



THERMO HYBAID

Omn-E Thermal Cycler

USER INSTRUCTION MANUAL

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OMN-E THERMAL CYCLER

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OMN-E THERMAL CYCLER

PCR Registration

This instrument, Serial No. is an Authorised Thermal Cycler. Its purchase price includes the up-front fee component of a Licence 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 Licence may be purchased from Perkin-Elmer or obtained by purchasing Authorised Reagents. This instrument is also an Authorised Thermal Cycler for use with applications Licences available from Perkin-Elmer. Its use with Authorised Reagents also provides a limited PCR Licence in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete Licence or right to perform the PCR process. Further information on purchasing Licences 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, USA. Tel No: + 415 638 5551. Fax No: + 415 638 6071.

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

OMN-E THERMAL CYCLER

Warranty

Thermo Hybaid guarantees that the Omn-E Thermal Cycling system you have received has been thoroughly tested and meets its published specification.

This guarantee is valid for 24 months only if the product and functions have been used according to the instruction manual. The tube thermistor assembly is guaranteed for 90 days only. No liability is accepted for loss or damage arising from the incorrect use of the Omn-E Thermal Cycling system. Thermo Hybaid's liability is limited to the repair or replacement of the unit or refund of the purchase price, at Thermo Hybaid's option. Thermo Hybaid is not liable for any consequential damages.

Thermo Hybaid reserves the right to alter the specification of the Omn-E without prior notice. This will enable us to implement developments as soon as they arise.

The Thermo Hybaid Omn-E is for research use only.

Read the Instruction Manual carefully before using the Omn-E to ensure that you obtain the best possible results from the machine.

CHAPTER 1

OMN-E THERMAL CYCLER

Introduction

Thermo Hybaid's Omn-E is a compact thermal temperature cycler. It is available with one of two possible block formats.

The 0.5ml version (HB-TRE-05) can either hold up to 48 x 0.5ml tubes, 96 x 0.3ml tubes or 1 x 96 well plate. The 0.2ml Block version (HB-TRE-02) can hold either up to 96 x 0.2ml tubes or 1 x "V" well 96 well plate, (e.g. Thermo Hybaid's OmniPlate 96).

The Heated Lid, if fitted, allows the Omn-E to be used for temperature cycling protocols without the need for mineral oil overlays. Such vapour barriers are normally required to prevent evaporation of reaction constituents during thermal cycling.

The Omn-E has a large display screen, which, in combination with its advanced software, allows protocols to be programmed and run with ease. The unit can store up to 36 programs, each program is identified either by a name and/or a number.

The Omn-E can be used for static temperature sample incubations or multi-step temperature cycling protocols, within a sample temperature range of ambient +10°C-99°C. The Omn-E can be run at an ambient operating temperature of +4°C-35°C.

Safety Information

The Omn-E has been designed for reliability and ease of maintenance. The Omn-E should only be used by suitably qualified and trained personnel. The unit should only be used for its intended purpose in accordance with the instructions and safety warnings contained in this manual.

Refer to the installation instructions contained in this manual before connecting to the mains supply. Before use, ensure that the unit has been set for the appropriate mains supply voltage.

WARNING: THIS APPLIANCE MUST BE EARTHED

The Omn-E is a class 1 appliance. To minimise the risk of electric shock, the unit must be connected to a protective earth via the supplied mains cord.

Replacement fuses must be of the correct rated current, voltage and type.

Do not operate the unit in an explosive environment.

Do not operate the unit if it appears to have been damaged or if a liquid or foreign object has entered the enclosure. Disconnect from the mains supply and consult your Thermo Hybaid representative.

Do not attempt to dismantle the unit. To avoid the risk of personal injury, and to ensure that the safety features of this unit are maintained, servicing should only be carried out by Authorised personnel.

Thermo Hybaid continues to offer full service and technical support for all its products.

International Sales, Service & Technical Support	UK +44 (0) 1784 425000
UK Sales & Technical Support	UK 0500 95 55 72
UK Service Support	UK 0500 49 22 43

Alternatively, please contact your local authorised distributor.

CHAPTER 2

OMN-E THERMAL CYCLER

Unpacking & Installation

Please retain this packaging for future use; it is important that the Omn-E is always transported in this packaging. Thermo Hybaid cannot accept responsibility for any damage incurred unless the instrument has been correctly packaged.

2.1 PACKING LIST

1. Omn-E Thermal Cycler (Heated Lid optional)
2. Power cable
3. User instruction manual
4. One control thermistor probe
5. Extension lead for control thermistor probe
6. One tube mineral oil
7. One Consumables Selection Pack (0.2ml or 0.5ml)
8. 0.5ml/0.2ml Heat shield

If any item is missing or damaged, contact Thermo Hybaid, or Thermo Hybaid's authorised distributor.

NB: If the Omn-E Thermal Cycler is not used as specified in this manual, the protection provided by the equipment may be impaired.

2.2 DESCRIPTION OF THE OMN-E

Remove the Omn-E from the packaging and place on the bench; do not connect to the mains supply immediately.

Open the lid to expose the 0.2ml or 0.5ml sample block. The control thermistor probe should be inserted into the jack socket to the left of the block towards the rear of the instrument. (The extension lead for the thermistor should not be used) Insert the thermistor probe into the block in position 'A2'. Ensure that the tube contains the same volume of mineral oil as the intended reaction volume, and that the probe is immersed in the oil.

The Omn-E is cooled by a fan and therefore should be situated with at least 15cm clearance at the rear and 8cm on either side of the unit. Do not place the Omn-E in direct sunlight or strong air currents, which may affect the performance of the unit.

Connect the Omn-E to a suitable mains power supply via the cable provided. The voltage and power rating labels of the instrument are found on the rear of the unit above the power socket. Avoid connecting the unit to a circuit that may be subject to mains fluctuations such as that shared by Ultracentrifuges, freezers or refrigerators.

The Omn-E may be used in a cold room (down to 4°C). However, when removed it should be allowed to equilibrate for at least two hours before being switched on.



If this is not done, there is a risk that condensation may form within the unit and cause a short circuit.

Switch on the Omn-E at the power switch at the rear of the unit. When first switched on the unit will complete a start up and self test routine. The self test routine checks the block thermistor, heating elements, the cooling fan and the Heated Lid (if fitted). This takes approximately 2 minutes.

This feature can be by-passed by pressing 'CONTINUE' whilst the Thermo Hybaid Omn-E Thermal Reaction screen is displayed.



Since the block will become hot during the self-test procedure, Thermo Hybaid recommends that the lid is kept closed to protect the user.

If the start up procedure has been successful the Main Menu will be displayed. If a fault has been detected one of a series of error screens may be displayed.

The Omn-E is very easy to operate and program. Whilst the software is designed to be intuitive, the following table gives a summary of the primary functions of each of the keys.

ENTER KEY	- Moving between program positions. - Selecting program choices.
MENU KEY	- Always moves to the Main Menu - can be utilised as an "escape" key.
NUMBER KEYS	- Used for entering parameters and selecting programs.

ARROW KEYS	- Moving cursor between program positions and in alphabetic naming of programs. - Used for rapid scrolling through programs and for moving between run screens.
STOP KEY	- Cancels instructions/operations. - Used to delete programs.
CONTINUE KEY	- Used to progress from “hold”, “pause” or “self-test” instructions. - Also used to access detailed editing functions.
PAUSE KEY	- Pauses a cycling program at current step.
BLOCK KEY	- Provides access to current/historical information on block status screens.

