コスモ・バイオ株式会社

Primus 96 advanced®/ Primus 96 advanced® Gradient

Innovative PCR-Technology

Instruction manual



peqlab Biotechnologie GmbH

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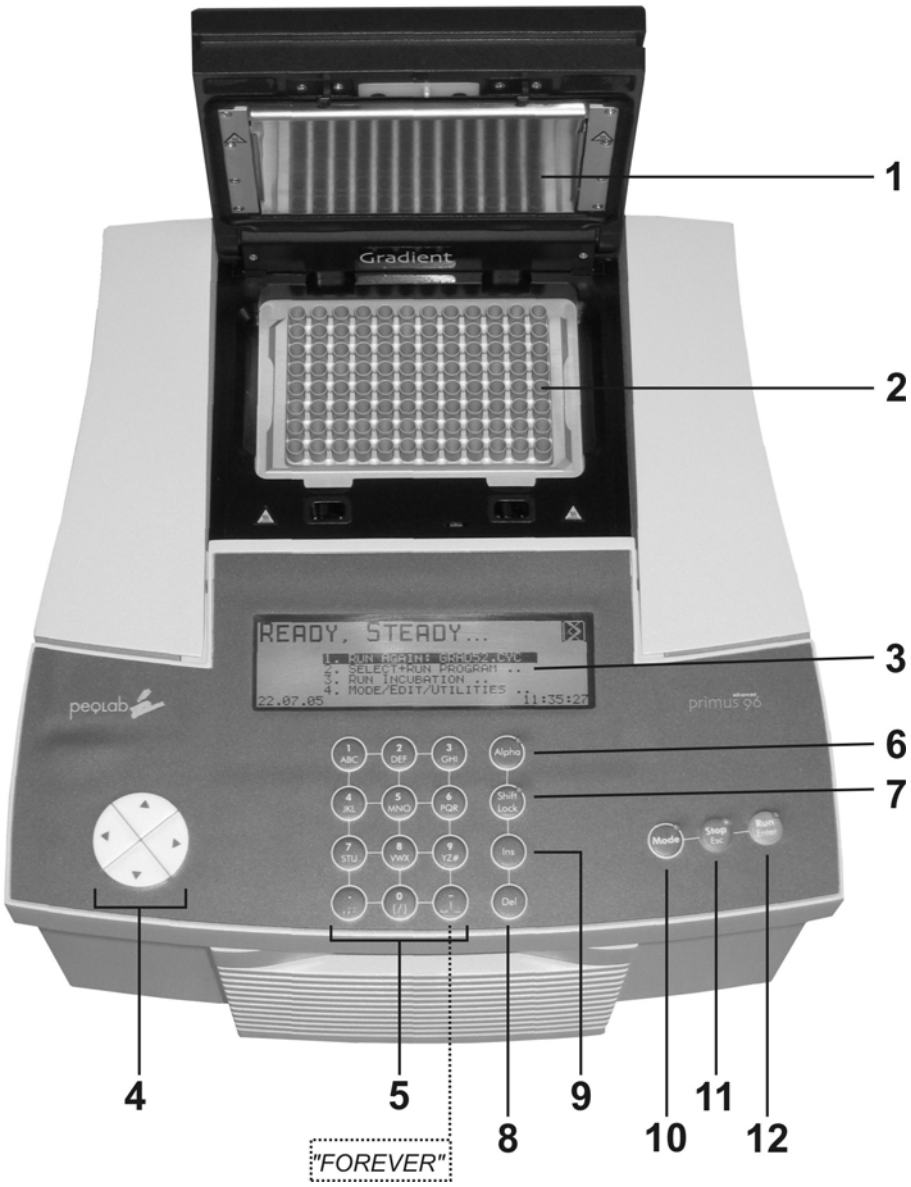
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* only available in Primus 96 advanced ® Gradient

1. Operating elements of the Primus 96 advanced®



1	Heated lid	With automatic height adjustment
2	Universal block	for 96 x 0.2 ml tubes; 96 well microplates; 48 x 0.5 ml tubes with flat caps (in standard block only)
3	Display	Displays menus and programs
4	Cursor key pad	Moves the cursor on the display
5	Alphanumeric key pad	For entering parameters
6	Alpha key	Switches between numbers and letters
7	Shift lock key	Switches between lower and upper case
8	Delete key	Deletes parameters or functions
9	Insert key	Inserts parameters or functions
10	Mode key	Selects edit and display options
11	Stop / Esc key (red)	Exits a menu option to return to a higher menu level
12	Run / Enter key (green)	Selects menus and program options

2. Installation of the Primus 96 advanced®

Before using the Primus 96 advanced® for the first time, check the voltage range and be certain it corresponds to your power supply.

