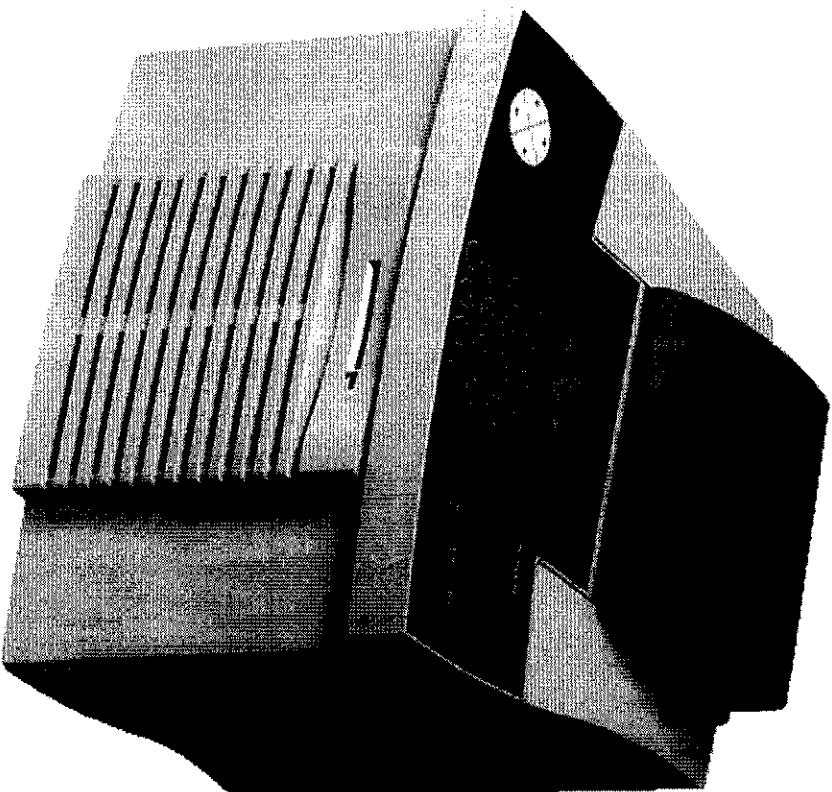


Primus Legal PCR Systems

*Primus 25, Primus 96, Primus 96 HPL,
Primus 384 HPL, Primus InSitu*

THERMOCYCLER

Operating instructions



MMW **MWGAG**
BIOTECH
THE PCR COMPANY

Primus 25, Primus 96 HPL, Primus 384 HPL, Primus InSitu

The Primus range of thermal cyclers from MWG Biotech AG currently includes the following devices:

Primus 25 Legal PCR-System

Primus 96 Legal PCR-System

Primus 96 Legal PCR-System mit High Pressure Lid

Primus 96 Legal PCR-System mit High Pressure Lid und Motordeckel

Primus 384 Legal PCR-System mit High Pressure Lid

Primus 384 Legal PCR-System mit High Pressure Lid und Motordeckel

Primus In Situ Legal PCR-System

Primus Z6 Legal PCR-System

Primus Multiblock Legal PCR-System (Primus HT)

Obtainable with:

- 96-well High Pressure Lid Blöcken (with / without motorlid)
- 384-well High Pressure Lid Blöcken (with / without motorlid)
- blocks freely choosable

All Primus thermal cyclers (exception: Primus In Situ) are fitted with **lid heating systems** with adjustable temperature. Coating of the samples using oil or wax may be omitted.

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1. Commissioning

The following items must be observed for you to operate your Primus Thermo-cycler optimally and safely:

- before commissioning the Primus device, check if the voltage range set on the device corresponds to your power supply.
- the device should be set up in such a manner that at a distance of at least 25 cm to the next wall or the next item is observed. Two Thermocycler should not be standing directly one behind the other. Only this ensures perfect cooling of the thermal cycler.
- the Primus devices have been developed for operation in enclosures (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%.
- the Primus thermo cycler should be protected against the ingress of liquids like for instance spray water, because otherwise the function of the device 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 power supply, on the back of the device.