Each of these functions is discussed fully in the following chapters.

CHAPTER 3

OMN-E THERMAL CYCLER

Programming & Operation

3.1 INTRODUCTION

The programming interface on the Omn-E has been designed to be easy to use. The memory will hold up to 36 complete thermal cycling programs. These are identified on the screen by a number (01-36) and a user defined name (up to 7 characters).

To run a program you may select an existing program, edit an existing program or create a new program. Additionally the Omn-E can be run in 'Manual' mode at a single temperature for static incubations.

The Omn-E interface also includes a number of advanced program features detailed in Chapter 4.

3.2 STEPS & STAGES OF A PROGRAM

The programs in the Omn-E are divided into 'stages' and 'steps' when displayed on the screen. A stage typically includes three steps where each step refers to the temperatures and times associated with a typical thermal cycling protocol i.e. 95°C, 55°C and 72°C for 1 min each. In addition each stage can be repeated for up to 99 cycles, and a 'hold' added to the end of the stage.

The Omn-E allows you to program up to 10 separate stages each with up to 10 separate steps. This means that any thermal cycling protocol may be saved in a single program space. The Omn-E can store 36 such programs, which may be labelled with a number and a name.

3.3 SELF TEST CHECK

A screen will display "Omn-E going through a self check procedure". For further details refer to Section 2.2 of this manual.

3.4 MAIN MENU

After successful start up the first menu to be displayed is the 'Main' Menu. It is possible to return to this menu at any time by pressing 'MENU'. The Main Menu displays the current date and time and the status of the block (active, inactive, interrupted or active resumed)

All aspects of the programming are accessed through the Main Menu.

RUN	Select 'RUN' to run an existing program.
MAN	Select 'MAN' to run a manual static incubation.
PROG	Select 'PROG' to edit, view or create a new program.
CLOCK	Select 'CLOCK' to alter the way the time and date are displayed and to set the date and time.

3.5 MANUAL OPERATION

This option is used for static incubations when thermal cycling is not required, e.g. hot start protocols, probe denaturation, enzyme reactions.

1. Select 'MAN' from the Main Menu and press 'ENTER'.
2. Enter the name of the user or protocol if desired using the  and  arrows to scroll through the characters. Move to the next/previous character using the  and  arrow keys. Press 'ENTER' only when the name is complete. If no characters are entered, the default "NO NAME" will be entered.
3. Enter the temperature using the numeric keys. Press 'ENTER' to continue.
4. Select the method of control. Scroll through the options using the  and  keys. The following methods of control are available: -
BLOCK
TUBE
SIM TUBE
(See Chapter 5 for details)

For machines fitted with Heated Lids, the Lid can be selected on or off using the ➔ key.

5. Press 'ENTER' to start the incubation. The screen displays the temperature of the block and/or the tube thermistor together with the elapsed time.

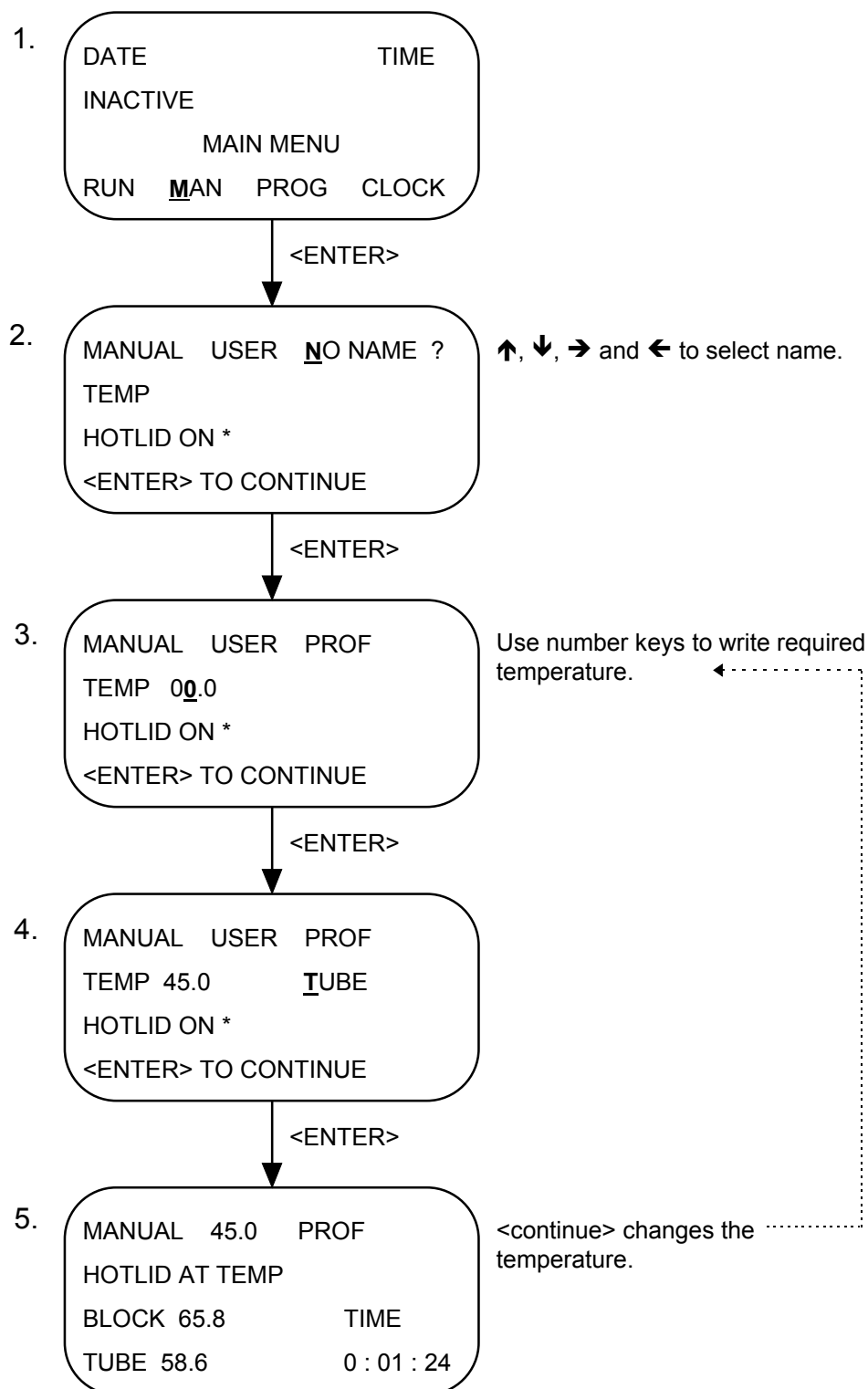
NB: If the machine is started from 'cold', approximately seven minutes will be required before the Heated Lid will be at operating temperature (based on 20°C ambient and 230V supply. This may vary under different power conditions.) Condensation may be experienced if samples are loaded before the Lid is at operating temperature.

6. Press 'STOP' twice to cancel the manual program.
7. Press 'CONTINUE' to change the set temperature or control method (and Lid setting if fitted).

(Please see *Figure 1* on following page.)

Figure 1:

Using the Omn-E for static temperature incubations (manual control)



* HOTLID display only appears if machine is fitted with a Heated Lid.