Place your Primus 96 advanced® in an adequate position in the lab. The unit should always be surrounded by at least 25 cm of free space to ensure proper cooling. Place several units beside each other, not behind each other.

Please avoid running the Primus cycler without having tubes or plates placed inside the block.

3. Main menu: Ready, Steady

The software for the Primus 96 advanced® 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 initialisation routine, then the main menu appears from which all other functions of the Primus 96 advanced® are accessible.

3.1. RUN

To start the last run program select **RUN**. The program starts immediately.
Attention: The modifications in the program after the last run will be ignored. In this case, select the program with SELECT + RUN PROGRAM and press enter to start.
Programs can be interrupted at any time during a run with the **Stop/Esc** key. Follow the instructions on the display.

3.2. SELECT + RUN PROGRAM

To run a previously stored program select **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. 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). Switch between YES und 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

The Primus 96 advanced® may be used as incubator with the INCUBATION function. Choose the desired temperature (**INCUB**) and switch the lid heating on or off (**LIDHT**). The current temperature, the status of the lid heating and the total time are displayed. Use the Stop/Esc key to exit the INCUBATION function.

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 (NEW), changed (EDIT), copied (COPY) or deleted (DELETE). Additionally, in Primus 96 advanced® Gradient the temperature distribution over the twelve columns of a distinct program containing a gradient step can be displayed and adapted (GRAD, see 4.3. for details).

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 of the Primus 96 advanced® is entered via **SETUP**.

- **KEYBORD LAYOUT:** For technical service only.
- **SOUND (USER-CALL-MODE):** For setting up acoustic signals if program pauses or the end is reached. One can choose between *repetitive*, *once* or *never*.
- **INTERFACE:** For remote control via a PC the RS232 port is activated. If the unit is equipped with a LAB BUS (RS485), this port can be selcted here.
- **LAB BUS:** If several units should get connected to each other via **LAB BUS**, the adress of the unit will be entered here.
- **CLOCK:** For setting date and time.
- **DISPLAY:** For inverting the colours of the display.

4. Programming

Thanks to the convenient user interface of the Primus 96 advanced®, it is easy to create PCR programs of various degrees of complexity. In the main menu, move the cursor to **MODE/EDIT/UTILS** and press Enter twice, or use the MODE key and choose **PROGRAM**.
The following options are available:

NEW	For creating a new program.
EDIT	For changing an existing program.
COPY	For copying a previously stored program (e.g. to modify a program).
DELETE	For deleting a certain or all programs.
GRAD*	For displaying or editing of the temperature distribution of a gradient step (see 4.3.).

4.1. Program steps

The following program steps are available for programming the Primus 96 advanced®:

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" can be chosen
d) Time change within a program loop. The sign indicates whether the time is increased or decreased.

RAMP The ramp starts at the last temperature defined and goes to the desired temperature at the desired heating/cooling rate (from 0.1 to 2.0 °C/s).

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 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 heated lid may be switched on or off. The stated temperatures are kept constant within the program after the Lidheat step. Temperatures from 70 °C to 99 °C are valid.

STORE Keeps the desired block temperature (default: 8 °C) for the desired time (minus key for "Forever") while the lid heat will automatically be switched off.

LIDOP The electromechanical lid locking is disabled. The user can open the lid.
With this program step the lid of a Primus with a motorized lid opens automatically.

LIDCL The electromechanical lid locking is enabled. Additionally, for Primus HPL instruments, lid pressure may be programmed. Lid pressure is programmable between 100 N and 250 N. With this program step the lid of a Primus with a motorized lid closes automatically.

GRAD* Configuration of a temperature gradient. Enter the temperature in the middle of the block and the range of the gradient.

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 .

* only available in Primus 96 advanced ® Gradient

4.2. Sample program

Select MODE, then PROGRAM and create a new program with NEW. Enter a program name and confirm with Enter to access the programming functions.