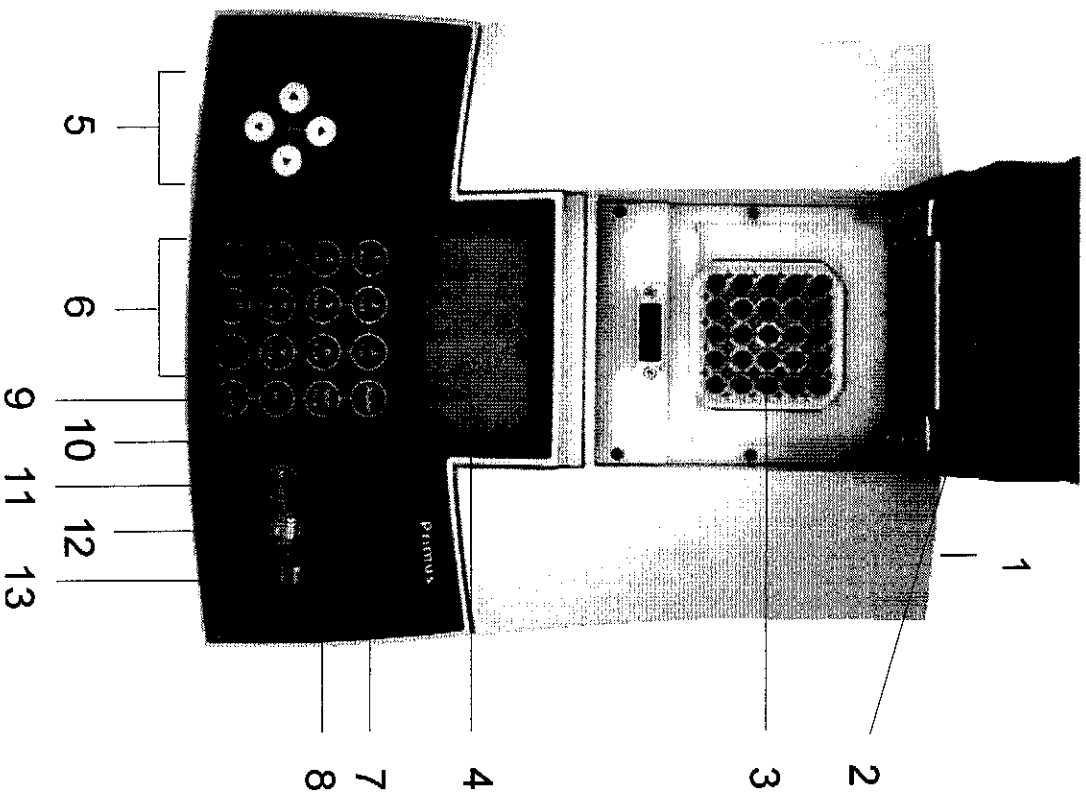
Shortly after startup, the software version is displayed on the display.

For example:

```
Welcome
Primus 96 + V1.19R (c) RW 3399
          model      software version
```

Then, the main menu appears.

2. Diagram of the Primus-Devices



| position | | description |
|----------|-------------------------|--|
| 1 | Mainswitch | |
| 2 | lid | integrated lid heating, for the high pressure lid with adjustable pressure |
| 3 | thermoplate | for reaction vessels, like singular tubes and microtiterplates |
| 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 small and capital letters |
| 9 | del key | deletes parameters or functions |
| 10 | ins 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 the Primus devices 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 you 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 you switch on the equipment, the screen first displays an initialization routine, then the main menu appears from which all functions of Primus thermal cyclers are accessible.

3.1. RUN PROGRAM..

To run a previously stored program select **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. Then press **Enter** to start. Before each run, you can enter a user name via the alphanumeric key pad.

At this point you can also decide to print a GLP report via the parallel printer interface (**GLP ONLINE PRINTS**) 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.

3.2. RUN INCUBATION..

You can use your Primus-device as an incubator 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 will be displayed.

3.3. SET MODE..

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

3.3.1. PROGRAM..

With the submenu **PROGRAM** you can create and change your own PCR programs.