3.6 CREATING A NEW PROGRAM

1. To create a new program select 'PROG' from the Main Menu, and select 'NEW' from the sub-menu.
2. The Omn-E will automatically display the number of the next free program space. You are now able to enter the name of the user or protocol, if desired, using the **↑** and **↓** arrows to scroll through the characters. Move to the next/previous character using the **←** and **→** arrow keys. Press 'ENTER' only when the name is complete. If no characters are entered, the default 'NO NAME' will be entered.

Note if there are no free program spaces the Omn-E will prompt you to overwrite an existing program.

3. Enter the temperature and time of the first step of your protocol. The arrow keys can be used to scroll backward or forward to correct a mistake. **Note** that the program will not allow you to advance to step 2 until a valid temperature and time have been entered.
4. Press 'ENTER' to move to step 2 of the protocol.
5. Continue to enter the remaining times and temperatures of this stage. Up to ten steps may be entered, although three steps are more typical.
6. Enter the number of cycles required.
7. Enter the hold temperature if required (see Chapter 5), or leave blank. Press 'ENTER'.
8. Repeat for each stage required. Up to ten stages may be entered. One is usual although three are needed if initial denaturation and final extension steps are required. For complex programs such as Touchdown PCR*, temperature decrements may be programmed in using the full 10 stages. Press 'ENTER' when complete.
9. Before saving this program you may alter the program number but not the name. Press 'ENTER' to save the program.

* See Appendix II

3.7 VIEWING AN EXISTING PROGRAM

It is possible to view existing programs without altering the information contained within them. It is not possible to edit the program in this mode and so the program remains unchanged.

1. Select 'PROG' from the Main Menu and 'VIEW' from the sub menu.
2. Use the 'ENTER' key and the  and  arrows to move through the program.

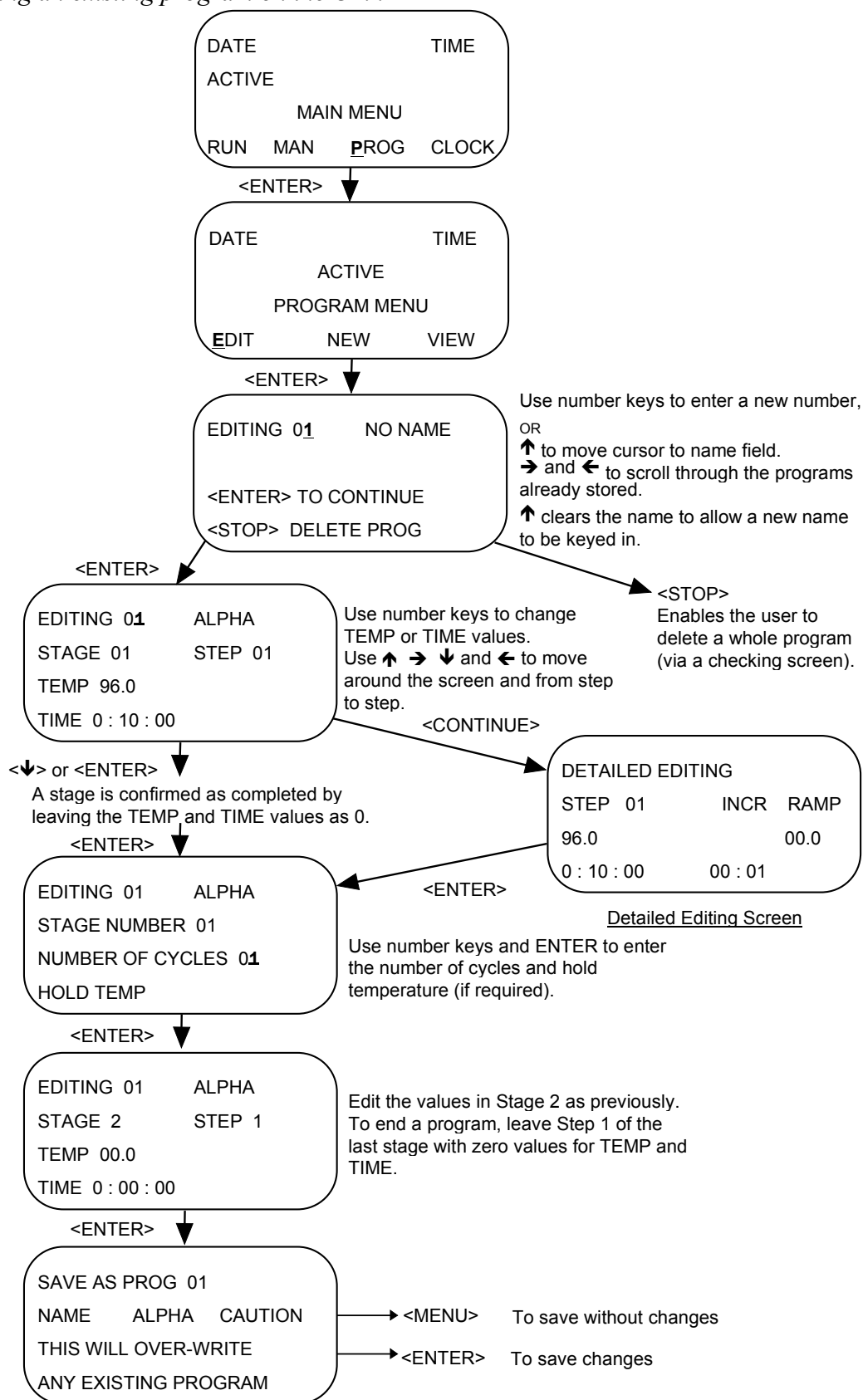
3.8 EDITING AN EXISTING PROGRAM

(See *Figure 2.*)

1. To edit an existing program select 'PROG' from the Main Menu and 'EDIT' from the sub menu.
2. Select the number of the program to be edited.
3. The entire program entry may be deleted by pressing 'STOP'. A caution message is displayed. The program name may be edited by pressing  which activates the alphanumeric function.
4. Press 'ENTER' to edit the program.
5. Use the  and  arrow keys to advance through each stage. Proceed through each time and temperature and alter as desired using the number keys.
6. Save program with edits by pressing 'ENTER' at "save as" screen. Also save program without edits by pressing 'MENU' at "save as" screen.

Figure 2:

Editing an existing program on the Omn-E



3.9 ***RUNNING A PROGRAM***

You can run any stored program by selecting 'RUN' from the Main Menu. (Also see *Figure 3*.)

1. The Omn-E will display '00 INVALID'. Scroll through the various program numbers and names by using the ◀ and ▶ arrow keys. Press 'ENTER' to select the desired program number.
2. For machines fitted with a heated lid, select Hotlid 'ON' or 'OFF'. Then press 'ENTER'.
3. For machines fitted with a Heated Lid, select load alarm 'ON' or 'OFF'. Then press 'ENTER'.
4. Turn the alarm 'ON' or 'OFF' by using the ◀ and ▶ arrow keys, then press 'ENTER'.
5. Select the method of temperature control.
Use the ◀ and ▶ arrow keys to scroll through the possible methods of control available. These include:

BLOCK
TUBE
SIM TUBE

(See Chapter 5 for details)

NB: If the "SIM TUBE" control method is chosen, the unit will require a calibration factor. Please refer to Chapter 5 for a detailed explanation.

6. Press 'ENTER' to begin the program.
Once running the normal selection of three run screens are able to be displayed (see *Figure 4*). It is possible to scroll through these by using the ↑ and ↓ arrows.
7. Where a Heated Lid is fitted, the Lid will require 5-7 minutes to heat up from cold once a program run is started. The load samples screen will then be displayed.