01	LIDHT	ON	99 °C	Heated lid on, temperature 99 °C

Press **Enter** to insert a new program step. Move from each parameter to the next with the cursor keys (←→) or with **Enter**.

02	TEMP	94.0°C	+0.0 °C	Denaturation at 94°C
		0:02:00	+0:00	For 2 minutes

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

03	LOOP[	30x		Open a program loop - 30 cycles

Continue by entering a temperature profile.

04	TEMP	94.0°C	+0.0 °C	Denaturation at 94°C
		0:00:20	+0:00	For 20 seconds
05	GRAD*	50.0°C	+/- 5°C	Annealing between temperatures from 45°C to 55°C from left to right
		0:00:30	+0:00	for 30 seconds
06	TEMP	72.0°C	+0.0°C	Elongation at 72 °C for 30 seconds in cycle 1
		0:00:30	+0:05	Using a time increment of 5 s will lead to a stepwise increase of elongation time of 5 seconds per cylce. This makes sense if the polymerase activity is expected to decrease during long PCRs (>30 cycles).

End the cycling step with **LOOP].** A final Elongation step will ensure complete synthesis of all PCR products.

07	LOOP]			Close the program loop
08	TEMP	72 °C	+0.0 °C	Final elongation at 72 °C for 3 minutes
		0:03:00	+ 0:00	

Starting from the actual temperature (72 °C in this case) the block will be cooled slowly to 8 °C using RAMP. last Ramps may be inserted into any program. The ramp starts from the last programmed temperature (here 72 °C) and decreases temperature to the set temperature (4 °C) at a constant rate.

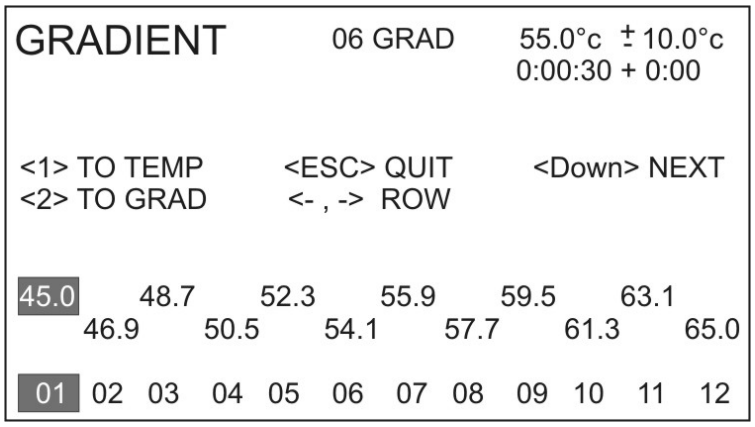
09	RAMP	8.0 °C	Target temperature 8 °C with a cooling rate of 1 °C/s
		1.0 °C/s	At this stage the usage of RAMP is not necessary but is used as an example. Decreasing the cooling rate makes sense for example when annealing complementary oligonucleotides quantitatively or for improved annealing of difficult primers to difficult templates.
10	STORE	8.0 °C	Final temperature of 8 °C for infinite time while lid heat is automatically
		Forever	switched off. Use minus key for "Forever"!
			During long incubations at low temperatures it is of advantage to use STORE since switching off the lid heat automatically will avoid block and lid working opposed to each other. This will increase the durability of the Peltier elements and will reduce power consumption.
11	End		End of program

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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.
Exit the program editor with **Stop/Esc**. Edited or newly created PCR programs are tested in a simulation run before being saved and before running.
You can only exit the editing mode if the program makes sense. If not, the editor will indicate possible error sources. A calculated program run time can be displayed (**CALCTIM**), and you can save your newly created PCR program with **Enter**.

4.3. Displaying and editing of the temperature distribution of a gradient step*

For displaying the temperature distribution in the single columns of the block after programming a PCR program containing an gradient step choose **MODE/EDIT/UTILITIES -> PROGRAM -> GRADIENT**. A list of programs containing at least one gradient step will appear. Select the respective program and press ENTER. In principle the following dialogue will appear:



The first two lines are containing the information that step No 6 of this program is a gradient step (06 GRAD) having a central temperature of 55 °C, a temperature spread of +/- 10 °C and a duration of 30 s. In the next two lines the available operating functions are explained. If the program is containing several gradient steps one can jump between respective steps using the cursor key <Down>.

