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THE MWG PRIMUS THERMAL CYCLER

Instruction Manual

This instruction manual includes operating instructions for the following Primus Thermal Cyclers:

Primus 25 PCR-System

Primus 96 PCR-System

Primus 96 PCR-System with High Pressure Lid

Primus 96 PCR-System with motorized High Pressure Lid

Primus 384 PCR-System with High Pressure Lid

Primus 384 PCR-System with motorized High Pressure Lid

Primus In Situ PCR-System

An additional instruction manual is available for the Primus Multiblock PCR- Systems (Primus HT)

- 96-well High Pressure Lid blocks (with/without motorized lid)
- 384-well High Pressure Lid blocks (with/without motorized lid)

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1. Start-up

The following items must be followed for optimal and safe operation of the Primus thermal cycler:

- ❖ Before starting the Primus, check the voltage range and be certain it corresponds to your power supply.
- ❖ The instruments should be set up so that a distance of at least 25 cm to the next wall or item is maintained. Two cyclers should not be standing directly behind one another. Only this ensures perfect cooling of the systems.
- ❖ The Primus instruments were developed for operation in laboratories, in which there is no explosive atmosphere and normal ambient pressure. The ambient temperature should be between 4°C and 30°C, the humidity between 10% and 90%.
- ❖ Primus cyclers should be protected from exposure to liquids. Otherwise, the function of the instrument might be impaired. Smaller quantities of condensing water, which might build up on the thermo-block, should be removed using a cloth.

Startup

The main switch is above the connection for the power cord, on the back of the instrument.
Shortly after startup, the software version is displayed.

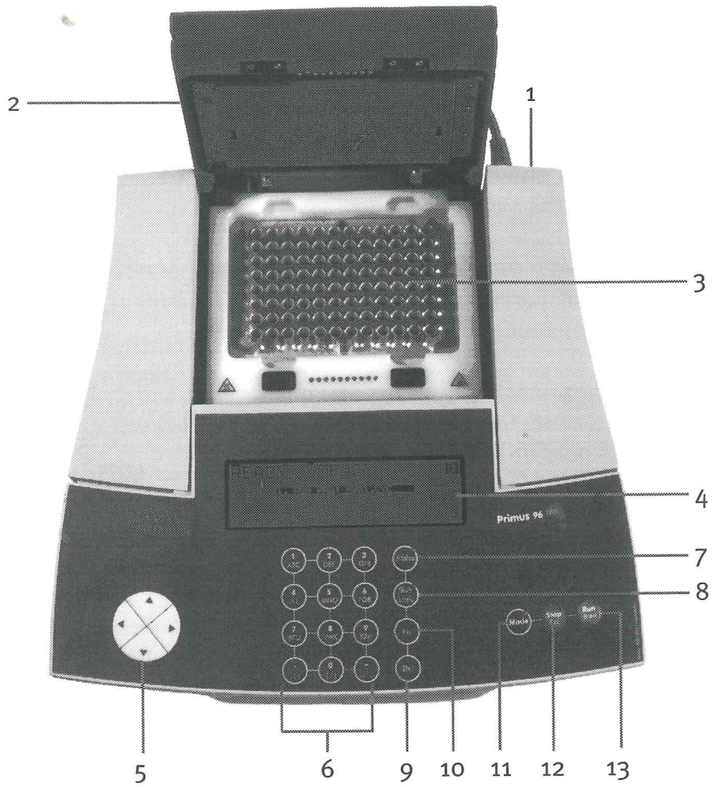
For example:

Welcome
Primus 96+ **V1.19R** (c) RW 3399

Gerätetyp Softwareversion

Then, the main menu appears.

2. Diagram of a Primus thermal cycler:



1	Mainswitch	
2	Lid	integrated lid heating, (optional high pressure lid on 96 and 384 models)
3	Thermal plate	for reaction vessels (single tubes and microplates)
4	Display	displays menus and programs
5	Cursor key pad	moves the cursors on the display
6	Alphanumeric key pad	for entering parameters
7	Alpha key	switches between numbers and letters
8	Shift lock key	switches between lower case and capital letters
9	Delete key	deletes parameters or functions
10	Insert key	inserts parameters or functions
11	Mode key	selects edit and display options
12	Stop/Esc key (red)	exits a menu option to return to a higher menu level
13	Run/Enter key (green)	selects menus and program options

3. Main menu

The software for Primus thermal cyclers is designed for simple and intuitive use. Generally, the arrow keys are used to move the cursor on the display screen. Text is entered using the alphanumeric key pad. The position of a letter on the key tells how many times the key must be pressed (in rapid sequence) to obtain that letter. Use the **Run/Enter** key to access the various function menus, and the **Stop/Esc** key to exit the function menus and return to the main menu.
When switching on the instrument, the screen first displays an initialization routine, then the main menu appears from which all other functions are accessible.