NB: It is advisable to start a program before assembling reaction mixes in order to avoid completed reaction mixtures being kept at inappropriate temperatures during the heat up time.

Figure 3a:

Running a program on the Omn-E with the Heated Lid

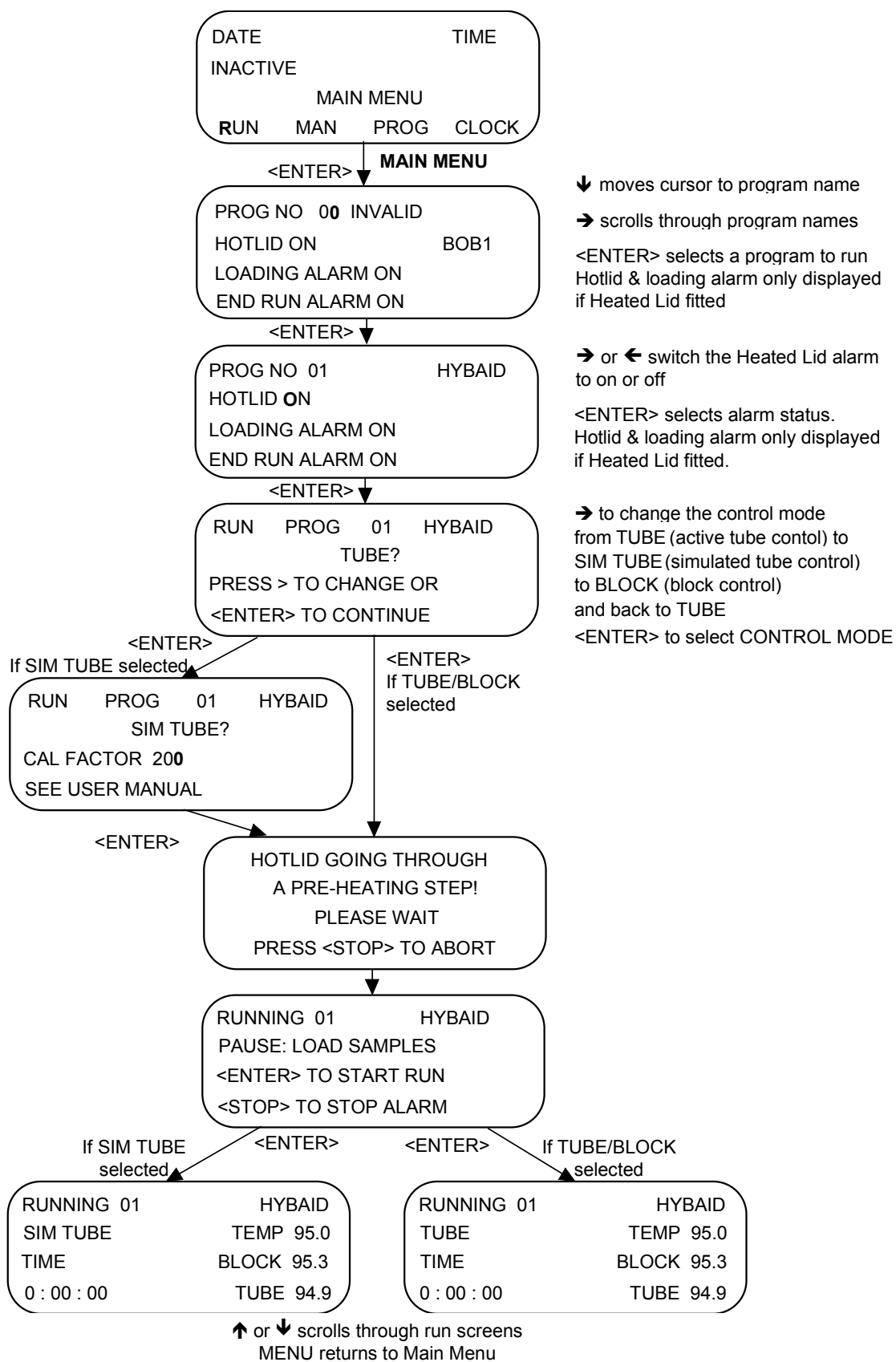
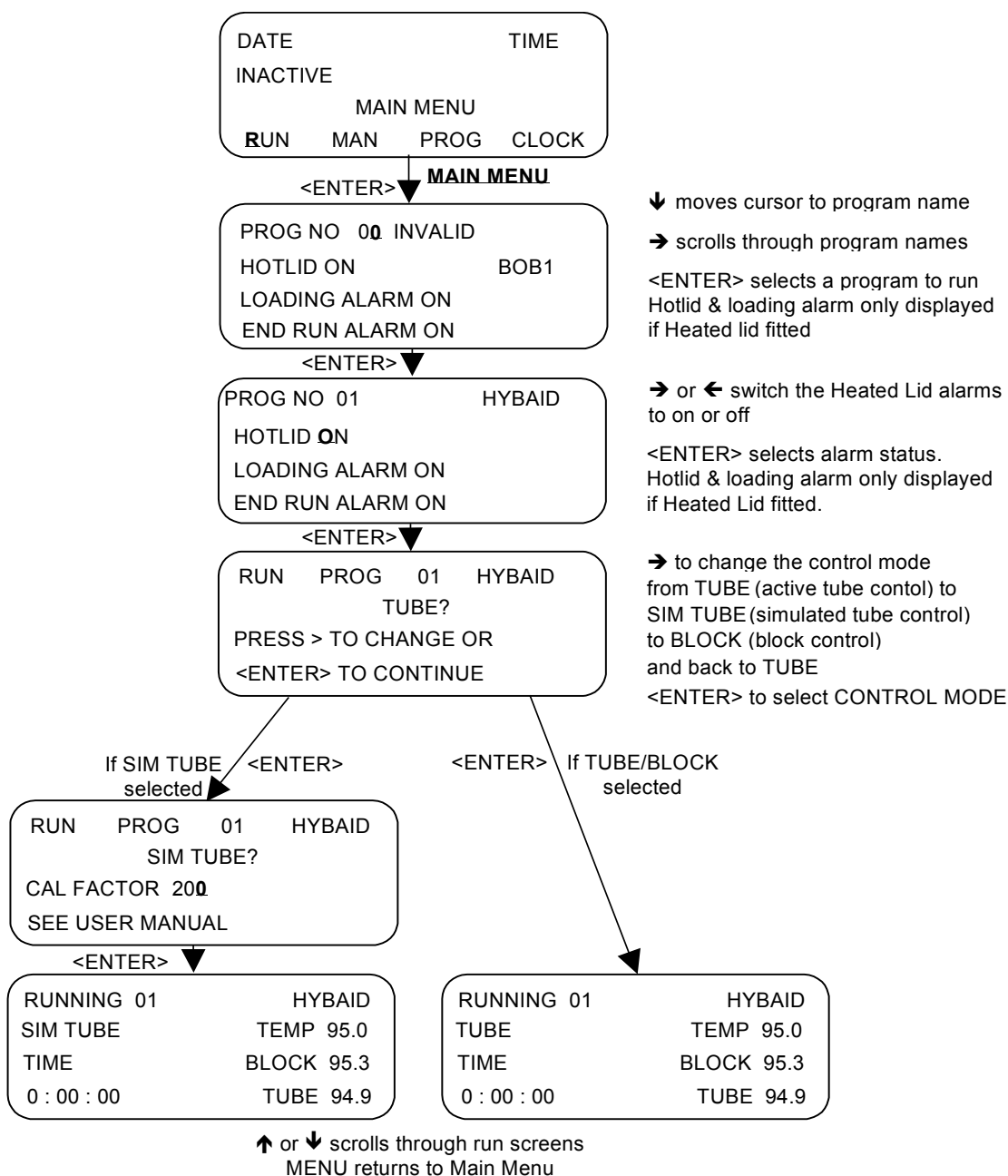