The following parameters are available: **NEW; EDIT; COPY; DELETE**

3.3.2. VIEW..

With the **VIEW** option you can get an overview of the stored programs without risking an accidental alteration of the data. The GLP report of the last program run can be viewed on the display. You can obtain information about setup, system, and block.

3.3.3. PRINT..

With the **PRINT** menu you can print programs, program lists, GLP reports and equipment information via the parallel printer interface.

3.3.4. SETUP..

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

4. Programming

Thanks to the convenient user interface of the Primus-device, you can easily 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. You have the following options:

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 (↑↓) you can access the **HEADER** of the program where you can enter a comment (**CMNT**), the author (**AUTH**), and the user (**USER**), and also a denaturing step in the case of power failure (**PWRFAILDENAT**).

4.2. Steps of the program

The following program steps are available to you for programming the Primus devices and may be selected using the cursor keys (↑↓). But it must be observed that only Primus high pressure lid devices are fitted with a chemical actuator and are able to perform programmed bearing pressure. Primus 25 has a self-adjusting lid heating system (no high pressure lid is available).

| | |
|--------------|--|
| TEMP | <p>a. temperature</p> <p>b. temperature change within a program loop. The sign states if the temperature is raised or lowered.</p> <p>c. period of time for which the preset temperature is to be held. Using the minus key, a time "FOREVER" may be input.</p> <p>d. time change within a program loop. The sign states if the time is to be extended or shortened.</p> |
| RAMPL | <p>the ramp always starts at the temperature defined last and runs with constant speed °C/s to the stated target temperature.</p> |
| PAUSE | <p>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 user is acoustically called when the pause is reached. After pressing the key Run/Enter, the program continues with the next step.</p> |
| LOOP1 | <p>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!</p> |
| LOOP1 | <p>end of a program loop.</p> |
| LIDHT | <p>the lid heating system may be switched on or off. The temperature within the range of 70°C to 120°C is kept constant within the program after the Lidheat step.</p> |
| LIDCL | <p>the program sequence is stopped and the operator is requested to close the lid. Additionally, for Primus HPL devices:</p> <p>Control of the chemical actuator in the high pressure lid. Using this step, the bearing load of the lid is set. The bearing load is variably adjustable in the range from 100 N to 300 N. When entering 0 N, the chemical actuator is not activated and the pressure is only applied by the lid spring.</p> <p>For HPL, the bearing pressure recommended by us is:</p> <ul style="list-style-type: none"> • for micro plate 96 well 100 N to 150 N • for micro plate 384 well 200 N to 300 N <p>No bearing pressure is used for individual tubes! (LIDCL 0 N)</p> |

| | |
|--------------|--|
| LIDOP | <p>the program sequence is stopped and the operator is requested to open the lid. Additionally, for Primus HPL devices: Using the step LIDOP, the chemical actuator is unloaded and the lid pressure is released. When the actuator reaches its unloading level, then the user is called to the device.</p> |
| STORE | <p>using the step STORE, the current program is completed. The step store includes the following steps:</p> <ul style="list-style-type: none"> • 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 device |
| END | <p>the END step cannot be programmed or deleted, but is generally always found at the end of a program. When reaching this step, the device is actively run to ambient temperature and call the operator to the device.</p> |

- During the cooling phases (target temperature < ambient temperature), it is sensible to switch off the lid heating system off (LIDHT off).

Specialities for programming of high pressure lid devices

- when using individual tubes, then no lid pressure may be applied (**LIDCL 0 Nl**).
- the programs may be temporally optimized by setting the step **LIDHT** before the step **LIDCL**. Then, simultaneously the lid heating system is activated and bearing pressure is built up.
- if the step **STORE** is not inserted at the end of the program, then 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 bearing pressure **repipetting** has to be performed, then the following steps must be programmed:
LIDOP: the pressure of the actuator is released. After the pressure is completely released, the user is called to the device and he may open the lid and pipet.
→ the step must be confirmed using **enter**
LIDCL: using this step and the corresponding pressure setting, the actuator pressure is built up again
→ 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**.