3.1. RUN PROGRAM: XXX.CYC

With this option, the last run cycling script can be started again.

3.2. SELECT AND RUN PROGRAM

To run previously stored programs select **SELECT AND RUN PROGRAM**. A list of all stored programs (up to 90) is displayed. Select the desired program with the arrow keys, or enter the program number directly. Press **Enter** to start. Before each run, a user name may be entered via the alphanumeric key pad.

At this point you can decide whether a GLP report shall be printed via the parallel printer interface (**GLP ONLINE PRINTS; GLP** = Good Laboratory Practice) or determine that in the case of a power failure the samples will be denatured before the program resumes (**POWER FAILURE DENATURATION**). Switch between YES and NO with the (↓↑) cursor keys. A predicted program run time will be displayed before the start.

Programs can be interrupted at any time during a run with the Stop/Esc key. Follow the instructions on the display.

3.3. RUN INCUBATION

Primus cyclers may be used as incubators with the **INCUBATION** function. Choose the desired temperature **(INCUBATION-TEMPERATURE)** and switch the lid heating on or off **(LIDHT)**. The current temperature, the status of the lid heating and the total time are displayed.

3.4. MODE/EDIT/UTILITIES

With this menu option you can edit and review all programming and operating functions.

3.4.1. PROGRAM

With the submenu **PROGRAM** programs may be created and changed.
The following parameters are available: NEW; EDIT; COPY; DELETE

3.4.2. VIEW

With the **VIEW** option, an overview of the stored programs may be viewed without risking an accidental alteration of the data. The GLP report of the last program run can be viewed on the display. Information about setup, system, and block are available here.

3.4.3. PRINT

With the **PRINT** menu programs, program lists, GLP reports and equipment information may be printed via the parallel printer interface.

3.4.4. SETUP

The basic setup (HARDWARE-OPTIONS) of the cycler is entered via the **SETUP** sub-menu. To enter the time and date use **CLOCK**. The temperature control at the option **SENSOR** must be set on 'block'. If several Primus thermal cyclers are connected via the lab bus (RS485), enter the machine's address here.

4. Programming

Thanks to the convenient user interface of the Primus, it is easy to create PCR programs of various degrees of complexity. In the main menu, move the cursor to **SET MODE** and press Enter, or go to the **PROGRAM** menu with the **Mode** key. The following options are available:

NEW..:	Creates a completely new program.
EDIT..:	Changes an existing program.
COPY..:	Copies a previously stored program.
DELETE..:	Deletes one or all programs.

4.1 Program header

With the cursor keys (↓↑) the **HEADER** of the program is accessible. Here, a comment **(CMNT)**, author **(AUTH)**, and user **(USER)** may be input. Also, a denaturation step in case of power failure **(PWRFAILDENAT)** may be programmed.

4.2. Steps of the program

The following program steps are available for programming the Primus. They may be selected using the cursor keys (↓↑). Only Primus High Pressure Lid (HPL) instruments are fitted with a chemical actuator and are able to perform programmed bearing pressure. Primus 25 and Primus 96 models do not have a High Pressure Lid.

TEMP
a) Temperature
b) Temperature change within a program loop.
The sign states if the temperature is increased or decreased.
c) Period of time for which the preset temperature is to be held.
Using the „minus" key, "FOREVER" may be input.
d) Time change within a program loop. The sign indicates whether the time is increased or decreased.

RAMP The ramp always starts at the temperature defined last and runs with constant speed °C/sec to the stated target temperature.

PAUSE The process is paused for the stated time at the preset temperature. The pause may be interrupted at any time by pressing a key. The thermal cycler emits an audible signal when the pause is reached. After pressing the key Run/Enter, the program continues with the next step.