Figure 3b:

Running a program on the Omn-E without the Heated Lid



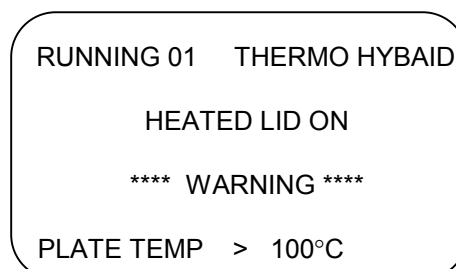
3.10 RUN SCREENS

When a program is running three separate run screens can be displayed. These contain information about the progress of the run together with the temperatures achieved during the run. This allows the user to monitor the performance of the Omn-E and to determine the time of the end of the program.

On machines fitted with a Heated Lid, screen 1 will alternate with a Heated Lid warning screen on some software versions. This serves the dual purpose of a safety warning and confirming the correct function of the Heated Lid during the run.

Figure 4:

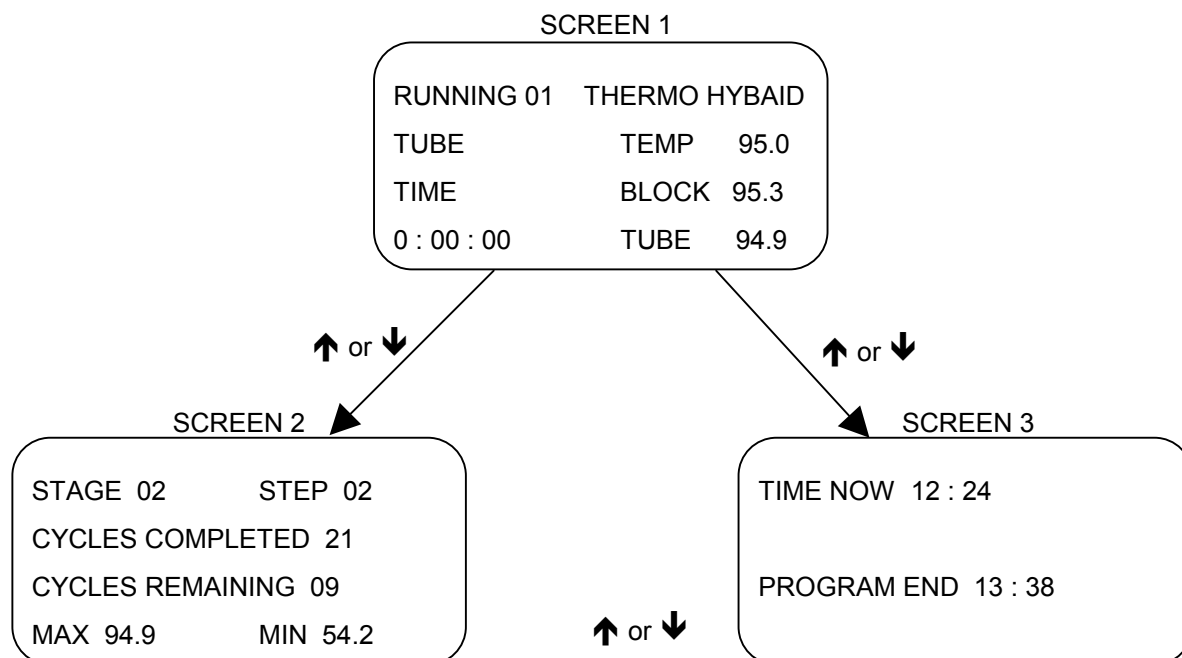
Hotlid warning screen



After a program has been activated the three run screens can be displayed by pressing the $\uparrow$ and $\downarrow$ arrows, scrolling through each screen in turn. A fourth screen becomes available if the run has been interrupted by a power failure (see Chapter 4). This screen can be accessed from the Main Menu by pressing the 'BLOCK' key.

Figure 5:

Main run screens displayed by the Omn-E



Screen 1

Screen 1 is the main run screen, which displays the following information: -

- Program number and name.
- Control method i.e. 'Tube', 'Sim Tube' or 'Block'.
- Current block and tube temperatures.
- Set (programmed) temperature.
- Time.

Screen 2

Screen 2 displays the following information: -

- Time now.
- The calculated run end time (an estimated value which is updated throughout the run).

Screen 3

Screen 3 displays the following information: -

- The current stage and step of the running program.
- The total number of cycles completed and remaining.
- The maximum and minimum temperatures achieved during the run.

Power failure screen

This additional screen appears if the run has been interrupted by a power failure or power fluctuation severe enough to affect the unit. (See advanced features for further information.) It contains the following information: -

- The duration of the power failure.
- The stage in the run when the power failure occurred.

End of run screen

The Omn-E displays a further screen at the end of the run, this provides the following information: -

- The program name and number.
- The total run time.
- The maximum and minimum temperatures recorded during the run.

Block idle screen

If 'BLOCK' is pressed and there is no run in progress, the block idle screen is displayed.

Error screens

A number of error screens may be displayed if a fault has been detected in the unit. Always contact the Thermo Hybaid Service Department or your local supplier for advice before attempting to use the instrument further.

3.11 HOLD & PAUSE FUNCTIONS

When entering a program the Omn-E will give you the opportunity to enter a 'Hold' temperature at the end of each stage. The samples will be held at this set temperature indefinitely. The screen will read 'HOLD' during a run. If a "Hold" is inserted between stages the program can be advanced to the next stage by pressing 'CONTINUE'. If a "Hold" is inserted at the last stage, pressing 'CONTINUE' will switch to the run summary screen (via an automatic cooling step in the case of instruments fitted with a Heated Lid).

Common uses of the Hold step include the following: -

- Inserting an initial 95°C incubation at the start of a protocol to perform the 'Hot Start' procedure. After the enzyme has been added, pressing 'CONTINUE' will advance the program into the cycling part of the protocol.
- A final 72°C incubation to ensure completion of the final extension step of a reaction.

NB: Extended "Hold" stages should not be used at the end of reactions using the Heated Lid, as the Heated Lid will remain switched on with potential for sample evaporation.

Pressing 'PAUSE' during a cycling program will stop the program at the target temperature for the step. Pressing 'CONTINUE' will continue the countdown for the step.

CHAPTER 4

OMN-E THERMAL CYCLER

Advanced Features

The Omn-E includes a number of advanced features beyond the basic programming discussed previously.

4.1 *CLOCK*

The time and date format of the clock display can be modified. To set the clock or alter the format select 'CLOCK' from the Main Menu. Use the ➡ keys to alter the format and press 'ENTER'. Use the numeric and ➡, ⬅ keys to enter new time/date. Scroll to 'SET' and press 'ENTER' to save.

