf North America, Inc. One Cantiague Road Westbury, NY 11590-0207	Brinkmann Instruments (Canada) Ltd. 6670 Campobello Road Mississauga, ONT L5N 2L8
Website: **Email:**	www.eppendorf.com info@eppendorf.com	www.brinkmann.com canada@brinkmann.com
Customer Support:	800-645-3050, menu option 2 custserv@eppendorf.com	800-263-8715, menu option 1 custserv@brinkmann.com
Repair:	800-645-3050, ext. 2404 service@eppendorf.com	800-263-8715, ext. 232 service@brinkmann.com
Applications Lab:	800-645-3050, ext. 2258 apps@eppendorf.com	800-645-3050, ext. 2258 (U.S.) bioapps@brinkmann.com

For more information contact your Eppendorf North America Sales Representative at 800-645-3050.

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