LOOP[Start of a program loop which is closed by a subsequent Loop] step. Input the number of cycles. No "loop within a loop" (nested loops) are possible!

LOOP] End of a program loop.

LIDHT The lid heating system may be switched on or off. Temperatures within the range of 70°C to 120°C are kept constant within the program after the Lidheat step.

LIDCL The program sequence is stopped and the operator is requested to close the lid. Additionally, for **Primus HPL instruments**, lid pressure may be programmed: Control of the chemical actuator in the High Pressure Lid. Lid pressure is adjustable between 100 N and 300 N. If 0 N (zero Newtons) is entered, the lid pressure is not activated and the only pressure applied is the lid spring.

For HPL models, the recommended pressures are:
- 96 well microplate: **100 N to 150 N**
- 384 well microplate: **200 N to 300 N**

No lid pressure is used **for individual tubes! (LIDCL 0 N)**

With this program step the lid of a Primus with a motorized lid closes automatically.

LIDOP The program sequence is stopped and the operator is requested to open the lid. Additionally, **for Primus HPL instruments**:
The lid pressure is released with this step.
When the lid has released its pressure, the user is called to the instrument.

With this program step the lid of a Primus with a motorized lid opens automatically.

STORE The step **STORE**, can be used to complete a program:
This step includes the following functions:
❖ switching the lid heating system off
❖ pressure release of the High Pressure Lid
❖ cooling to 8°C; (from software version V1.19T, the time is programmable)
❖ the operator is called to the instrument

END The **END** step cannot be programmed or deleted, but is always found at the end of a program. When reaching this step, the instrument is actively run to ambient temperature and the operator is called to the instrument.

During the cooling phases (target temperature ‹ ambient temperature), the lid heating system should be switched off.

4.2.1. Additional Instructions For Programming of High Pressure Lid Instruments

❖ When using individual tubes, no lid pressure should be applied **(LIDCL o N)**

❖ Programs may be optimized by setting the step **LIDHT** before the step **LIDCL**. This causes simultaneous lid heating and pressurization to occur.

❖ If the **STORE** step is not inserted at the end of the program, the following steps must be input:

LIDOP for release of pressure of the High Pressure Lid
LIDHT off for switching the lid heating system off

❖ If, during the course of a program using lid pressure, **repipetting** has to be performed, the following steps must be programmed:

LIDOP: The pressure of the lid is released. After the pressure is completely released, the user is called to the instrument. At this point, the lid may be opened and pipetting enacted.
➔ the step must be confirmed using **Enter**

LIDCL: Using this step and the corresponding pressure setting, the lid pressure is re-established.
➔ the step must be confirmed using **Enter**

To temper the samples during the pipetting process, a specific holding temperature of the block may be set between the two steps LIDOP and LIDCL.

4.2.2 Additional Instructions for the Operation of Motorized High Pressure Lid Instruments:

The motorized lid can only be opened with the (←→) cursor keys or with the LIDOP/LIDCL function.

4.3. Sample program

After selection of **MODE**..., then **PROGRAM**... create a new program with **NEW**. Enter a name for the program file and confirm it with **Enter** to access the programming functions.

With the cursor keys (↓↑) access the **HEADER**. Move from each parameter to the next with the cursor keys (←→) or with **Enter**.

00	HEADER	COMMENT: PCR PROGRAM AUTHOR: Example USER: Example PWRFAILDENAT: Yes 95.0°C 0:02:00		After power failure the instrument starts with a denaturation step (95°C, 2min) before the program continues with the next programmed step

With the cursor keys (↓↑) or **Enter** the first program step is highlighted.

01	LIDHT	ON	110°C	Lid heat on, temperature 110°C

With **Enter** a new program step may be input. Move from each parameter to the next with the cursor keys (←→) or with **Enter**.

02	LIDCL		100 N	Chemical actuator on; pressure 100 N
02	LIDCL		0 N	Chemical actuator off; pressure 0 N With the use of singular tubes

Again, enter another program step with **Enter**. Choose one of the steps listed above by using the (↓↑) cursor keys, e.g., **TEMP**.

03	TEMP	94.0°C 0:02:00	+0.00°C +0:00	Denaturation at 94°C For 2 minutes

To add a thermal cycling step press **Enter** and select Loop[.