4.3.1. Creating a new PCR program with a homogeneous block temperature

If the analysis of the PCR products obtained by running a PCR program containing the above displayed gradient step gives best results in column No 10 for example, select the respective column/temperature by using the cursor-keys (<- , -> ROW). By pushing the button „1" (<1> TO TEMP) all columns will adapt to the choosen temperature (in this case 61.3 °C). For saving the adapted version under a new name push the „Run" key.

4.3.2. Creating a new gradient program for further analysis

If the analysis of the PCR products obtained by running a PCR program containing the above displayed gradient step rises the assumption that optimal annealing temperature will be found in a lower or higher temperature range, select the lowest (in this case 45 °C/column No 1) or highest temperature, respectively, by using the cursor-keys (<- , -> ROW). By pushing the button „2" (<2> TO GRAD) the gradient step will get adapted, having now the previously selected temperature in it's center (in this case 45 °C +/- 10 °C). For saving the adapted version under a new name push the „Run" key.

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5. Reaction vessels

As reaction vessels you can use 0.2 ml tubes and '96 well'-PCR-plates. The 0.5 ml tubes can be used only with standard lid. For optimum results, only use thin-walled tubes with a slim cone.

Article	Abstract	Order number
ThermoTube™ PCR Tubes 0.2 ml (domed cap)	natural, 1 bag à 1000	82-0337-A
	natural, 5 bags à 1000	82-0337-B
	assorted colours, 1 bag à 1000	82-0491-A
	assorted colours, 5 bags à 1000	82-0491-B
ThermoTube™ PCR Tubes 0.2 ml (flat cap)	natural, 1 bag à 1000	82-0620-A
	natural, 5 bags à 1000	82-0620-B
	assorted colours, 1 bag à 1000	82-0622-A
	assorted colours, 5 bags à 1000	82-0622-B
ThermoTube™ PCR Tubes 0.5 ml (flat cap)	natural, 1 bag à 1000	82-0350-A
	natural, 5 bags à 1000	82-0350-B
	assorted colours, 1 bag à 1000	82-0533-A
	assorted colours, 5 bags à 1000	82-0533-B
Thermo-Fast® 96 PCR Plates (Standard)	natural, 1 pack à 25 plates	82-0600-A
	natural, 5 x 1 pack à 25 plates	82-0600-B

6. Block exchange

The Primus 96 advanced® has been equipped with a newly developed precision lock for a change of the thermal block within seconds. 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.

7. Technical specifications

Temperature-range of block	4.0 °C to +105.0 °C
Control accuracy of block	+/- 0.1 °C
Block uniformity (at 72 °C)	+/- 0.35 °C
Cooling rate of block	2 °C/s
Heating rate of block	4 °C/s
Temperature-range of heated lid	70 °C to 99 °C
Minimal setting steps heated lid	1 °C
Maximum gradient*	+/-19.9 °C (= 39.8 °C)
Temperature range of gradient*	35 °C to 105 °C
Control accuracy of gradient *	+/- 0.1 °C
Capacity of 96 well block	96 x 0.2 ml tubes 96 well-plates 48 x 0.5 ml tubes with flat caps only
Additional available blocks	Block for 384 well plates *In situ* block for 4 microscope slides with integrated buffer sponge
Heated lid	Automatic height adjustment for various tube heights (Standard Lid) Electromechanical lid locking (optional) disables opening of lid, when a program is started Optionally with High-Pressure Lid (HPL) or Motorized Lid (ML)
Internal Memory	90 programs with a maximum of 99 steps/program
Programming steps	Temp, Ramp, Pause, Loop[, Loop], LidHt, LidOp, LidCl, Store
Time (hours:min:sec)	0:00:01 to 9:59:59, unlimited ("forever")
Time increment/decrement (min: sec)	0:01 to 9:59
Temperature increment/decrement	0.1 °C to 9.9 °C
Additional functions	GLP report, instant Incubation, Comment, author, user of each program, Power failure recognition and auto-restart, Internal real time clock;
Ports	RS232 port for remote control via PC (9600 Baud) Parallel printer port
Power consumption	500 VA
Power supply	230V / 115V AC, 50 / 60 Hz
Dimensions (W x H x D)	315 x 320 x 315 mm
Weight including block	approx. 12 kg

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We are always there to help!

For technical and use-related questions or for service, please contact PEQLAB:

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