4.2 *ALARM*

The Omn-E is equipped with an audible alarm, which sounds at the end of the run. On machines fitted with a Heated Lid, the alarm will also sound at the end of the Hotlid pre-heat cycle. It will sound intermittently for the first minute and then briefly until switched off. To silence the alarm press 'STOP'

The alarm may be disabled before starting a run. To change the alarm setting, select 'RUN' from the Main Menu, and use the arrow and 'ENTER' keys to enable or disable as required.

4.3 *AUTO POWER FAILURE RESTART*

If there is a power interruption during a run the Omn-E will re-start automatically. The Main Menu screen will display 'RESUMED' to indicate the run was interrupted. An additional run screen will display the duration of the power failure and the exact stage in the run that it occurred. This information can be accessed by pressing 'BLOCK' and using the ⬆ and ⬇ arrow keys. This information will allow the user to determine if the samples have been amplified reliably.

NB: If there are several breaks in the power supply, only the most recent interrupt is recorded.

4.4 TOTAL RUN TIME/MAXIMUM & MINIMUM TEMPERATURE

At the end of the run the Omn-E will display the maximum and minimum temperatures recorded during the run. The specifications of the Omn-E dictate that the maximum temperature should not exceed 1°C above the set temperature and the minimum not less than 1°C below the set temperature.

4.5 RUN END TIME CALCULATION

During a run the Omn-E will display the calculated time of the completion of the run. This screen is displayed by scrolling through the run screens using the **↑** and **↓** arrows. The run screens can be accessed from the Main Menu by pressing the 'BLOCK' key.

4.6 TEMPERATURE RAMPING

The ramp rate (in °C/sec) controls the rate of change of sample/block temperature. At the "annealing" step it is useful in some applications to limit the rate of increase in temperature to allow extension of short or degenerate primers. To adjust the ramp rate simply go into the Edit or New program mode. Enter both the temperature and dwell time values that you wish to alter the ramp rate on. You must enter both of these values in order to access the detailed editing screen. Press 'CONTINUE' to forward to the detailed editing screen. The cursor is moved around this screen using the four arrow keys or the 'ENTER' key. Move the cursor until it highlights the ramp rate value. The rate ramp is stated in °C/sec. The number range that can be entered is 0.01 to 1.00. The default value is 1.00. If no figure is entered, the machine ramps as quickly as possible. Press **↓** and 'ENTER' to refine the main editing program.

4.7 TIME INCREMENTS

Time increment may be used to add a time interval to each repeat cycle of a specific step. E.g. an increment of 10 seconds on a 60 second step will give time intervals of 60, 70, 80, 90, 100 seconds. Useful to increase an incubation step to allow for depletion of an enzyme, for example. In the Edit mode, the time increment can be accessed via the detailed editing screen. From the temperature step to be incremented, press 'CONTINUE' to access the detailed editing screen and **↓** to move the cursor to the increment position. The maximum increment, which can be added, is 9 minutes 59 seconds. Press 'ENTER' to rejoin the main editing program.

CHAPTER 5

OMN-E THERMAL CYCLER

Modes of Control used in the Omn-E

5.1 *ACTIVE TUBE CONTROL*

Active tube control makes use of the Tube Thermistor provided with the unit. This is designed to locate into position A2 in the block of the Omn-E. The thermistor acts as a sample mimic, monitoring the sample temperature as it changes during cycling, feeding back this information to the Omn-E's processor. This feedback allows the unit to respond to the sample temperature and hence ensure that the samples achieve the exact temperatures and times programmed. To bring the sample to temperature rapidly, the block is heated/cooled to beyond the set temperature for the sample (*Figure 5a*). This can be observed on the display of the Omn-E during cycling.

NB: The tube thermistor should be filled with the mineral oil provided to a volume equivalent to the sample plus oil overlay. It is important to change the thermistor oil when performing new protocols with different volumes. It is advised that the volume is checked regularly, to maintain performance of your unit.

NB: The thermistor tube itself should be changed every 5-10 cycles, ideally using the same tube as is used for the samples.



The tube thermistor is delicate and must only be used with standard research grade mineral oil (e.g. Sigma). Contact with aqueous solution will damage the thermistor and may affect the subsequent performance of the instrument.

Extension lead for the Tube Thermistor.

The Omn-E is provided with an extension lead for the thermistor. This should only be used for transferring protocols from instruments that cannot use Active Tube Control. See Chapter 6 for further details.

5.2 *BLOCK CONTROL*

Block control is the method of control used by many thermal cyclers. The cycler simply monitors the temperature of the block and ensures that the block is heated to the set temperatures and times. It takes no account of the 'lag time' between the temperature of

the block and the sample. As a result the sample may not be exposed to the exact programmed temperature (*Figure 5b*). In addition the number of samples in the block and the volume in each sample may also affect, and can be affected by the performance of the block.

Block control is available on the Omn-E if desired (see ‘Programming the Omn-E’). Block control is effective for simple incubations, robust cycling protocols or manual control, but is not recommended for high performance cycling.

5.3 ***SIMULATED TUBE CONTROL***

In ‘Simulated Tube’ control mode the Omn-E uses an algorithm to calculate the changing temperature of a sample rather than relying on the feedback from the thermistor (*Figure 5c*).

‘Simulated Tube’ control is used for cycling with 96 well plates and racked tubes.

Calibration Factor

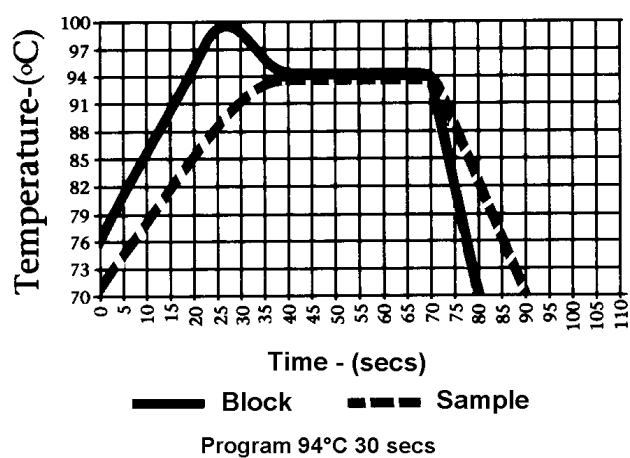
In ‘Simulated Tube’ control mode a calibration factor is needed to reflect the volume of sample in each well. The calibration factor can be entered after selecting a program to run under the ‘RUN’ menu.

NB: The appropriate calibration factor will depend on the block type (0.5ml or 0.2ml) and the consumable being used. Please refer to the following guidelines when entering calibration factors.