04	LOOP[	25x		Open a program loop – 25 cycles

Continue by entering a temperature profile. The next program shows a typical cycling setup for touchdown PCR.

05	TEMP	94.0°C 0:00:30	+0.00°C +0:00	Denaturation at 94°C For 30 seconds
06	TEMP	50.0°C 0:00:30	-0.2°C +0:00	Annealing at 50°C with stepwise Temperature decrement by 0,2° C (touchdown)
07	TEMP	72.0°C 0:00:30	+0.0°C +0:02	Elongation with stepwise time increment of 2 seconds

End the cycling step with **LOOP]**.

08	LOOP]			End the program loop

With the multifunctional step **STORE** the program is ended.

09	STORE	AT 8.0°C	1:00:00	-lid heating off -releasing the pressure -cooling to 8°C for one hour -user is called by a signal

When a **STORE** step is not used, the following single steps should be input:

LIDHT off: lid heating off
TEMP: enter the target temperature
LIDOP: releasing the lid pressure; the user is called by a signal

The last program step, **END**, cannot be programmed or deleted.

10	END			End of the program

Programming is completed by pressing the **Stop/Esc** key. The program may be stored by subsequent pressing of the **Run/Enter** key.

Ramps may be inserted into any program. A ramp starts from the last programmed temperature and increases or decreases temperature to the next programmed temperature at a constant rate (°C/sec).

RAMP	60.0°C 1,0°C/s	+0.0°C +0:00	Target temperature 60°C with a Speed of 1°C per second

4.4. Storage of programs

After writing or editing a program, exit the program editor using **Stop/Esc**. Edited or newly created PCR programs are tested during storage and before running the program in a simulation run. It is only possible to leave the editing mode if the program is error free. Possible sources of errors are shown to the operator. A calculated program run time **(CALCULATED TIME)** is stated. The new PCR program is stored using **Enter (SAVE PROGRAM)**.

5. Operation of the High Pressure Lid

In order to operate the Primus High Pressure Lid (HPL) optimally, the following conditions must be observed:

Only use microplates when lid pressure is programmed. Activating lid pressure will crush individual tubes.

The „Thermosprint“ microplate was developed for applications in the Primus thermal cycling systems. If low profile microplates (for instance, Costar Polycarbonate) are used, a spacer plate must be inserted into the lid. It is enclosed as an accessory.

While the pressure of the lid is increasing, the display flashes as follows:

Lid Pressure:	xxxN
300 mA	xxxs

The lid pressure set during programming allows for calculation of the pressing time. This pressing time is only shown after the lid has made contact with the consumable in the block.

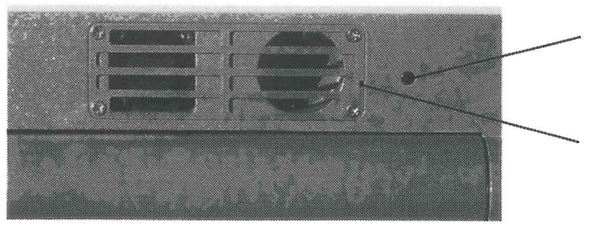
If the chemical actuator does not make contact after 2 minutes, it is automatically switched off and the following fault message appears:

System Message
HPL Actor Fault
(Press)

While running a program (heating + cooling), the lid of the Primus HPL may not be opened!!
Before opening, the lid must first have released pressure and contracted. With the lid open, the pressure cannot be released.

If the lid should be opened inadvertently, there is a reset switch for releasing the lid pressure. There is a small hole in the ventilation grid on the back of the lid. Insert a paperclip into this hole to depress the reset switch. During emergency pressure release, a red diode is lit on the back of the lid.

Backside of High Pressure Lid



While pressure is being released, the following text flashes on the display:

PLEASE WAIT
Releasing lid pressure
LEAVE LID CLOSED

During pressure release, the lid may not be opened and new programs may not be started.