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

```
00  HEADER          COMMENT: PCR Program      After Powerfail the
      AUTHOR: Example      USER: Example        device starts with a
      PWRFAILDENYAT: Yes 95.0°C 0:02:00  denaturation-step (95°C,
                                          2min).
```

With the cursor keys (↑↓) or **Enter** you get the first program step.

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

With **Enter** you can fed in a new program step. 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 in the above table by using the (↑↓) cursor keys, e.g., **TEMP**.

```
03  TEMP          94.0°C +0.00°C          denaturation at 94°C
      0:02:00 +0:00          for 2 minutes
```

Go on with **Enter**

```
04  LOOP1        25x           open a program loop - 25 cycles
```

Continue by entering a temperature profile. The next program shows typically temperatures of a PCR reaction.

```
05  TEMP          94.0°C +0.00°C          denaturation at 94°C
      0:00:30 +0:00          for 30 seconds
```

```
06  TEMP          50.0°C -0.5°C          annealing at 50°C with stepwise
      0:00:30 +0:00          temperature decrement by 0,5 °C
                                   (touchdown)
```

```
07  TEMP          72.0°C +0.0°C          elongation with stepwise time
      0:00:30 +0:05          increment
                                   by 5 seconds
```

End the program loop with **LOOP1**.

08 LOOP]

end the loop program

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

09 STORE

-lid heating off
-releasing the pressure
-cooling to 8°C Forever
-user is called by a signal

Alternatively to the **STORE**-Step also the following singular steps can be fed in:

LIDHT off: lid heating off

TEMP: enter the target temperature

LIDOP: releasing the lid pressure; the user is called by a signal

The last programstep **END** can't be deleted neither programmed.

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.

There is furthermore the option of inserting ramps (**RAMP**) into the program. Starting from the temperature defined last, during a certain time interval, heating respectively cooling is performed during a specific time interval.

| | | | |
|------|---------|--------|-------------------------------|
| RAMP | 60.0°C | +0.0°C | targettemperature 60°C with a |
| | 1,0°C/s | +0:00 | speed of 1°C per second |

4.4. Storage of the programs

After completion respectively editing of the program, leave the program editor using **Stop/Esc**. Edited or newly created PCR programs are tested during storing and before running the program in a simulation run. It is only possible to leave the editing mode if the program is sensible. Possible sources for faults are shown to the operator. A calculated program running time (**CALCULATED TIME**) is stated. You store your newly created PCR program using **Enter** (**SAVE PROGRAM**).

5. Operation

To be optimally able to operate the Primus high pressure lid (HPL), the following conditions must be observed.

Operation of the Primus high pressure lid thermo cycler (with corresponding bearing pressure) is only sensible using microtitration plates in the thermo cycler block.

The "Thermosprint" microtitration plate was especially developed for application of the Primus 96 Thermocycler. If low profile microtitration plates (for instance Costar Polycarbonate) are used, then a spacer plate must be inserted in the lid. It is enclosed as accessory.

A selection of microtitrapiates / seals tested and recommended by us are listed under item 6., reaction vessels and seal.

For as long as the bearing pressure of the lid is being increased, the display flashes as follows:

| | |
|---------------|-------|
| Lid Pressure: | xxxxN |
| 300 mA | xxxxs |

The desired pressure was set during programming. The respective pressing time (xxx s) is calculated from this.

This pressing time is only shown after the actuator has surmounted the idle stroke and has made contact.

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

System Message
HPL Actor Fault
(Press)

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

If the lid should be opened inadvertently, then on the lid to the left beside the ventilation grid, there is a bore for unloading the actuator. The key enclosed therein may for instance be pressed using a paper clip and emergency unloading may be performed. During emergency unloading, a red diode is lit to the left beside the unloading bore.

For as long as pressure is being unloaded, the following text flashes on the display:

PLEASE WAIT
Releasing lid pressure
LEAVE LID CLOSED

At the time of pressure unloading, the lid may not be opened and no new program may be started.