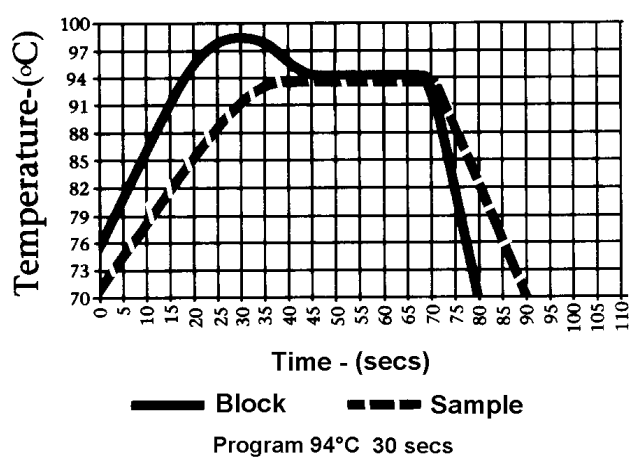
	Block Type	
Consumable Type	0.5ml Block	0.2ml Block
96 well plate	vol x 10	vol
0.5ml tube	vol	-
0.3ml tube	vol	-
0.2ml tube	-	vol

Figure 6:
Modes of control used by the Omn-E

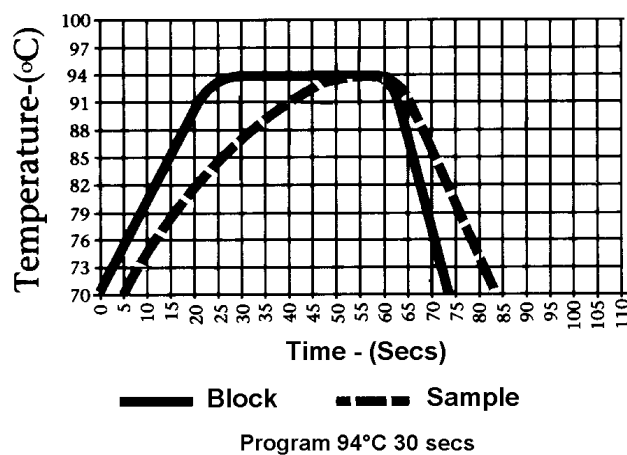
5a. Active Tube Control



5b. Simulated Tube Control



5c. Block Control



CHAPTER 6

OMN-E THERMAL CYCLER

Troubleshooting for Successful Amplifications

There are many variables within a typical PCR reaction including the constituents of the reaction mix itself and the cycling conditions used. It is therefore not possible to publish a standard amplification protocol, which will be successful in every situation. However, the following guidelines may help to resolve some of the more common issues in the optimisation of use of Thermo Hybaid instruments.

6.1 TRANSFERRING PROTOCOLS

As thermal cyclers vary considerably in their method of operation it is necessary to consider the mode of control when transferring protocols between different machine types.

As an example consider the following protocol: -

95°C	1 min	cycled 30 times
65°C	1 min	

Using a block control machine the sample may actually experience only 30 seconds at each temperature. On a tube control machine the sample will experience exactly the programmed time and temperature. Even though the tube control machine executes the program more accurately, transferring a protocol directly may result in lower yields. This is because the enzyme has been exposed to high temperatures for longer compared to the block control machine, reducing its activity significantly in later cycles.

The most accurate way of transferring protocols to a Thermo Hybaid instrument is to use the tube thermistor as a probe. Set the Thermo Hybaid machine to run on Block Control in the Manual Mode. In this mode the temperature of the tube thermistor is displayed on the run screen, but is not being used to control the instrument. It is therefore possible to remove the thermistor and place it into a second block, connecting it to the Omn-E with the extension lead provided with the unit. The second cycler can then be cycled and the actual temperatures monitored via the tube thermistor. This information can be noted and used to build up the temperature profile of the second machine and program the Omn-E accordingly. It is wise to monitor a number of cycles to detect any variation.

NB: It is not possible to use the tube thermistor to monitor the temperature of samples in 96 well plates.

6.2 OPTIMISING PROTOCOLS

In addition to the differences between block and tube control it is important to optimise the protocol itself.

In general, thermal cycling protocols consist of three distinct steps: -

1. **Denaturation** of the template at an elevated temperature, typically between 90-95°C.
2. **Annealing** of the primers to the template dictated by the melting temperature of the primers, typically between 35-60°C.
3. **Enzymatic extension**. This is dictated by the optimum temperature for polymerase enzyme activity, typically 72°C.

These three steps are repeated for 20 to 30 cycles.

Denaturation Step

The denaturation temperature at each cycle must be sufficient to denature the template completely including any secondary structure and G-C rich regions. However, the effect on enzyme activity of repeated high temperature incubations should also be considered. An initial extended denaturation step of 95°C for 5 minutes before enzyme addition will serve to denature complex high molecular weight DNA. In later cycles this should be reduced to a maximum of 30 seconds at up to 92-95°C. The use of shorter times at lower temperatures, e.g. 10 seconds at 90-92°C has been used successfully in some applications (e.g. cycle sequencing).

Annealing Step

The annealing temperature depends on the size and the composition of the oligonucleotides used as primers. In general this varies between 50-70°C and as a rough guide should be 5°C below the T_m, calculated approximately as follows: -

$$T_m = 2 \times (A + T) + 4 \times (C + G)$$

Because a difference of as little as 1°C in the annealing temperature can affect the reaction, it is recommended that a range of temperatures is tested to optimise the

annealing temperature for each primer set. In general terms, the higher the annealing temperature, the more specific the reaction, i.e. increased stringency.

Extension Step

The extension temperature is largely dependent on the optimum temperature of the enzyme chosen, and is typically within the range 70-72°C. The time required depends on the length of the product being synthesised. A time of one minute per kilobase is more than sufficient to begin with. Several protocols (called biphasic) dispense with a separate extension step altogether, and rely on the temperature of the enzyme during the annealing step, and during the transition between the annealing and denaturation steps.

6.3 OTHER HELPFUL HINTS

1. Check the volume in the thermistor tube

The thermistor must always be immersed in mineral oil. Do not use aqueous solutions in the thermistor tube, which will damage the probe.

Too much oil in the tube relative to the sample tubes will result in the samples overshooting. Repeated overshoots will gradually reduce the activity of the enzyme and affect yield.

Too little oil will result in the samples undershooting the target temperatures. Repeated undershoots may decrease efficiency of annealing as temperatures are not reached by the sample.

The volume in the thermistor tube must match the total volume in the sample tubes including any oil overlay.

The correct position for the tube thermistor is A2.

Check location of thermistor within the control tube

Always ensure the thermistor probe is located centrally in the control tube and immersed in the liquid. If it is pushed against the side it will be measuring the temperature of the microcentrifuge tube and not the sample temperature.

2. **Ensure the samples have been brought to the same temperature as the tube thermistor before beginning the run.**

If samples have been placed directly from the freezer or fridge into the block the tube thermistor will not accurately reflect the correct sample temperature. A brief incubation at 35°C for 2 minutes will bring all samples to the same starting temperature.

3. **Tube thermistor readout of ***** on the run screen**

This indicates that the thermistor is not connected correctly or is damaged. Check the location of the thermistor in the unit and if the fault persists contact Thermo Hybaid's Service Department or your local distributor.

4. **Check the fit of the tube in the block**

The Omn-E block will neatly fit several types of reaction tubes. The tube thermistor supplied with the Omn-E is mounted in an appropriate OmniTube, and this tube should be changed periodically as the fit of the tube will deteriorate with time. The reaction tubes should be distributed evenly in the block. If you have opted to use a reaction tube other than one of those recommended, have its fit in the Omn-E block checked. Also remount the thermistor probe in this tube to ensure your samples and the control tube are well matched.

5. **Check through the program** after it has been entered using the VIEW function. Have you entered the correct combination of program number and block number? Has the correct block type been entered using the BLOCK SETUP function?

6. **Runs using Simulated Tube Control**

For tubes, the calibration factor entered is the same as the total volume in one tube including overlay.

For OmniPlates running on machines with 0.5ml well Blocks, the calibration factor entered is total well volume times 10 to a maximum of 500. This accommodates the non-optimal fit of the OmniPlate in the 0.5ml block. Other 96 well plates, for example the Costar ThermowellH may be used with lower multiples of volumes, but these need to be individually determined.