6. System Messages

In case the user has made a fault or during the operation of the Primus an operational fault or unexpected event occurs the system announces with "System Message" and an event code number. The meaning of the event code number is subsequently listed

No.	Error description	Acknowledged by
0	Errorfree	System
1	Wrong firmware in device	System
2	Keyboard not found	System
3	LCD not found	System
4	Device overheated	User
5	12 V not found	System
6	(+ 12 V) not found	System
7	(VL/12 V) not found	System
8	Fuse on power unit blown	System
9	Controller EEPROM defective	System
10	Battery voltage too low	User
11	Block sensor defective	User
12	Tube sensor defective	User
13	Lid heating sensor defective	User
14	Heatsink sensor defective	User
15	Peltier reading too low	User
16	Lid heating defective	User
17	General Peltier defect (failure)	System
18	Only Primus96: poor Peltier synchronism	User
19	Block temperature deviates more than ± 5° from setpoint	User
20	Only Primus96: malfunction locking magnet	User
21	Only Primus96: malfunction pressure buildup	User
22	Only Primus96: applied pressure actor power consumption too low	User
23	Only Primus96: applied pressure actor power consumption too high	User
24	Only Primus96: malfunction pressure decrease	User
25	Block EEPROM defective	User
26	Power-down	User
27	Illegal LaborBus address entered	User
28	Illegal date entered	User
29	Illegal time entered	User
30	Temp entry error (illegal value)	User
31	Temp entry error (illegal value)	User
32	Time entry error (illegal value)	User
33	Timel entry error (illegal value)	User

34	Illegal value for ramp gradient	User
35	Illegal loop[counter	User
36	Illegal file name	User
37	Memory full	User
38	Printer offline	User
39	Printer paper empty	User
40	Printer fault	User
41	LidHt entry illegal	User
42	Denat time entry error	User
43	Temp block not found	User
44	Tube sensor card not found	User
45	Nonsensical reading from tube sensor	User
46	No memory card in slot	User
47	Wrong memory card in slot	User
48	Memory card battery low	User
49	Memory card battery defective	User
50	Memory card write-protected	User
51	Lid open (should be closed)	User / autom.
52	Lid closed (should be open)	User / autom.
53	General write error memory card	User
54	Write protection memory card or file activated	User
55	Only Primus96: illegal applied pressure entry	User
56	Only Primus96: malfunction unlock	User / autom.

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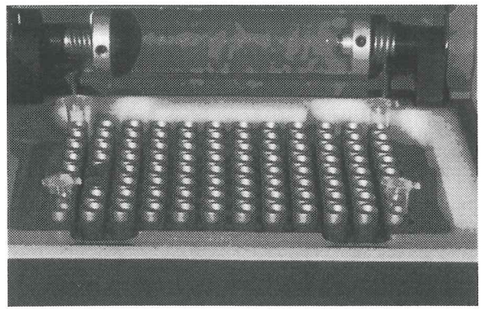
7. Reaction vessels and seals

Both 0.2 ml and 0.5 ml tubes, strips and microplates can be used in the Primus 25 and 96 models with standard lid. For optimum results, only use thin-walled tubes with a slim cone. The Primus 96HPL, 96ML can be used with 0.2 ml tubes and microplates while the 384HPL and 384 ML can only be used with microplates.

To achieve the best results with respect to seal and performance of PCR, the following microplates / seals should be used.

Singular tubes
For the use of singular tubes (0.2 ml) the chemical actuator must remain switched off. This means during programming in the step LIDCL o N must be input. Empty .2ml tubes must be placed in each corner of the block in order for the spring action of the lid not to be hampered. Single tubes may bind the lid at an angle causing the lid not to retract properly, crushing your tubes in the process.



96 well plates:

Microplatte	*Seal*
Thermosprint IN-2596	Adhesive film HB-0558
Thermosprint IN-2596	Cycle-seal blue RO-CYS1
Thermosprint IN-2596	Silicon knub mat IN 7000
Thermosprint IN-2596	Aluminium adhesive film IN 7201

Note:
For all low profile microplates, a spacer plate must be inserted into the lid. It is enclosed with the thermal cycler as an accessory.