6. Reaction vessels and seals

For reaction vessels, it is possible to use 0.2 ml tubes, strips and microtitration plates, for Primus 25 and Primus 96 with **standard lid** (not for the high pressure lid) also 0.5 ml tubes. For optimum results, only use thin-walled tubes with a slim cone.

To achieve the best results with respect to seal as well as performance of PCR, the following microtitration plates / seals should be used.

96-well plates:

| <i>Mikrotiterplate</i> | <i>Seal</i> |
|------------------------|----------------------------|
| Thermosp rint IN-2596 | adhesive film HB-0558 |
| Thermosp rint IN-2596 | cycle-seal blue D-1044-394 |
| Thermosp rint IN-2596 | silicon knob mat IN 7000 |

For all low profile microtitration plates, a spacer plate must be inserted in the lid. It is enclosed as accessory.

384-well Platten:

To safely and reliably close the 384 well microtitration plates, in addition to the cover film a **compression pad** (HB-CPD1) must be used. This compression pad is enclosed as accessory.

Mikrotiterplate

Seal

384-well MTP W-77031 1301

cycle-seal blue D-1044-394 +
compression pad

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

Further possible seals

384-well MTP W-77031 1301

adhesive film
compression pad

HB-0558

+

384-well MTP W-77031 1301

aluminium film
compression pad

IN 7201

+

Further combinations of microtiterplates and seal are being tested.

Individual tubes

For using individual tubes (0.2 ml), the chemical actuator must remain shut off, i.e. in the program, **LIDCL 0 N** must input for the step.

Order numbers:

| | |
|--------------|---|
| IN-2596 | mikrotiterplate ThermoSprint 96-well; transparent |
| W-77031 1301 | mikrotiterplate 384-well, white |
| HB-CPD1 | compressionpad, Si-Foam, natural, 3 mm |
| IN-7000 | silicon knob mat for 96 well MTP, transparent |
| IN-7201 | aluminium film, |
| D-1044-394 | silikone film; cycle-seal, blue |
| HB-0558 | sealing Tape, transparent |
| HB-0404N | strip of 8 Tubes with lid, 0.2 ml |
| HB-0620N | Tube, 0.2 ml, with lid |
| HB-0625 | mikrotiterplate, 25-well, 0.2 ml, transparent |

7. Block change

The Primus-devices has been equipped with a newly developed precision lock for a change of the thermoblock within seconds. To change the block, release it with the lever on the back of the apparatus. The block will be ejected to the back along the guide rails by a transmission wheel.

The block can be operated outside the apparatus, e.g., for work with radioactivity or pathogenic substances. In these cases it must be connected to the apparatus with a connection cable.

8. Specifications

Temperatur

| | |
|---------------------------------|------------------|
| Temperature range of block | 4°C bis +105.0°C |
| Control accuracy of block | +/- 0.1 °C |
| Uniformityt well-to-well | +/- 0.5°C |
| Cooling rate of block Ø | up to 3°C/sec |
| Heating rate of block Ø | 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 mikrotiterplate |
| Primus 96 | 96 x 0,2 ml tubes; 48 x 0,5 ml tubes, 96 well mikrotiterplate |
| Primus 96 HPL | 96 x 0,2 ml tubes; 96 well mikrotiterplate |
| Primus 384 HPL | 384 well mikrotiterplate |
| Primus In Situ | 4 objekt slides |
| Primus HT | Alternitively with Primus 96 HPL or Primus 384 HPL blocks |

Software

| | |
|--|---|
| Memory | 90 Programs with max. 99 Steps / Program optional |
| PCMCIA-Memorycard | TEMP, RAMP, PAUSE, LOOP, LOOPI, LIDHT, LIDOP, LIDCL |
| Programming steps | |
| Time | 0:00:01 to 9:59:59, unlimited ("forever") |
| Time increment / decrement | 0:01 to 9:59 |
| Temperature increment / decrement | 0.1°C to 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

9. General

Primus 25

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

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

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

Primus Multiblocksystem HT

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

Primus 25, Primus 96 HPL, Primus 384 HPL, Primus InSitu

Weight including 4 blocks

ca. 36 kg

We are always there to help!

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