For OmniPlate 96 running on machines with 0.2ml well Blocks, the calibration factor entered is total well volume times 1.

7. **Check maximum and minimum** values achieved during the run, which can give an indication of unusual temperature performance.

CHAPTER 7

OMN-E THERMAL CYCLER

Cleaning & Decontamination

7.1 *GENERAL CLEANING*

All surfaces of the Omn-E system and Heated Lid should be cleaned regularly with a soft cloth, hot water and a mild detergent (Fairy liquid or Teepol). It is important to thoroughly dry all surfaces after cleaning. On no account should organic solvents be used in the cleaning of any of this equipment.

7.2 *DECONTAMINATION*

It has recently been observed by researchers that when ^{35}S labelled nucleotides are thermally cycled they can break down into lower molecular weight forms which are highly volatile and can leach through the walls of tubes and microtitre plates thus contaminating the block and possibly the heater plate of the Heated Lid.

We do not recommend the use of ^{35}S labels, as replacing a dangerously contaminated block is expensive. This is not covered under our warranty agreement and requires special service arrangements.

If ^{35}S labels must be used, the following recommendations will assist in minimising contamination.

1. Use a mineral oil overlay in all reactions, even if using the Heated Lid.
2. If using tubes, use only the thick walled variety.
3. Use the thermal cycler in a fume hood, as air contamination is a possibility.
4. If using microtitre plates, coat the under surface with a thin layer of mineral oil.

All Omn-E and Heated Lid components, which may come into contact with radioactivity, should be decontaminated before re-use.

If radioactivity must be used the thermal cycling block and Heated Lid surfaces can be decontaminated using a 10 % v/v solution of Neutracon (Decon Lab Ltd, Conway Street Hove, East Sussex BN3 3LY Tel: +44(0) 1273-739241, Fax: +44(0) 1273-722088 or PCC-54 (Pierce Eurochemie B.V Holland). It is important to stress that complete

decontamination is unlikely but low level counts can be achieved by repeated application of a fresh solution of 10% v/v Neutracon to the "Hot" area.



Thermo Hybaid recommends that if radioisotopes are to be used, equipment MUST be located in a designated Radiation Area. Local Radiation Safety procedures MUST be followed at all times.

APPENDIX I

OMN-E THERMAL CYCLER

Technical Specifications & Consumables

SPECIFICATIONS

Description	Omn-E Thermal Cycler with 0.5ml Blocks	Omn-E Thermal Cycler with 0.2ml Blocks
Catalogue N°	HB-TRE-05-220/110	HB-TRE-02-220/110
Temperature		
Range	Ambient +10°C -99°C	Ambient +10°C-99°C
Control precision	±1.0°C	±1.0°C
Display resolution	0.1°C	0.1°C
Block uniformity	±0.5°C	±0.5°C
Control available	Tube control Simulated tube control Block control	Tube control Simulated tube control Block control
Block		
Block capacity	48 x 0.5ml tubes/ 96 x 0.3ml tubes 1 x 96 well plate	96 x 0.2ml tubes/ 1 OmniPlate 96
Standard Accessories	Removable heat shield 0.5ml tube thermistor	Removable heat shield 0.2ml tube thermistor
Power Requirements	220/240V, 50/60Hz or 110/120V, 50/60Hz	
Maximum Power	160W	
Input Fuse Rating	2 x 1.6A(T) 220V or 2 x 3.5A(T) 110V	
Programming		
No. of programs	36	
Max. no. of program stages	10	
Max. no. of steps per stage	10	
Max. programmed dwell time	9hr 59 min 59 sec	
Pause facility	Yes	
Temp. ramping	0.01°C/sec -1.0°C/sec	

Programming (contd.)				
Auto restart	Yes			
Run “end time” calculation	Yes			
Run end audible alarm	Yes			
File protection	Yes			
Alphanumeric program names	Yes			
Output	RS232			
Dimensions	Width	190mm	Depth	280mm
	Height	200mm	Weight	5kg

OMN-E WITH HEATED LID

Description	Omn-E Thermal Cycler with 0.5ml Block & Heated Lid	Omn-E Thermal Cycler with 0.2ml Block & Heated Lid
Catalogue N°	HB-TRE-05HL-220/110	HB-TRE-02HL-220/110
Heated Lid Operating Temp	110°C	110°C
Dimensions	Width 190mm Height 210mm	Depth 280mm Weight 5.5kg
Other specifications are as for non-Heated Lid versions.		

The Omn-E and Omn-E with Heated Lid are designed to comply with IEC1010-1 safety requirements and with EMC regulations.

Description	Heated Lid Retrofit Kit for 0.5ml Block Omn-E Units	Heated Lid Retrofit Kit for 0.2ml Block Omn-E Units
Catalogue N°	HB-TRE-HLO	HB-TRE-HLM
Heated Lid	Yes	Yes
Software Upgrade	Yes	Yes
Instructions	Yes	Yes

CONSUMABLES

	Catalogue N°	Description
OmniPlate 96	HB-TR3-MT	Polycarbonate 96 well plate (pack of 25)
OmniPlate 96 Lid	HB-TR3-MTL	Polycarbonate lids (pack of 25)
Heated Lid	HB-TRE-HLM	Heated Lid for Omn-E 0.2ml Block
Heated Lid	HB-TRE-HLO	Heated Lid for Omn-E 0.5ml Block

Other Consumables

	Catalogue N°	Qty	Description
OmniTube (0.2ml)	HB-TC-3372N	1000	0.2ml individual tubes with domed lids
OmniTube (0.2ml)	HB-TC-6202N	1000	0.2ml individual tubes with flat lids
OmniTube (0.5ml)	HB-TC-4895N	1000	0.5ml individual tubes with domed lids
OmniTube (0.5ml)	HB-TC-3505N	1000	0.5ml individual tubes with flat lids
OmniStrip (0.2ml)	HB-TC-2662N	2000	0.2ml strips of 8 tubes & caps
OmniStrip (0.3ml)	HB-TC-4043N	2000	0.3ml strips of 8 tubes & caps
Racked OmniTube 96	HB-TC-4073N	10	For 0.3ml 96 well racks (caps not included)

APPENDIX II

OMN-E THERMAL CYCLER

Abbreviations & Notes

α	Alpha
β	Beta
DNA	Deoxyribonucleic Acid
ml.....	Millilitres
mRNA	Messenger RNA
mg.....	Milligrams
ng.....	Nanograms
^{32}P	Radioisotope Phosphorus 32
^{33}P	Radioisotope Phosphorus 33
PCR	Polymerase Chain Reaction*
RNA	Ribonucleic Acid
rtPCR.....	Reverse Transcription PCR*
^{35}S	Radioisotope Sulphur 35
μg	Micrograms
μl	Microlitres
IS PCR.....	<i>In Situ</i> PCR

* The Polymerase Chain Reaction (PCR) is covered by U.S. patents #4,683,195 & #4,683,202, which are owned by Hoffman La Roche. Furthermore, the *In Situ* Amplification process is covered by patents that are currently pending before the U.S. Patent Office.

This document provides no Licence, nor does it encourage any reader to violate these or any other patents. However, there is a common antitrust law to provide a “research exemption” to U.S. patents for research that has no commercial content. Should a reader wish to use the processes here described for a remunerative or commercial application, we strongly urge that proper patent Licences be obtained.

OMN-E THERMAL CYCLER

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