384 well plates:

Microplate	*Seal*
384-well MTP W-770311301*	Cycle-seal blue RO-CYS1 + compression pad*
384-well MTP W-770311301	Adhesive film HB-0558 + compression pad
384-well MTP W-770311301	Aluminium film IN 7201+ compression pad
384-well MTP W-770311301	Compression pad II HB-CPD2

**The best results were achieved using this combination of consumables.*
A bearing pressure of 200 N is sufficient.

Note:
To reliably seal 384 well microplates, cover film and a compression pad (HB-CPD1) must be used together

Other 384 well microplates:

Microplate HB-0384-TF:	250 N-300N bearing pressure necessary; more robust than MP W-770311301
Microplate NU-264584:	250 N-300N bearing pressure necessary; more robust than MP W-770311301

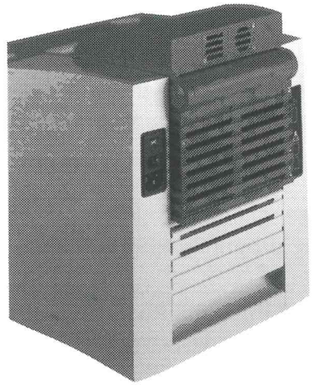
Seals for 384 well microplates:
Adhesive film HB-0558 + Compression pad HB-CPD1
Aluminium adhesive film IN 7201 + Compression pad HB-CPD1
Compression pad HB-CPD2

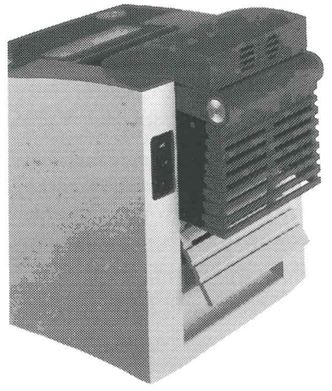
Order numbers:

IN-2596	96 well microplate, Thermosprint; transparent
W-770311301	384 well microplate, white
HB-0384-TF	384 well microplate, white
NU-264584	384 well microplate, white
HB-CPD1	Compression Pad, Si-Foam, natural, 3 mm
IN-7000	Silicon knub mat for 96 well MTP, transparent
IN-7201	Aluminium film, adhesive
RO-CYS1	Silicone film; cycle-seal, blue
HB-0558	Sealing tape, transparent
IN-7201	Aluminium adhesive film
HB-CPD2	Compression pad
HB-0266N	Strip of 8 Tubes with lid, 0.2 ml
HB-0620N	Tubes, 0.2 ml, with lid
HB-0625	25 well microplate, 0.2 ml, transparent

8. Instructions for removing blocks

The Primus cyclers are equipped with a newly developed precision lock for a change of the thermal block in seconds. Turn power off and interrupt powerline, otherwise hazard of dejustage. To change the block, release it with the lever on the back of the instrument. The block will be ejected to the back along the guide rails by a transmission wheel.





9. Safety Precautions

Electrical voltage: to	Ensure that the proper voltage is supplied the instrument before turning it on for the first time.
Explosive substances:	Explosive and reactive substances should never be cycled or incubated in any Primus instrument.
Fluids:	Reaction vessels should be filled outside the cycler so that no fluids penetrate the instrument.
Danger of burns:	Thermal block, inner side of heating lid and reaction vessels quickly attain temperatures of greater than 50°C. These parts are marked with the DIN sign. Keep the heated lid closed until temperatures of 30°C or lower are reached.

All repair work must be done by MWG service engineer! Only original MWG BIOTECH replacement parts may be used. Before opening the instrument the power cord should be removed from the power supply.

10. Memory Card Operation

MWG SRAM memory cards consist of very low power consumption CMOS SRAM IC's, decoder IC's and power control IC's mounted on a special printed circuit board by using advanced surface mount technology which offers portable and non-volatile memory storage.

When the optional memory card is inserted into the PCMCIA slot in the front of the thermal cycler, you will have the option of storing your cycling programs for use on multiple instruments or for back-up of your programs. You can store up to 50 programs on a single Primus memory card. Individual programs can be copied directly into the memory of the thermocycler for permanent storage.

If the memory card is used, many of the menus will change, giving you the option to run programs, write new programs, edit, copy and delete existing programs from either the internal memory or from the memory card.
The following screen will appear when you select "Run" from the main menu:

Run
1. Memory-Program
2. Card-Program

If no programs have been saved on the memory card (or on the internal memory), the following screen will appear:

Run
Programs on card: 0
No programs available!

When writing new thermal cycler programs, you have the opportunity to save directly on the memory card or in the thermal cycler memory. Simply choose the location where you want the program to reside. The following screen will appear when writing new programs if the card is present:

New Prg
Memory-Program
Card-Program

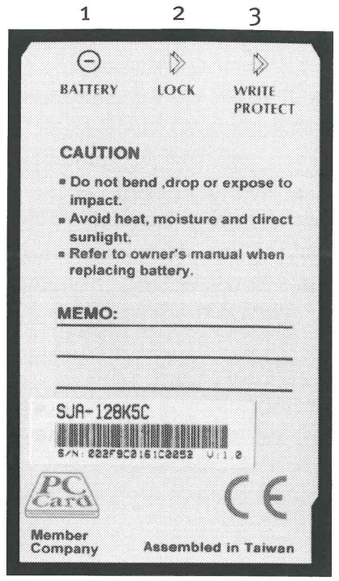
Copy functions are expanded to give you four new options:

1. Memory › Memory	Used for copying programs stored in the thermal cycler memory. The copied program is re-named and can be edited like a normal program.
2. Memory › Card	Transfers programs from the thermal cycler's memory to the PCMCIA memory card.
3. Card › Memory	Transfers programs from the PCMCIA memory card to the thermal cycler's internal memory.
4. Card › Card	Allows the user to copy the contents of one memory card to another memory card.

10.1 Memory card handling precautions:

- Do not bend or flex the memory card.
- Do not expose the memory card to moisture, water or fluids of any kind.
- Always carry the memory card in its plastic case when not inserted into the PCMCIA slot in the thermocycler.
- Never write to the memory card when the battery is low. The thermocycler will alert you if the battery in the memory card is low.
- Never eject the memory card when the thermocycler is writing to it.

4



1 Flash memory cards require a battery to store information.

2 The 3-volt CR2325 battery can be replaced by unlocking the lock tab on the end of the memory card and sliding out the battery holder.

3 If you do not want your programs deleted, modified or over-written, you can slide the write protect tab in the direction of the arrow. When the memory card is write protected, you can not write any new programs to the memory card. You can deactivate write protect any time by sliding the write protect tab in the opposite direction of the arrow marked on the bottom of the card.

4 Insert this end into the thermal cycler.

11. Specifications

Temperature

Temperature range of block	4°C to +105.0°C
Control accuracy of block	± 0.1 °C
Uniformity well-to-well	± 0.5°C
Cooling rate of block	up to 3°C/sec
Heating rate of block	up to 4°C/sec
Temperature range of heated lid	70°C to 120°C
Control accuracy of block	± 1 °C

Block

Primus 25	25 x 0,2 ml tubes; 13 x 0,5 ml tubes, 25 well microplate
Primus 96	96 x 0,2 ml tubes; 48 x 0,5 ml tubes, 96 well microplate
Primus 96 HPL	96 x 0,2 ml tubes; 96 well microplate
Primus 384 HPL	384 well microplate
Primus In Situ	4 slides
Primus HT	With Primus 96 HPL or Primus 384 HPL blocks

Software

Memory	90 Programs with a maximum of 99 Steps/Program
PCMCIA-Memorycard	Optional
Programming steps	TEMP, RAMP, PAUSE, LOOP[, LOOP], LIDHT, LIDOP, LIDCL
Time	0:00:01 bis 9:59:59, unlimited ('forever')
Time increment/decrement	0:01 bis 9:59
Temperature increment/decrement	0.1 °C bis 9.9°C
GLP report	
Instant incubation	
Autocalibration	
Comment, author, user for each program	
Remote control for use with PC	
Power failure recognition	
Internal real time clock	
Service control system	

Interfaces

RS232 interface (9600 baud)
Parallel printer interface

12. General

Primus 25

Power supply	230V/115V AC, 50/60 Hz
Dimensions	225 x 280 x 245 mm (width x depth x height)
Weight including block	approx. 6.3 kg

Primus 96, Primus 96 ML, Primus 96 HPL, Primus 384 ML, Primus 384 HPL, Primus InSitu

Power supply	230V/115V AC, 50/60 Hz
Dimensions	315 x 315 x 295 mm (width x depth x height)
Weight including block	approx. 12 kg

Primus Multiblock System HT

Power supply	230V/115V AC, 50/60 Hz
Dimensions	
Rack with 4 blocks	808 x 301 x 288 mm (width x depth x height)
Weight including 4 blocks	approx. 24,3 kg
Control unit	562 x 300 x 298 mm (width x depth x height)
	approx. 36 kg

Notes: