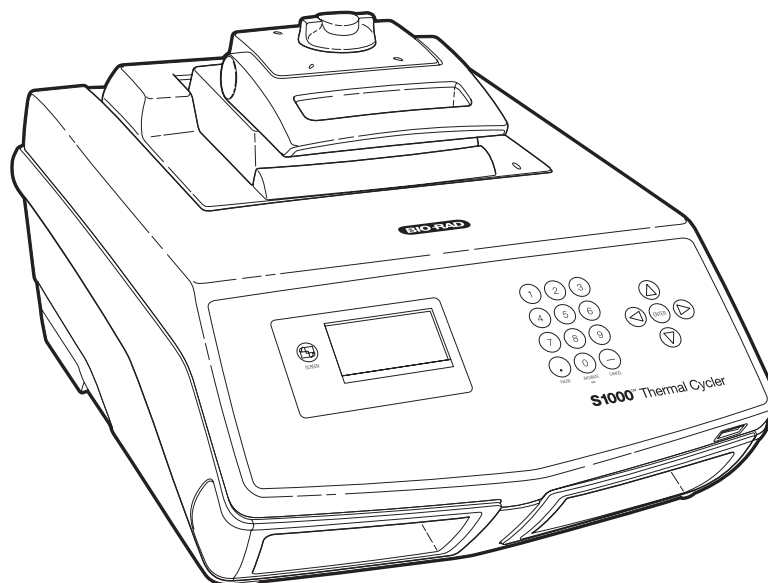

S1000™ Thermal Cycler

Instruction Manual

Catalog # 184-2000
185-2096
185-2048
185-2384



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Safety and Regulatory Compliance

The S1000™ thermal cycler heats and cools quickly during operation. We strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 1 to review the meaning of each safety warning label:

Table 1: Meaning of safety warning label.

	CAUTION: Risk of danger! This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled. Wherever this symbol appears, consult the manual for further information before proceeding.
	CAUTION: Risk of electrical shock! This symbol identifies components that pose a risk of electrical shock if improperly handled.
	CAUTION: Hot surface! This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

Instrument Safety Warnings

The following warnings labels display on the instrument, and refer directly to the safe use of this S1000 thermal cycler:

Table 2: Instrument safety warning labels.






Icon	Meaning
	Warning about risk of harm to body or equipment. Operating the S1000 thermal cycler instrument before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel should operate this instrument.
	Warning about risk of harm to body or equipment from electrical shock. Do not attempt to repair or remove the outer case of this thermal cycler base, power supply, heat pump, or other accessories. If you open these instruments you put yourself at risk for electrical shock and void your warranty. All repairs must be done by an authorized repair service.
	Never remove the outer case of a thermal cycler base because you might receive an electrical shock. This thermal cycler uses neutral fusing, which means that live power could still be exposed inside the instrument even when the fuse is blown or removed.
	Warning about risk of burning. A thermal cycler generates enough heat to cause serious burns. Wear safety goggles or other eye protection at all times during operation. Always allow the sample block to return to idle temperate before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns.

Table 2: Instrument safety warning labels.

Icon	Meaning
	Warning about risk of explosion. The sample blocks can become hot enough during the course of normal operation to cause liquids to boil and explode.

Safety Use Specifications and Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

Table 3: Safe use specifications

Safe use requirements		Specifications
Input power	Rated	100–240 VAC, 50–60 Hz.
Fuses		250 V, 10 A.
Temperature	Indoor use	Ambient temperature of 5°C to 31°C. Relative humidity maximum of 80% noncondensing.
Altitude		Up to 2,000 meters above sea level.
Overvoltage Categories		II
Pollution degree		2

SAFETY COMPLIANCE

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- UL Std No. 61010A-1 Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- UL Std No. 61010A-2-010 Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 1010.1-92 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements (includes Amendment 1)
- CAN/CSA C22.2 No. 1010.1B-97 - Amendment 2 CAN/CSA C22.2 No. 1010.1-92 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 1010.2.010A-97 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials, Amendment No. 1
- EC 61010-1 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials

ELECTROMAGNETIC COMPATIBILITY (EMC)

- F.C.C Title 47 Part 15B as a Class A digital device.

- **EN61326 Class A Electrical Equipment for measurement, control, and laboratory use - EMC Requirements.**

FCC WARNINGS AND NOTES

- **Warning: Changes or modifications to this unit, not expressly approved by the party responsible for compliance, could void the user's authority to operate the equipment.**
- **Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference, at his own expense.**
- **Note regarding FCC compliance: Although this design of instrument has been tested and found to comply with Part 15, Subpart B of the FCC Rules for a Class A digital device, please note that this compliance is voluntary, for the instrument qualifies as an "exempted device" under 47 CFR 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture.**
- **Note regarding Canadian EMC compliance: Le present appareil numerique n'emet pas de bruits radioelectrique depassant les limites applicables aux appareils numeriques de class A prescrites dans le reglement sur le brouillage radioelectrique edicte par le Ministere des Communications du Canada.**
- **Cables: Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.**
- **Use only Bio-Rad USB cable (Catalog No. 184-8000) with your 1000 Series cyclers.**

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1 Introduction

Congratulations on purchasing the S1000™ thermal cycler, a member of the Bio-Rad 1000 series thermal cycling platform. It combines powerful thermal performance with unsurpassed flexibility and expansion potential making it ideal for both large and small laboratories.

This chapter provides an introduction to the S1000 thermal cycler and more, including:

- The 1000 series platform of thermal cyclers (below).
- S1000 thermal cycler features and performance (page 2).
- Bio-Rad's PCR resources and support (page 3).
- Writing conventions for this manual (page 4).

The Bio-Rad 1000 Series Platform

The 1000 series line of PCR instrumentation is a flexible and modular platform designed to offer best in-class performance and advanced features in an easy-to-use, reliable package. With the 1000 series platform, you can get results faster by seamlessly integrating your workflows with functions such as PC compatibility, optimization, programming options, online support, and USB connectivity.

Table 4: 1000 Series Catalog Numbers

<i>Product</i>	<i>Catalog Number</i>	<i>Item Description</i>
C1000 Thermal Cycler	184-1000	C1000 thermal cycler, chassis only
C1000 Fast 96-well System	185-1096	C1000 chassis + Fast 96-well Rxn Module
C1000 48/48 System	185-1048	C1000 chassis + Dual 48/48 Rxn Module
C1000 384-well System	185-1384	C1000 chassis + 384-well Rxn Module
S1000 Fast 96-well System	185-2096	S1000 chassis + Fast 96-well Rxn Module
S1000 48/48 System	185-2048	S1000 chassis + Dual 48/48 Rxn Module
S1000 384-well System	185-2384	S1000 chassis + 384-well Rxn Module
S1000 Thermal Cycler	184-2000	S1000 thermal cycler, chassis only
96-well Fast Reaction Module	184-0096	96-well Fast Rxn Module for 1000 Series

Table 4: 1000 Series Catalog Numbers

<i>Product</i>	<i>Catalog Number</i>	<i>Item Description</i>
48/48-well Fast Reaction Module	184-0048	Dual 48/48 Rxn Module for 1000 Series
384-well Reaction Module	184-0384	384-well Rxn Module for 1000 Series
CFX96 Real-Time System	185-5096	C1000 chassis + CFX96 Rxn Module
CFX384 Real-Time System	185-5384	C1000 chassis + CFX384 Rxn Module
CFX Manager Software	184-5000	PC Software for use with Real Time PCR
CFX Software, Security Edition	184-5001	Single User License real time software

S1000 Thermal Cycler Performance and Features

The S1000 thermal cycler features superb thermal performance with the flexibility to expand throughput. It can perform standard polymerase chain reactions (PCR), cycle sequencing, or other techniques that require controlled temperature incubation.

The S1000 operates as a stand-alone thermal cycler with the following features:

- **Reduced Protocol Run Times:** The S1000 offers fast thermodynamics for shorter run times and expedited results.
- **Reliable Results with Precise Thermal Control:** Sample block uniformity of $\pm 0.4^{\circ}\text{C}$ at 90°C after 10 seconds with an accuracy of $\pm 0.2^{\circ}\text{C}$; adjustable lid temperature to further control reaction temperature and prevent evaporation.
- **Thermal Gradients:** Optimize reaction performance using temperature gradients across sample blocks.
- **Flexible Vessel Format with Interchangeable Reaction Modules:** Simply change reaction modules when you need 48, 96, or 384-well blocks with the same thermal cycler base. Uniform and repeatable microplate sealing with adjustable lid force.
- **Pre-installed, Customizable Protocols for Use as Templates:** Speed protocol writing by customizing pre-loaded protocols.
- **Ready for High-Throughput:** Use remote commands to control up to 32 thermal cyclers for automated robotic systems.

Bio-Rad Resources and References

Bio-Rad Laboratories provides many resources for scientists. The following websites contain useful information about running PCR experiments:

- **Gene Expression Gateway (bio-rad.com/genomics/)**
This site provides rich technical resources on a wide variety of methods and applications related to PCR and real-time PCR. This site also features tools, citations, technical support, and troubleshooting resources.
- **Life Science Research web site (discover.bio-rad.com)**
This site includes links to technical notes, manuals, product information, and technical support.

press the following links to download or request a copy of this manual or other Bio-Rad Laboratories literature:

- press the PDF icon to download a portable document format copy and open it using Adobe Acrobat Reader software (www.adobe.com).
- press the folder icon and order a printed copy.
- press the FAX icon to request a FAX copy.
- Phone your local Bio-Rad Laboratories office to request a printed copy. In the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the Literature option.

Use the following resources to locate what you need:



Table 5. Bio-Rad resources.

Resource	How to contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad Laboratories web site by selecting your country on the home page (bio-rad.com). Also find the nearest international office listed on the back of this manual.
Technical notes and literature	Go to the Gene Expression Gateway (bio-rad.com/genomics/) and locate the Search box in the upper, right corner of the web page. Type a search term in the box to find links to products, technical notes, and manuals.
Technical specialists	<p>Bio-Rad Laboratories provides quality technical support. We staff our Technical Support department with experienced scientists to provide our customers with practical and expert solutions. To find technical support on the web, go to the Gene Expression Gateway (bio-rad.com/genomics).</p> <p>To find local technical support, contact your nearest Bio-Rad Laboratories office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the technical support option.</p>

Writing Conventions Used in This Manual

This manual uses the writing conventions shown in Table 6 to quickly provide relevant information.

Table 6. Writing conventions

Convention	Meaning
TIP:	Provides helpful instructions
NOTE:	Provides important information
WARNING!	Explains crucial information about a topic that may lead to injury, instrument damage, data loss
Screen message	Indicates the one or more words on the screen the user should select.
NAME of control panel key	Indicates a key on the thermal cycler control panel. For example, these keys have the following names: <ul style="list-style-type: none"> The ENTER key  The RIGHT ARROW key 
Select X	Select X by using the arrow keys. For example, “select New” means “Use the arrow keys or to highlight the New option on the screen”
Select X > Y	From menu X, select Y. For example, “Select Main > RUN” means “Select the RUN option in the main menu”
Press X	Press X key on the control panel. For example, “press ENTER” means “Press the ENTER key on the control panel”

2 Get Started

The S1000™ thermal cycler is easy to set up and run: insert the reaction module, plug in the thermal cycler base, then begin a PCR experiment.

This chapter describes how to set up and operate the S1000 thermal cycler. Refer to the following sections for detailed instructions:

- Setting up the S1000 thermal cycler (below)
- Introducing the S1000 thermal cycler base and interchangeable reaction modules (page 7)
- Choosing and inserting a reaction module (page 9)
- Operating the lid of the reaction module (page 12)
- Operating the control panel (page 13)
- Selecting files and folders from the file tree (page 16)

Setting Up the S1000 Thermal Cycler

This section provides an overview of the steps needed to safely unpack and set up the S1000 thermal cycler. Follow these instructions to prepare to run the thermal cycler:

1. Unpack the S1000 thermal cycler base and reaction module

The S1000 thermal cycler base package includes the thermal cycler base, a power cord, quick guide, and this manual. Keep all packaging to safely move this instrument. Place the thermal cycler base in the desired location.

NOTE: A thermal cycler requires sufficient cool air flow to run precisely. To check for sufficient air flow before running the instrument, see page 91.

2. Insert a reaction module

The reaction module fits into the thermal cycler bay and locks in place for operation. For detailed instructions about loading the reaction module, see page 10.

3. Plug in the power cord and turn on the thermal cycler

Use the supplied power cord to plug the thermal cycler base into an electrical outlet. Use the power switch on the back of the base to turn on the power.

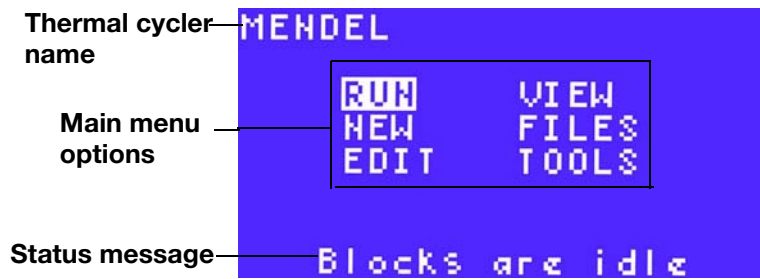
NOTE: Before operating the thermal cycler, be sure to read the safety warnings and operating requirements listed in the “Safety and Regulatory Requirements”.

As the instrument boots up, the thermal cycler runs a self-test to verify proper functions, and then displays the main menu.

Use the main menu to begin operating the thermal cycler. The main menu provides access to all thermal cycler operations and indicates cycler status. If the thermal cycler is named, the name displays in the top of the main menu.

For information about naming the thermal cycler, see page 82.

For example, the main menu shows the options listed below, the status message `Blocks are idle`, and the thermal cycler name `MENDEL`:



For more information about the options listed in the main menu, see page 16.

This thermal cycler, and associated accessories, are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for the details of the warranty. For more information about contacting your local Bio-Rad Laboratories office, see page 3.

Thermal Cycler Overview

When complete, the S1000 thermal cycler includes a base and reaction module. The following sections describe important parts of the thermal cycler and reaction modules:

- Front view of the base without a reaction module (below)
- Top view of the base with reaction module (page 7)
- Back view base with reaction module (page 8)

Front View without Reaction Module

This front view of the thermal cycler base shows a control panel and reaction module. The following image shows major features of the thermal cycler base, including the control panel, LCD screen and air vents.



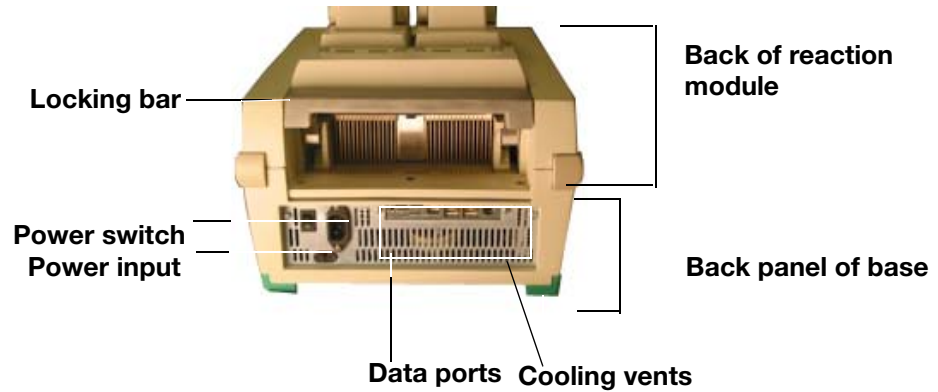
Top View with Reaction Module

This top view shows the reaction module docked in the bay of the thermal cycler base. The following shows major features of the reaction module, and thermal cycler base. For more information about reaction modules, see page 9.



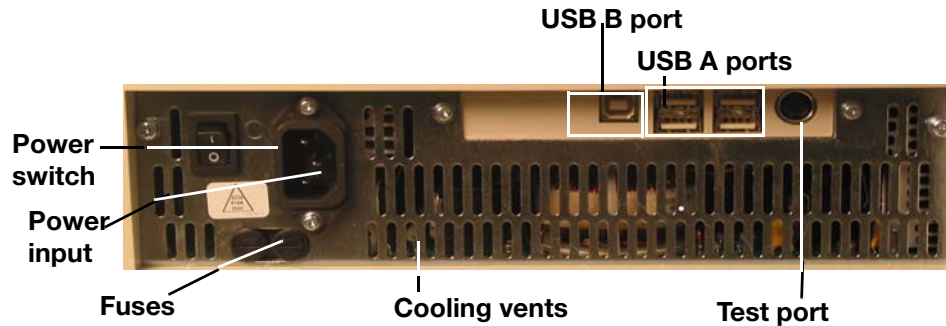
Back View with Reaction Module

The following shows the major components on the back of the thermal cycler with a reaction module, including the power switch, data ports, and reaction module.



The back panel includes data ports that transfer data to and from the thermal cycler.

- USB B port for connecting the S1000 thermal cycler to a C1000 thermal cycler.
- USB A ports for connecting to another S1000 thermal cycler that is connected to a C1000 thermal cycler or to a USB hub (page 88).
- Test port (used for service testing only).



Interchangeable Reaction Modules

The S1000 thermal cycler can run any of the 1000 series reaction modules. The reaction modules include fully adjustable heated lids that are capable of reliably running with a broad range of plastics.

Table 7: 1000 series reaction module size and limits

Number of wells	Number of blocks	Recommended sample volume (upper limit)
Dual-48	2	10-50 uL (50 uL limit)
96	1	10-50 uL (50 uL limit)
384	1	3-30 uL (30 uL limit)
CFX 96 Real-Time	1	10-50 uL (50 uL limit)
CFX 384 Real-Time	1	3-30 uL (30 uL limit)

All reaction modules are compatible with a large variety of PCR reaction vessels, in addition to the tested microplates, tubes, caps, and sealing tape listed on page 58.

All reaction modules include the features shown in the following image:

- **Heated inner lid:** Set the lid temperature for each protocol. The lid also applies the force that seals reaction vessels.
- **Lid force knob:** Adjust the knob to obtain appropriate lid force to seal the reaction vessel.
- **LED:** Turns on to show which block is selected and/or in use.
- **Locking lever:** Secures the reaction module to the base during operation.
- **Sample Thermal block:** Holds sample vessels and controls the temperature throughout the run.



Inserting and Removing a Reaction Module

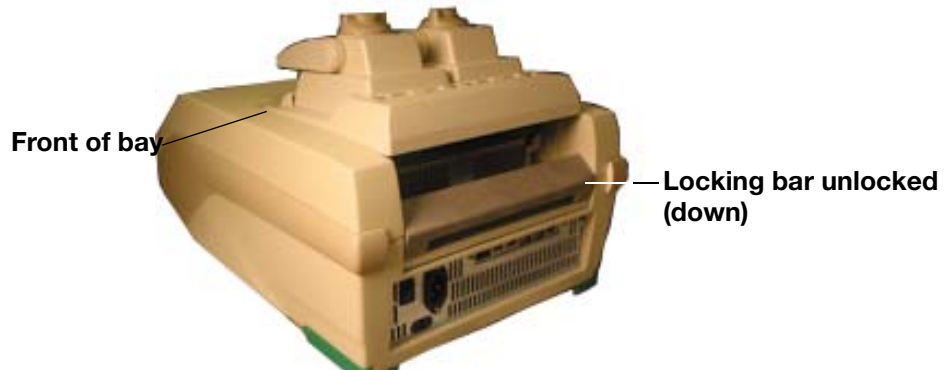
Inserting and removing a reaction module is quick and easy. When inserted, a reaction module locks into the thermal cycler base for safe operation. This section includes instructions for inserting and removing a reaction module.

INSERTING A REACTION MODULE

To insert a reaction module follow these instructions:

1. Align the reaction module in the bay

Lower the locking bar to the unlocked position. Then lift the module into the reaction module bay and leave about 2 cm of space in front:



2. Lock the reaction module in place

Pull up the locking bar to push the module forward, locking it in place.



TIP: Store the reaction module in the base when not in use.

REMOVING A REACTION MODULE

Follow these instructions to remove a reaction module from the base:

1. Unlock the reaction module

Push down the locking bar to unlock the module.



2. Lift the reaction module out of the bay

Grasp the front and back of reaction module, and lift it up and out of the bay.



3. Store the reaction module on a clean, flat surface

If you choose not to store the reaction module in the base, be sure to store it in a safe location.

WARNING! Damaging the ventilation fins on the bottom of the reaction module could compromise the ability of the module to heat and cool correctly.

Opening and Closing the Adjustable Lid

The inner sealing plate of the lid applies both force and heat, which help run consistent and successful reactions for these reasons:

- Force on the reaction caps or sealing tape helps prevent evaporation
- Heat on the caps or sealing tape prevent condensation

For recommended lid heat and force parameters, see page 33.

The following two sections describe how to open and close the lid:

- Opening the lid (below)
- Closing and adjusting the lid (page 13)

WARNING! After a run the heated lid may be hot. Use caution when opening and closing the lid. Be sure to avoid touching the heated lid or block unless it has cooled.

Opening the Lid

To open the lid, follow these instructions:

1. Release the lid force knob

Twist the lid force knob in a counterclockwise direction to release the lid.

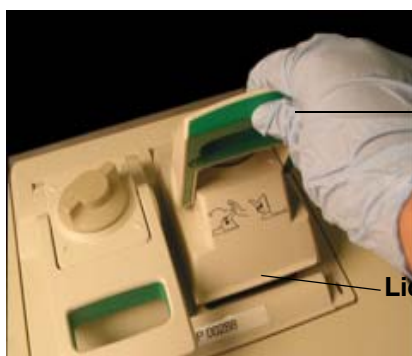


Lid force knob

Turn knob to release seal and force

2. Lift the lever

The lid lock is a lip on the lid. To release the lid, push back on the lid while lifting the lever.



Lid lever

Lid lock (unlocked)

3. Open lid completely

Continue lifting the lid lever toward the back of the reaction module so that it stays open independently.

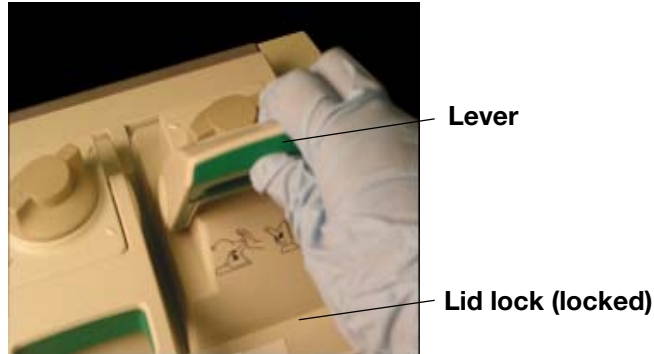
Closing the Lid and Setting Lid Force

To close the lid, follow these instructions:

1. Close the lid

Insert the reaction tubes or plate into the reaction module.

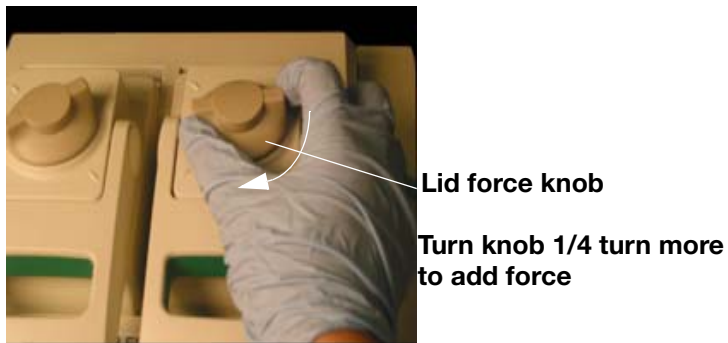
Push the lever down and back to close the lid and lock it in place.



2. Adjust lid force

Turn the lid force knob clockwise (to the right) to increase the lid force. Turn counter-clockwise (to the left) to decrease lid force.

TIP: First, turn the lid force knob clockwise until the lid makes contact with your plate or tubes (it will feel slightly tight). Then turn the knob 1/2-turn past contact if using a tape seal, or 1/4-turn past contact if using strip caps or individual tubes.

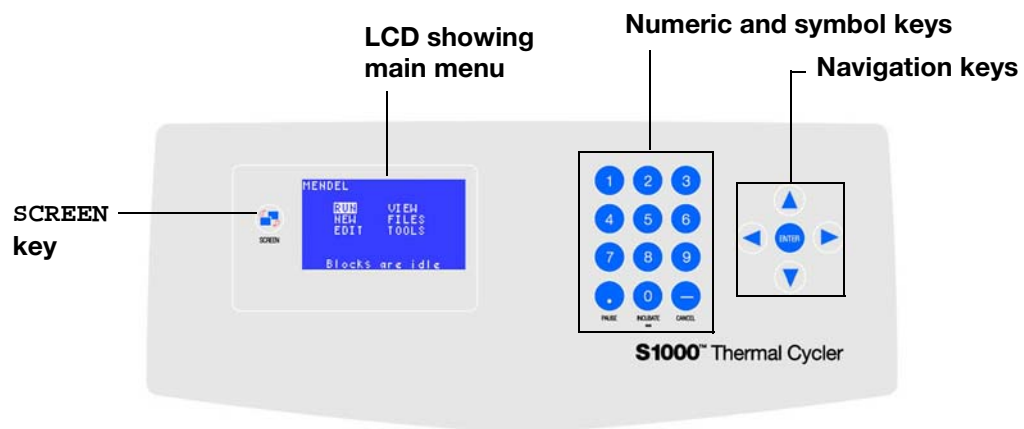


NOTE: Do not turn the lid force knob more than a 1/2 turn after the inner plate touches the vessels. Excessive force is not necessary.

Control Panel Keys and Main Menu

The control panel on the S1000 thermal cycler provides access to all the functions needed to run the thermal cycler. The control panel includes the following:

- Liquid crystal display (LCD): Displays the main menu and other screens
- Keypad & Navigation Keys: Enter commands with these keys (page 15)



Control Panel Keys

Table 8: Functions of keys on control panel

Keys	Function	Additional notes
SCREEN	Toggles between screens for alternate views	Press this key to view the status of a run
NUMERIC AND SYMBOL KEYS		
1 through 9	Enter numbers	Press each key to select numbers
PAUSE (.)	Enter a decimal point or pause a protocol	
INCUBATE (0, ∞)	Enter a zero, begin an instant incubation or enter an infinite hold	
CANCEL (-)	Enter a minus sign or cancel a function	Press this key to delete an entry, cancel a function, stop a protocol, end an incubation, or delete text on the screen
NAVIGATION KEYS		
RIGHT ARROW	Move cursor to the right	
LEFT ARROW	Move cursor to the left	
UP ARROW	Move cursor up	Press the up arrow key to scroll from A to Z on the screen. For example, press the up key three times to select the letter C
DOWN ARROW	Move cursor down	Press the down arrow key to scroll from Z to A on the screen. For example, press the down arrow key eight times to select the letter S
ENTER	Confirm a selection, a command, an entry, or to open a folder	

The Main Menu

The main menu provides access to all major functions that control the S1000 thermal cycler.



TIP: To access the main menu when the thermal cycler is not running a protocol, press the **CANCEL** key several times until you exit the current function. To access the main menu when a protocol is running, press the **SCREEN** key to toggle through a set of screens, including the main menu.

Select the options in the main menu to start these instrument functions:

- **RUN:** Select the **RUN** option to choose an existing protocol file to run
- **NEW:** Select the **NEW** option to create a new protocol file
- **EDIT:** Select the **EDIT** option to modify stored protocol files
- **FILES:** Select the **FILES** option to copy, move, rename, delete, or secure protocol files and/or folders
- **VIEW:** Select the **VIEW** option to review an existing protocol file
- **TOOLS:** Select the **TOOLS** option to change thermal cycler settings or to view the last protocol that was run

The File Tree

All protocol files are stored in the file tree. The file tree displays when you need to select a protocol to run, select a protocol to edit or select a protocol to view. For example, when you select **RUN** in the main menu, the file tree opens to allow you to select a file to run.

The file tree includes these folders:

- **MAIN folder:** This folder stores the preinstalled protocols. This folder cannot be deleted or renamed. This folder can also store user-created files, but this practice is not recommended.
NOTE: Protocol files stored in the **MAIN** folder are not secure
- **User folders:** These folders are created by S1000 thermal cycler users for storing user-created protocol files. User folders and all the files inside them can be secured with a password.

The following image shows an example of a file tree. In this example the **MAIN** folder is selected and the protocol files in that folder are listed on the right side of the screen. This protocol list includes the preinstalled protocol files and user-created protocol files, and the selected protocol file is **ITAQFST**. In this example, the folders named **EVA**

and GRANT are user-created folders. Notice that all folder names display with angle brackets (< and >) surrounding the name:



NOTE: If a folder contains more than six protocols, then it is necessary to scroll down to see all the protocols. To scroll through a folder, press the arrow keys.

The MAIN folder always contains the preinstalled protocols (page 101). The preinstalled files in the main menu can be run or copied by any user. When the main menu becomes full of files, it is difficult to find a specific protocol. Move any user-created protocol files to another folder for easy access (page 65).

3 Create and Edit Protocols

The main menu provides quick access to the options needed to create and edit protocol files.

This chapter provides instructions about using the **NEW**, **EDIT**, and **FILES** options:

- Creating a new protocol file by selecting the **NEW** option (below)
- Adding an increment (**INC**), an extend (**EXT**), a ramp rate change (**RATE**), or a signal (**BEEP**) parameter to protocol steps by selecting **OPTION** (page 28)
- Overview of Temperature Control modes, including Calculated and Block modes (page 33)
- Overview of lid temperature and manual lid settings (page 33)
- Changing a protocol file using the **EDIT** option (page 34).

Creating a Protocol File with the **NEW** Option

Select the **NEW** option in the main menu to create a new protocol file. A protocol file contains protocol steps and instrument instructions to create a cycle of steps.

NOTE: A protocol file can contain up to 99 protocol steps. The first step must be a temperature (**TEMP**) or gradient (**GRAD**) step, and the last step must be an **END** step.

Before you begin creating a new protocol, list the PCR protocol steps and conditions for your PCR experiment, including the temperatures and times for each temperature step. Then choose the protocol steps (page 63) and instrument settings (page 64) needed for the protocol. For information about optimizing PCR protocols, see page 53.

NOTE: If you are transferring a PCR protocol from another thermal cycler to the S1000 thermal cycler, review the suggestions in page 57.

This section includes step-by-step instructions for creating a new PCR protocol on the S1000 thermal cycler. These instructions assume you know how to operate the main menu and control panel (page 13).

1. Select **NEW** to create a new protocol file

Press the arrow keys to select **NEW** in the main menu. Press **ENTER** to confirm the selection and continue to the next screen.

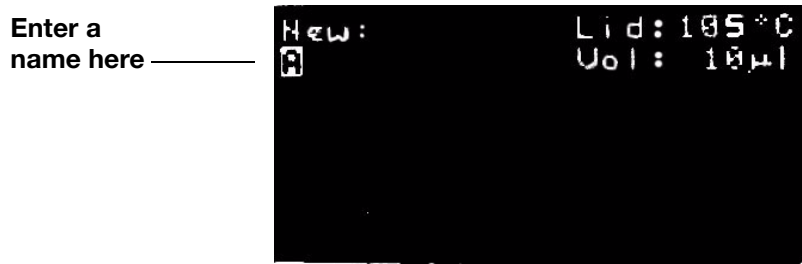


NOTE: The S1000 thermal cycler's internal memory can hold up to 400 typical 2-STEP protocols.

2. Name the new protocol file

Enter a protocol name composed of letters and numbers. To type a number, press the numbered key. To select a letter of the alphabet, press the up or down arrow key. Press **ENTER** to continue to the next space.

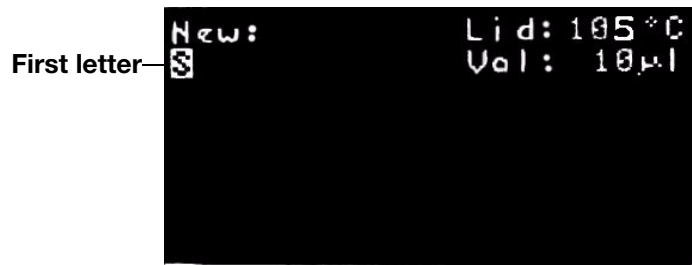
For example, to select the letter **C** press the **UP** key 3 times. To select the letter **S**, press the **DOWN** key eight times. In this example, the default letter **A** is selected:



NOTE: On the S1000 thermal cycler, a protocol file name can contain from one to eight characters. The characters are numbers or capital letters. Each protocol name must be unique to all folders.

Enter the first letter.

In this example the letter **S** is entered:



TIP: For more information about how to enter a letter of the alphabet, see “Control Panel Keys” (page 15).

In this example, the characters **STD3** are entered, and the cursor is highlighting the next space:



TIP: To delete or change a letter, press **CANCEL** and select a new letter. To delete the entire name, press **CANCEL** multiple times.

When the protocol name is complete, press **ENTER** to continue to the next screen:



3. Enter a lid temperature (optional)

Press the numeric keys to type a new lid temperature. Press **ENTER** to accept the lid temperature and continue to the next screen. The lid temperature can range from 0 to 110°C. The default S1000 thermal cycler lid temperature for new protocols is 105°C.

NOTE: When the block is running an infinite hold at a temperature below the **Turn off below** parameter, the lid heater maintains 31.0°C.

To change the default **Turn off below** parameter, select **TOOLS > DEFAULTS** (page 83).

In this example the lid temperature is changed to 110°C.



4. Enter a sample volume in microliters (uL)

Press the number keys to type the volume of your sample. Press **ENTER** to continue to the next screen. The default sample volume for a new protocol is 10uL.

NOTE: Entering a sample volume between 1 and 50 selects **Calculated** temperature Control mode (standard). Entering zero (0) selects **Block**

mode. Calculated mode is the recommended mode because it most accurately represents the actual sample temperature. For more information about Temperature control modes, see page 33.

TIP: The sample volume can be changed each time you run a protocol.

5. Enter a temperature step in the protocol file

Press the arrow keys to choose a step type. Select **TEMP** to enter a temperature step. To select **GRAD** and enter a gradient temperature step, see Step 6 on page 23 for instructions. Press **ENTER** to continue to the next screen.

In this example, **TEMP** is selected:



NOTE: The first step in a protocol must be a **TEMP** or **GRAD** step.

Enter the target temperature between 0.0 (zero) and 100.0°C. Press the number keys to type the target temperature for the temperature step. Press **ENTER** to continue to the next item.

In this example the target temperature is 95:



Enter the hold time between 0:01 (one second) and 18:00:00 (18 hours). Enter 0 (zero) to add an infinite hold and hold this step **FOREVER**. Press the number keys to type the hold-time (**TIME**) in minutes and seconds.

For example, to enter four minutes (4:00), type 400, or to enter 30 seconds type 30. Press **ENTER** to continue to the next field. In this example, the hold time is four minutes:



Confirm the parameters. The next screen displays three choices: **YES**, **NO**, and **Option**. Select one of the choices by pressing the right and left arrow keys, then press **ENTER** to continue to the next screen:

- Select **YES** to confirm the current parameters for this protocol step.

- Select **No** to change any parameters in this protocol step.
- Select **Option** to add more parameters to this protocol step. For more information about entering options, see “Adding INC, EXT, RATE, and BEEP Parameters” (page 28).

In this example, **YES** is selected:

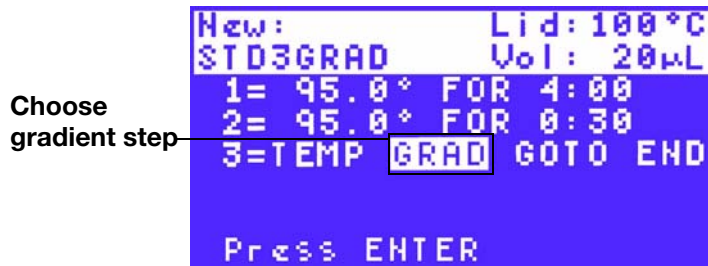


6. Enter a gradient temperature step (optional)

Press the right arrow key to select **GRAD** and enter a gradient temperature step. Press **ENTER** to continue to the next screen.

NOTE: A temperature gradient is limited to a 24°C spread. The lowest possible “Lower” temperature in the gradient is 30°C and the highest “Upper” temperature is 100°C. Therefore the lowest gradient is 30-54°C, and the highest possible gradient is 76-100°C.

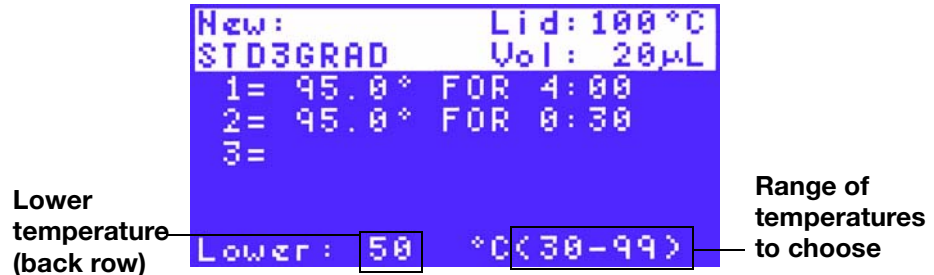
In this example, **GRAD** is selected:



TIP: Check the temperature in each row of the block in a gradient by selecting the gradient calculator tool (**TOOLS > GRADCALC** on page 84).

Enter the lower temperature in the gradient. The lower temperature is at the front (Row H) of the block.

In this example the lower temperature is 50°C:



Enter the upper temperature in the gradient. The upper temperature is at the back (Row A) of the block.

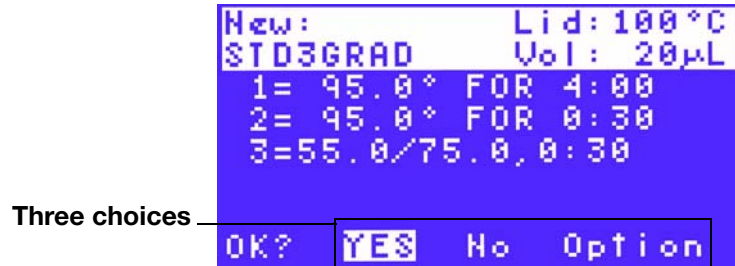
NOTE: The range of temperature is limited by the widest available range for gradient, which is 24°C. The highest **Upper** temperature is 100°C.

Enter the hold time between 0:01 (one second) and 18:00:00 (18 hours).

Confirm the parameters. The next screen displays three choices: YES, No, and Option. Select one of the choices by pressing the right and left arrow keys, then press ENTER to continue to the next screen:

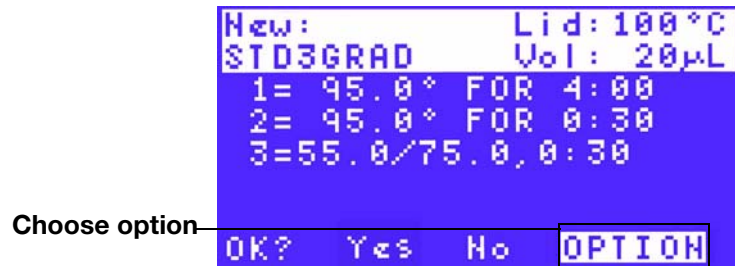
- Select YES to confirm the current parameters for this protocol step.
- Select No to change any parameters in this protocol step.
- Select Option to preview the temperature gradient (PREVIEW, described next), or to add a hold time extension (EXT described on page 30).

In this example, YES is selected to confirm the current parameters:



Select Option to preview the gradient before the run (optional)

In this example, OPTION is selected:



Select PREVIEW to view the gradient

In this example, PREVIEW is selected:



Press ENTER to preview the gradient; press again to return to the previous screen.

In this example, the gradient is formed on a 96-well block with a range from 55 to 75°C. This screen displays the approximate temperature of each row of the block, and labels the front and back rows:

Rows in block	A=	75.0 °	FRONT ROW
	B=	73.8 °	
	C=	71.5 °	
	D=	67.6 °	
	E=	62.9 °	BACK ROW
	F=	59.1 °	
	G=	56.4 °	
	H=	55.0 °	

Temperature in each row (approximate)

7. Continue entering the protocol steps

Repeat the instructions in Step 5 (page 22) to continue entering temperature steps.

In this example, four steps were entered:

Entered steps	New:	Lid: 100 °C
	STD3GRAD	Vol: 20 µL
	1=	95.0 ° FOR 4:00
	2=	95.0 ° FOR 0:30
	3=	55.0/75.0, 0:30
Choose new step type	4=	72.0 ° FOR 0:30
	5=	TEMP GRAD GOTO END
	Press ENTER	

NOTE: A protocol can contain up to 99 protocol steps. The first step must be a temperature (TEMP) step and the last step must be an END step.

8. Enter a GOTO step immediately after the set of steps that cycle (optional)

The GOTO step creates the cycle in the PCR protocol. For more information about how the GOTO step creates a cycle, see "Protocol Steps" (page 61).

Press the arrow keys to choose GOTO.

Press ENTER to continue to the next screen.

In this example, step 5 will be a GOTO step:

Choose GOTO	New:	Lid: 100 °C
	STD3GRAD	Vol: 20 µL
	1=	95.0 ° FOR 4:00
	2=	95.0 ° FOR 0:30
	3=	55.0/75.0, 0:30
4=	72.0 ° FOR 0:30	
5=	TEMP GRAD GOTO END	
	Press ENTER	

NOTE: The GOTO step cannot be the first or the last step in the protocol. A protocol always starts with a TEMP or GRAD step and ends with an END step.

Enter the step number for the first step in the GOTO repeats.

Press the number keys to enter a step number.

Then press ENTER to continue to the next screen.

In this example the first step is 2. The GOTO step instructs the thermal cycler to return to step 2 and repeat all the steps between step 2 and step 5 (the GOTO step):

Steps that repeat during GOTO step

First step in GOTO repeats

```

New: Lid: 100 °C
STD3GRAD Vol: 20 µL
1= 95.0 ° FOR 4:00
2= 95.0 ° FOR 0:30
3= 55.0/75.0, 0:30
4= 72.0 ° FOR 0:30
5= GOTO
GOTO STEP: 2
    
```

Enter the number between 1 and 9999 for the additional repeats (ADDTNL REPEATS) in the GOTO step. Then press ENTER to continue to the next screen.

NOTE: The GOTO step adds additional cycles to the PCR protocol. The first cycle is not included in the GOTO step. For example, to run a PCR protocol with 31 cycles, enter 30 repeats in the GOTO step.

In this example the number of repeats is 30, and the total number of cycles is 31:

Steps that repeat in the GOTO step

Number of additional repeat in GOTO step

```

New: Lid: 100 °C
STD3GRAD Vol: 20 µL
1= 95.0 ° FOR 4:00
2= 95.0 ° FOR 0:30
3= 55.0/75.0, 0:30
4= 72.0 ° FOR 0:30
5= GOTO 2
ADDTNL REPEATS: 30
    
```

Confirm the parameters. Press the arrow keys to select Yes or No. Select Yes to accept the GOTO step parameters, or select No to return to the beginning of this step and change the GOTO step parameters. Then press ENTER to continue to the next screen.

Confirm GOTO parameters

```

New: Lid: 100 °C
STD3GRAD Vol: 20 µL
1= 95.0 ° FOR 4:00
2= 95.0 ° FOR 0:30
3= 55.0/75.0, 0:30
4= 72.0 ° FOR 0:30
5= GOTO 2, 30 TIMES
OK? YES No
    
```

9. Enter the remaining steps in the protocol file

Enter the remaining steps by choosing the step type and adding parameters. Then press ENTER to continue to the next screen.

NOTE: A protocol can contain up to 98 TEMP, GRAD, and GOTO steps. The first step is always a TEMP or GRAD step. The last step is always an END step.

TIP: To instruct the thermal cycler to emit a sound at the end of the protocol, include a BEEP option in the final temperature step (page 32).

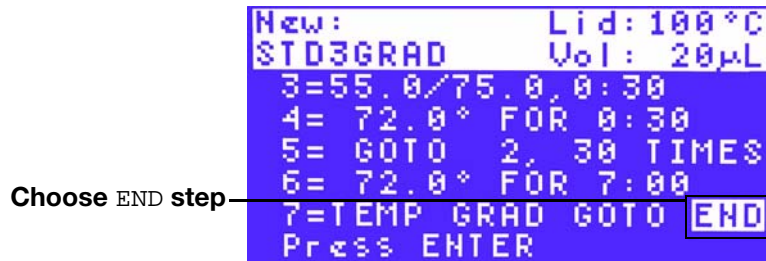
10. Enter an END step to finish the protocol file

The END step instructs the thermal cycler to finish the protocol file.

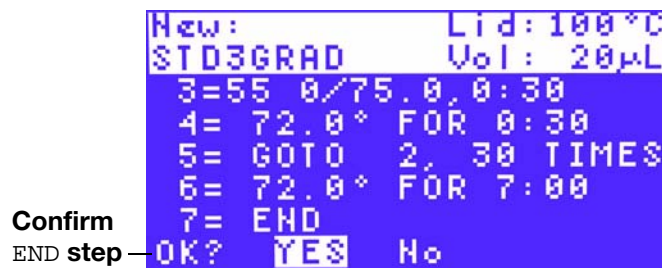
NOTE: The END step must be the last step of a protocol; a protocol can only contain one END step.

To enter an END step, press the arrow keys to select END. Then press ENTER to continue to the next screen.

In this example, the END step type is selected:



Confirm the END step. Press the arrow keys to select Yes or No. Select Yes to accept the protocol step parameters, or select No to return to the beginning and select a different protocol step.

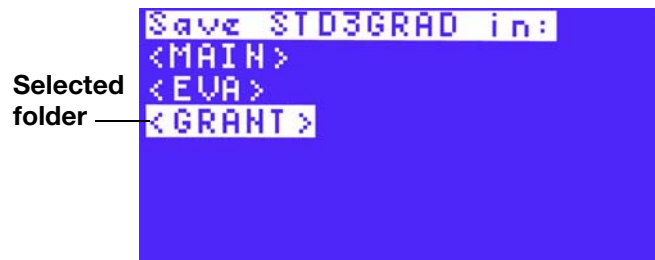


11. Save the new protocol file in an existing folder in the file tree

If there are no user-created folders (i.e. there is only one folder: MAIN), the file tree folder screen will not appear.

Press the arrow keys to choose a folder from the list of folders on the S1000 thermal cycler. Store the protocol file in a folder that you created, or in the MAIN folder. Press ENTER to save the protocol file in the selected folder and return to the main menu.

In this example, the selected folder is called GRANT:



TIP: If you store the protocol in the MAIN folder, you can move it to a specific folder with the FILES > MOVE (page 67).

To run a protocol, follow the instructions in "Running a Protocol With the RUN Option" on page 41.

Adding INC, EXT, RATE, and BEEP Parameters

A new temperature step can contain up to four additional parameters. Add these parameters by selecting **OPTION** (Step 5 on page 22).

Table 9 describes the parameters and where to find instructions about adding them to protocol steps:

Table 9: Additional parameters for temperature steps.

Parameter name	Instructions	Description	Range and limitation
Increment (INC)	page 28	Changes the target temperature of a step by the specified increment each cycle.	The range is -10 to 10°C/cycle in tenths of a degree within the temperature limits of a temperature step (0-100°C). A step with an increment must be within a GOTO repeat in order to increment the temperature with each cycle.
Extension (EXT)	page 30	Extends the hold time by the specified number of seconds each cycle.	The range is -60 to 60 seconds/cycle in whole numbers within the time limits of a temperature step (1 second to 18 hours). A step with an extend must be within a GOTO repeat in order to extend the step with each cycle.
Ramp rate (RATE)	page 31	Changes the ramping rate.	The range is 0.1 to 5°C/second in tenths of a degree for temperature steps. Ramp rate can only be changed on the dual 48- and 96-well reaction modules. The default ramp rate is the thermal cycler's maximum rate for the reaction module (see "Interchangeable Reaction Modules" on page 9).
Beep (BEEP)	page 32	Instructs thermal cycler to emit a sound when it reaches the target temperature in a temperature step.	No range. Only applies to temperature steps.

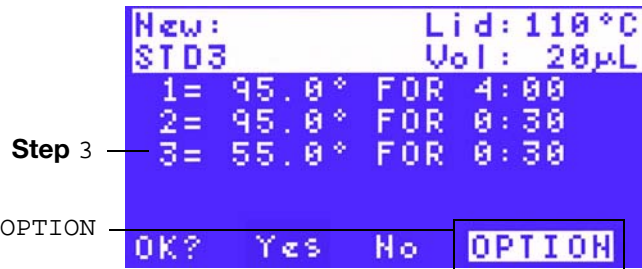
Adding an Increment (INC) to a Temperature Step

The **INC** parameter changes the target temperature of a protocol step. The increment can increase or decrease the target temperature with each cycle in the protocol.

To add an increment, follow these instructions:

1. Choose **OPTION** to add more parameters to a temperature step
Press the right and left arrow keys to choose **OPTION**. Then press **ENTER** to continue to the next screen.

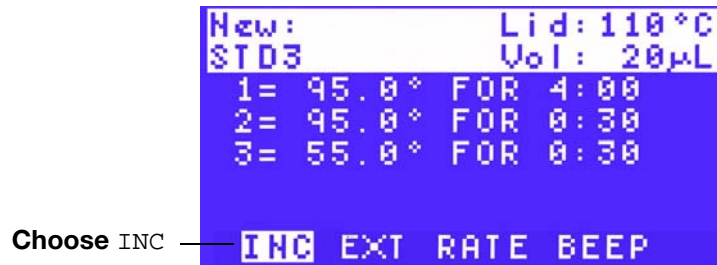
In this example, the **OPTION** will be added to step 3 parameters:



NOTE: The **INC** parameter must be added to a step within the **GOTO** repeats in order to increment with each cycle of the reaction.

2. Choose **INC to add an increment to the protocol step each cycle**

Press the right and left arrow keys to choose the **INC** parameter (if it is not already selected). Then press **ENTER** to continue to the next screen.

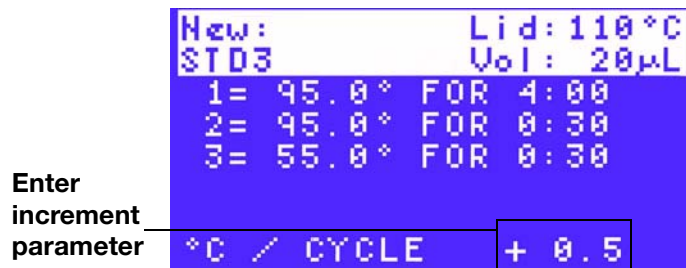


3. Enter the increment temperature

Press the number keys to type an increment parameter. To decrease the temperature each cycle, enter a negative number by pressing the **CANCEL (-)** key. Press the **PAUSE (.)** key to enter a decimal point. Then press the **ENTER** key to continue to the next screen.

NOTE: Enter an increment from -10.0 to 10.0°C per cycle in tenths of a degree, and within the limits of a temperature step (0-100°C).

In this example, the increment is + 0.5 °C. The target temperature for step 3 will increase by 0.5°C each cycle:



4. Confirm the step parameters

Press the arrow keys to select **YES** or **No**. Select **YES** and press **ENTER** to confirm the parameters for protocol step 3. Select **No** and press **ENTER** to change the parameters.

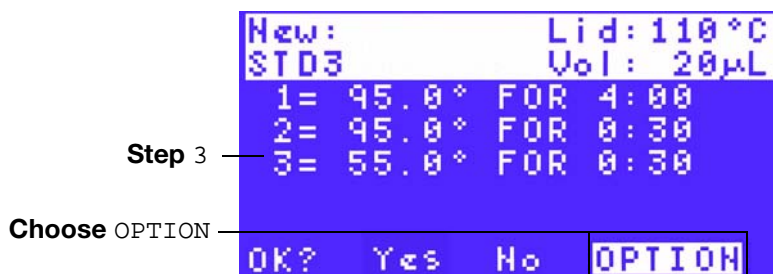
Extending (EXT) the Hold Time in a Temperature Step

The **EXT** parameter changes the hold time for a temperature or gradient temperature step. The extension increases or decreases the hold time with every cycle.

To add an extension, follow these instructions:

1. Choose **OPTION** after entering the initial temperature step parameters

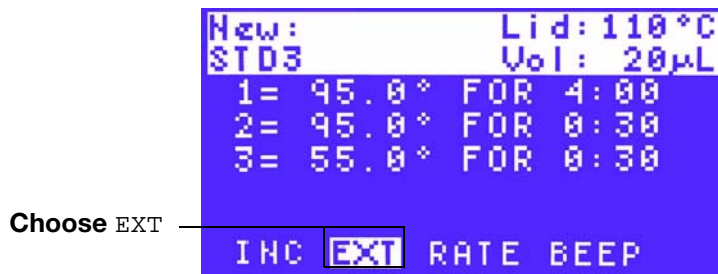
Press the right and left arrow keys to choose **OPTION**. Then press **ENTER** to continue to the next screen. In this example, the **OPTION** will be added to step 3 parameters.



NOTE: The **EXT** option must be added to a step within a **GOTO** repeat in order to extend with each cycle of the reaction.

2. Choose **EXT** to extend the hold time each cycle

Press the right and left arrow keys to select the **EXT** parameter. Then press **ENTER** to continue to the next screen.

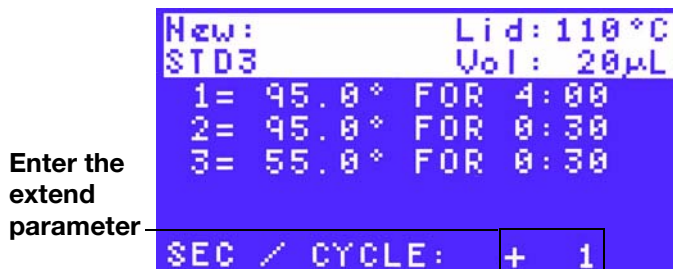


3. Enter the extension time in seconds

Press the number keys to type a parameter. To decrease the hold time each cycle, press the **CANCEL (-)** key to enter a minus sign. Then press the **ENTER** key to continue to the next screen.

NOTE: Enter an extension time between -60 and 60 seconds/cycle in whole numbers, and within the time limits of a temperature step (from 1 second to 18 hours).

In this example, the increment is +1 second. The hold time for step 3 will increase by +1 second each cycle:



4. Confirm the step parameters

Press **ENTER** to confirm the increment temperature and finish entering the step parameters. Press the right arrow key to select **NO** and change the parameters for this protocol step. Press **ENTER** to confirm the selection.

Changing the Ramp Rate (RATE) in a Temperature Step

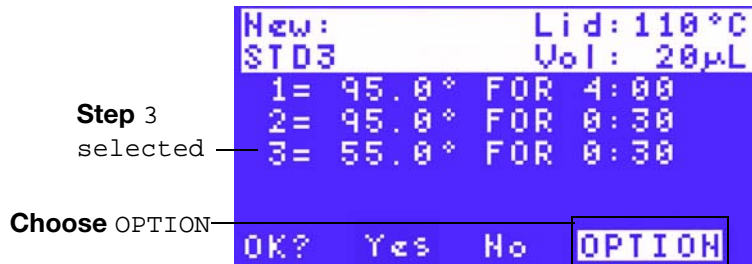
The **RATE** parameter changes the ramp rate of a temperature step. The ramp rate is the rate at which the thermal cycler heats or cools to the target temperature of a step.

To change the rate, follow these instructions:

1. Choose **OPTION** after entering the initial temperature step parameters

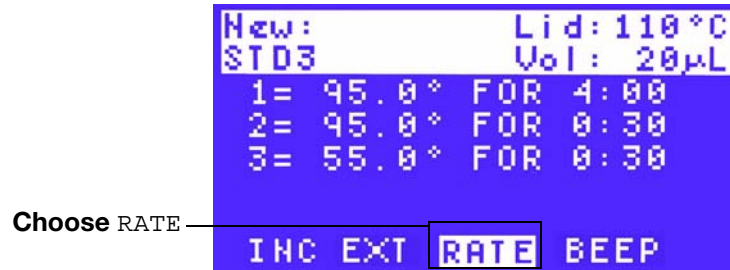
Press the right and left arrow keys to choose **OPTION**. Then press **ENTER** to continue to the next screen.

In this example, the **OPTION** will be added to step 3 parameters:



2. Choose **RATE** to change the ramp rate

Press the right and left arrow keys to choose the **RATE** parameter. Then press **ENTER** to continue to the next screen.

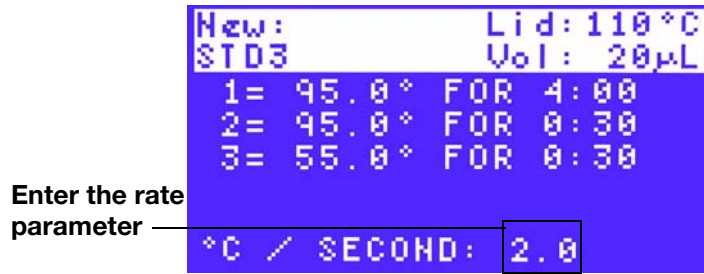


3. Enter a ramp rate

Press the number keys to type the parameter (in °C/second). Press the **PAUSE (.)** key to enter a decimal point.

NOTE: Enter a ramp rate between 0.1 and 5°C/second in tenths of a degree.

In this example the rate is 2.0. The thermal cycler will ramp to the target temperature for step 3 at 2.0°C/second:



4. Confirm the step parameters

Press the arrow keys to select YES or No. Select YES to confirm the ramp rate and finish entering the parameters for step 3. Select No and change the parameters for this protocol step.

Adding a Beep (BEEP) to a Temperature Step

The BEEP parameter instructs the thermal cycler to emit a sound when the temperature reaches its target. A BEEP can be added to any temperature step.

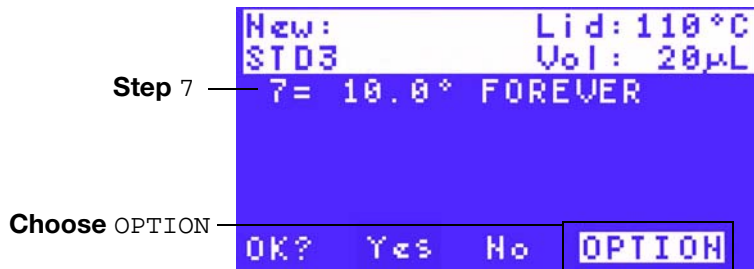
TIP: Add the beep step to a temperature step, such as an infinite (FOREVER) hold when you want the thermal cycler to signal that it has initiated the step.

To add a beep, follow these instructions:

1. Choose OPTION after entering the initial temperature step parameters

Press the right and left arrow keys to choose OPTION. Then press ENTER to continue to the next screen.

In this example, the OPTION will be added to step 7 parameters.



2. Choose BEEP to signal the end of the protocol step

Press the right and left arrow keys to choose the BEEP parameter.

In this example, the option will be applied to step 7.



NOTE: The `BEEP` parameter can only be added to a temperature step.

3. Confirm the step parameters

Press the arrow keys to select `YES` or `NO`. Select `YES` to confirm the selection and finish entering the parameters for step 3. Select `NO` and change the parameters for this protocol step. Then press `ENTER` to continue to the next screen.

Setting Temperature Control Mode

The S1000 thermal cycler uses one of two temperature control modes to determine when the sample reaches the target temperature. The thermal cycler determines the sample temperature by utilizing one of the following temperature control modes:

- **Calculated mode.** When you enter a sample volume between 1 and 50 uL (or 30 uL for 384-well reaction modules), the thermal cycler calculates the sample temperature based on the sample volume. This mode is the recommended temperature control mode, because it most accurately represents the actual sample temperature.
- **Block mode.** When you enter a sample volume of zero (0) uL, the thermal cycler assumes the sample temperature is the same as the measured block temperature.

To select a temperature control mode while creating a new protocol, see Step 4 (page 21). To select a temperature control mode when starting a protocol, see Step 4 (page 43).

Choosing Lid Temperature

The adjustable heated lid allows the user to control lid temperature and force. Heating the lid prevents condensation from forming inside the tubes and plates.

When running, the S1000's heated lid maintains the lid temperature specified for the protocol being run. Without a heated lid, water can be lost from the reagents to condensation, concentrating the reactants in the tube or plate.

The thermal cycler's default lid temperature is 105°C.

NOTE: When the block is running an infinite hold at a temperature below the `Turn Off Below` parameter, the lid heater maintains 31.0°C. The default `Turn Off Below` setting is 30.0°C. To change the default `Turn Off Below` parameter, select `TOOLS > DEFAULTS`.

Changing an Existing Protocol with the EDIT Option

Select the **EDIT** option when you want to change an existing protocol. Here are some reasons to choose the **EDIT** option:

- Make small changes to a protocol
- Edit a copy of a protocol
- Change a copy of a preinstalled protocol

Follow these instructions to change an existing protocol:

1. Select EDIT from the main menu

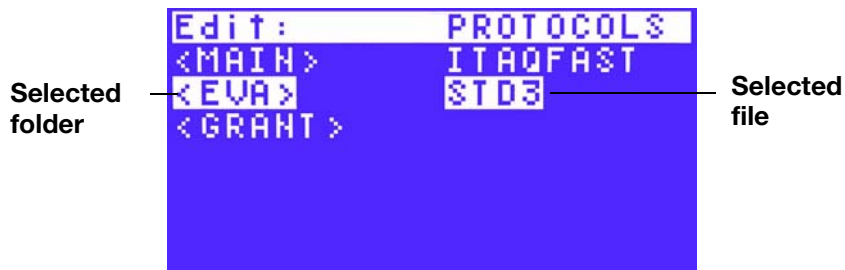
Press the arrow keys to select **EDIT** in the main menu, then press **ENTER** to confirm the selection.



2. Select a protocol to edit from the file tree

Press the arrow keys to select a folder, and press the right arrow key select a file within that folder. Then press **ENTER** to continue to the next screen.

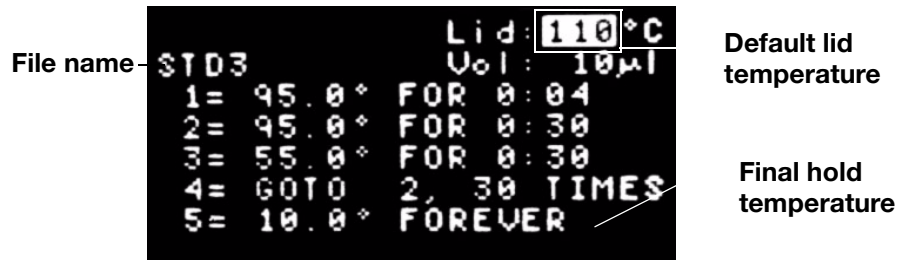
In this example, the file named **STD3** is selected in the folder named **EVA**:



3. Change the default lid temperature for this protocol (optional)

Press the number keys to enter a new lid temperature. Then press **ENTER** to continue to the next item.

In this example the lid temperature is changed to **110°C**.

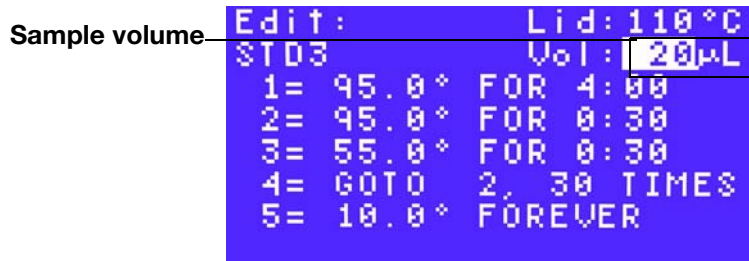


4. Change the default sample volume (optional)

Press the numeric keys to enter a sample volume. Then press **ENTER** to continue to the next item.

NOTE: Entering a sample volume between 1 and 50 selects Calculated mode (standard). Entering zero (0) selects Block mode. For more information about these temperature control modes, see page 33.

In this example, the sample volume is 20 ul, and is unchanged:

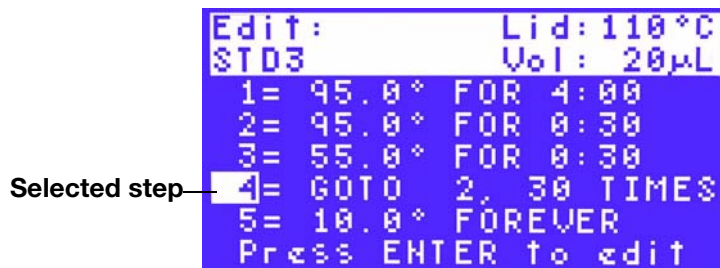


TIP: The sample volume can be also changed each time you run a protocol.

5. Select a protocol step to edit

Press the up or down arrow keys to select a protocol step. Press **ENTER** to begin editing the step.

In this example, step 4 is selected:



6. Choose an editing method

This screen displays four editing methods. Press the arrow keys from this screen to choose one of these methods:

- Choose **INS** to insert a step above the selected protocol step (see Step 7 on page 36).
- Choose **DEL** to delete the selected protocol step (see Step 8 on page 37).
- Choose **EDIT** to change the parameters in the selected protocol step (see Step 9 on page 38).
- Choose **OPTION** to add more parameters to the selected protocol step (see Step 10 on page 39).

TIP: To replace a temperature step with a gradient step, choose **INS** to insert the gradient step, and then choose **DEL** to delete the temperature step.

To stop editing the protocol, press the **CANCEL** key several times.

(If asked “Save Changes?”, press **YES** to save. Press **NO** to discard edits.)

In this example, **INS** is selected to insert a step above the selected step:

The screenshot shows a terminal window with the following text:

```

Edit:                               Lid: 110°C
STD3                                Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 55.0° FOR 0:30
4= GOTO 2, 30 TIMES
5= 10.0° FOREVER
Choose a method— INS DEL EDIT OPTION
    
```

Labels on the left indicate that step 4 is the "Selected step" and the **INS** option is chosen.

7. Insert a protocol step (optional)

Choose **INS** to insert a protocol step above the selected step. Press **ENTER** to continue to the next screen.

In this example, a new step will be inserted above step 4:

The screenshot shows the same terminal window as above, but with the **INS** option highlighted in the "Choose a method" line.

```

Edit:                               Lid: 110°C
STD3                                Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 55.0° FOR 0:30
4= GOTO 2, 30 TIMES
5= 10.0° FOREVER
Choose INS — INS DEL EDIT OPTION
    
```

Labels on the left indicate that step 4 is the "Selected step" and the **INS** option is chosen.

Choose the type of protocol step to insert, then press **ENTER** to continue to the next screen.

In this example, a temperature step (**TEMP**) is selected:

The screenshot shows the terminal window with the following text:

```

Edit:                               Lid: 110°C
STD3                                Vol: 20µL
3= 55.0° FOR 0:30
4= TEMP GRAD GOTO
5= GOTO 2, 30 TIMES
6= 10.0° FOREVER
7= END
Press ENTER to edit
    
```

Labels on the left indicate that step 4 is the "Choose step type" and the **TEMP** option is selected.

Enter the step parameters. Then press **ENTER** to confirm each parameter.

In this example, the target temperature of 72°C is entered:

The screenshot shows the terminal window with the following text:

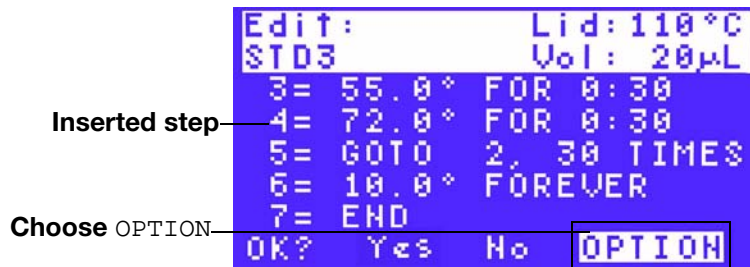
```

Edit:                               Lid: 110°C
STD3                                Vol: 20µL
3= 55.0° FOR 0:30
4=
5= GOTO 2, 30 TIMES
6= 10.0° FOREVER
7= END
Enter temperature parameter — TEMP°C: 72
    
```

Labels on the left indicate that step 4 is the "Inserted step" and the temperature parameter "72" is entered.

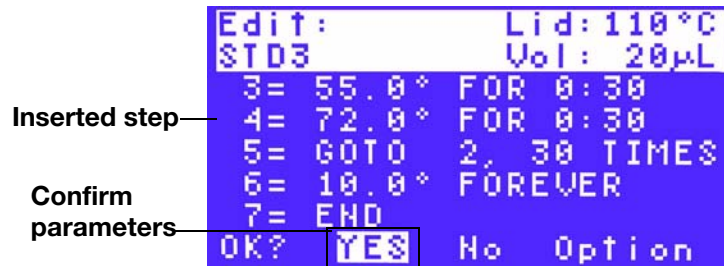
Enter more step parameters by choosing **Option** (optional). For example, add an increment or extension to this temperature step. For more instructions about entering additional parameters to a step, see "Adding INC, EXT, RATE, and BEEP Parameters" on page 28.

In this example, **OPTION** is selected to add parameters to step 4:



Enter the parameters of the new step. Then press **ENTER** to confirm each parameter.

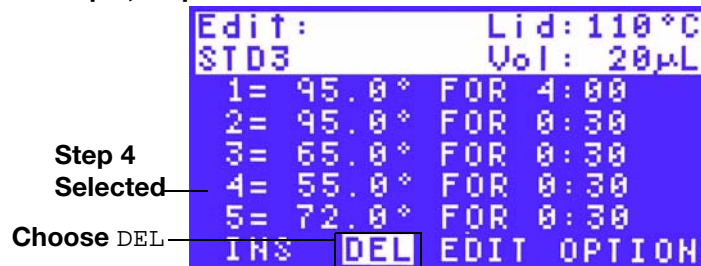
In this example, the target temperature of 72°C is entered:



8. Delete a protocol step (optional)

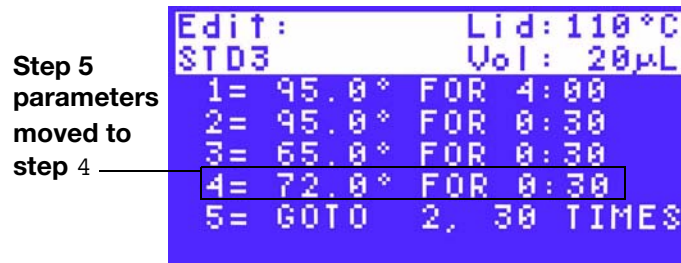
Choose **DEL** to delete the selected protocol step. In this example, step 4 is selected to delete. Press **ENTER** to continue to the next screen.

In this example, step 4 is selected to be deleted.



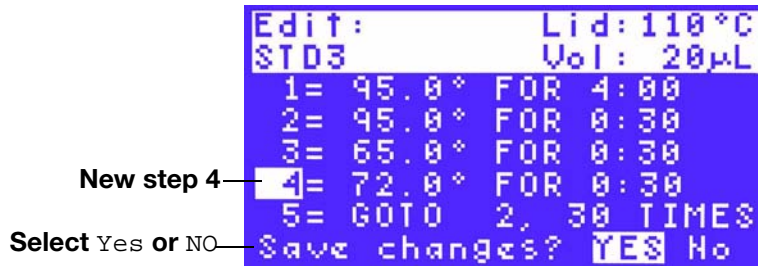
Delete the selected step. Press **ENTER** to delete the step and continue to the next screen. Notice that the deleted step parameters are replaced with the parameters of the next step.

In this example, step 4 was deleted, so step 5 parameters moved to step 4, and step 6 parameters moved to step 5:



Confirm the deletion. When prompted with **Save Changes?**, press the right and left arrow keys to select **Yes** or **NO**. Select **Yes** and press **ENTER** to delete the step. Select **NO** and press **ENTER** to return to the beginning of this step.

In this example, YES is selected:

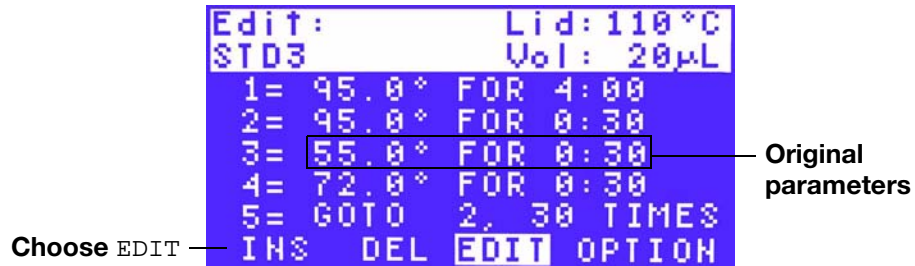


9. Edit a protocol step (optional)

Choose **EDIT** to change the parameters in the existing protocol steps.

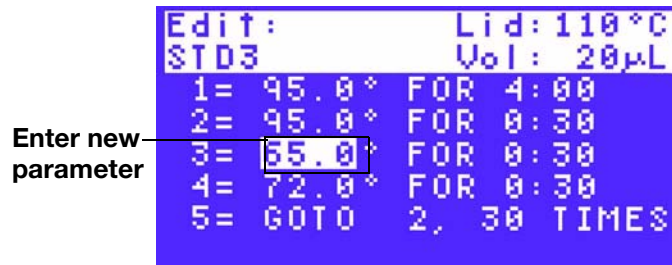
TIP: When you first select **EDIT**, you can edit the parameters in the selected protocol step. To edit the parameters in a different protocol step, press the arrow keys.

In this example, step 3 is selected. Notice the original parameter for temperature is 55.0°C:



Change the first parameter in the step. Press the keys on the control panel to enter the temperature. Then press **ENTER** to continue to the next parameter.

In this example, the temperature is changed from 55 to 65°C.



Change the second parameter in the step. Press the number keys to type a new parameter. Then press **ENTER** to continue to continue to the next step.

In this example, the hold time is changed to 35 seconds.



Continue editing the existing parameters in each step (optional). Press the up and down arrow keys to choose an earlier or later step in the protocol.

In this example, the temperature parameter is changed to 70.0°C in step 4:

Enter new parameter in next step

```

Edit:                               Lid: 110°C
STD3                                 Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 65.0° FOR 0:30
4= 70.0° FOR 0:30
5= GOTO 2, 30 TIMES
    
```

Press **ENTER** to finish changing parameters in the step and to continue editing the protocol. In this example, step 3 is finished and step 4 is selected:

Step 3 parameters edited

Step 4 selected

```

Edit:                               Lid: 110°C
STD3                                 Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 65.0° FOR 0:30
4= 72.0° FOR 0:30
5= GOTO 2, 30 TIMES
Press ENTER to edit
    
```

10. Add an additional parameter to an existing protocol step (optional)

Select a protocol step, then choose **OPTION** to change the parameters of the selected protocol step. Then press **ENTER** to continue to the next screen.

Step 3 selected

Choose OPTION

```

Edit:                               Lid: 110°C
STD3                                 Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 55.0° FOR 0:30
4= 72.0° FOR 0:30
5= GOTO 2, 30 TIMES
INC DEL EDIT OPTION
    
```

Choose **INC** to add an increment to the selected step. Then press **ENTER** to continue to the next screen.

In this example, step 3 is selected:

Step 3

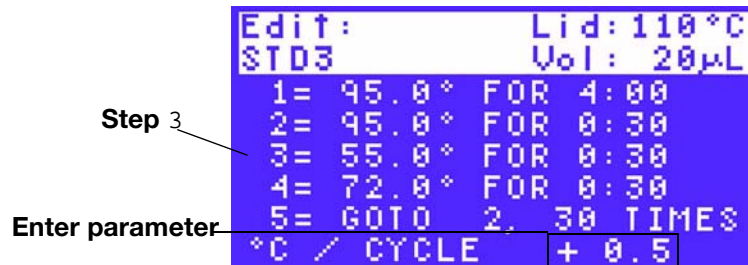
Choose INC

```

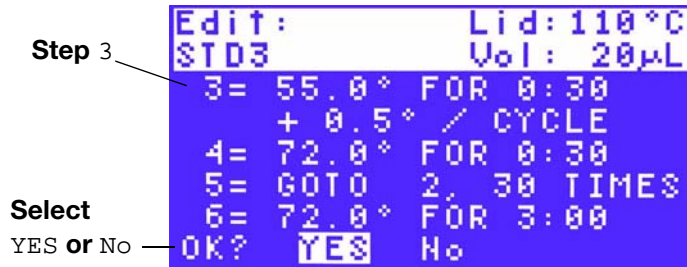
Edit:                               Lid: 110°C
STD3                                 Vol: 20µL
1= 95.0° FOR 4:00
2= 95.0° FOR 0:30
3= 55.0° FOR 0:30
4= 72.0° FOR 0:30
5= GOTO 2, 30 TIMES
INC EXT RATE BEEP
    
```

Enter the increment parameter by pressing the appropriate keys. The press **ENTER** to continue to the next screen.

In this example, an increment of +0.5°C/cycle is entered:



Confirm the parameter. Press the right and left arrow keys to select Yes or NO. Select Yes to save the changes or select NO to return to the beginning of this step. Then, press ENTER to continue to the next screen.

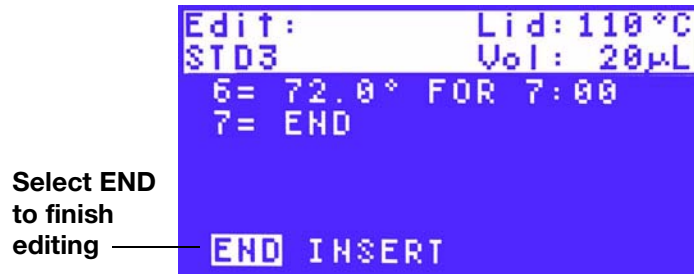


11. Finish editing the protocol

To finish editing, select the final step in a protocol, the END step. Press the down arrow key to select the END step number. Then press ENTER to continue to the next screen.

Choose END to finish the protocol. Press the left arrow key to select END. Then press ENTER to finish editing the protocol.

In this example, END is selected.



4 Run Existing Protocols

The S1000 thermal cycler runs protocols intuitively. Select the `RUN` option in the main menu and choose a protocol file to run. For your convenience, use the preinstalled protocols to quickly run a standard protocol.

This chapter provides instructions about how to run a protocol and using the `INCUBATE` key to incubate samples at a single temperature:

- Run and monitor a protocol (below)
- Monitor a protocol by pressing the `SCREEN` key (page 46)
- Pause and resume a protocol by pressing the `PAUSE` key (page 48)
- Skipping a step during a run (page 49)
- Stop a protocol by pressing the `CANCEL` key (page 50)
- Incubating samples at a single temperature by pressing the `INCUBATE` key to run an instant incubation (page 50)

The instructions in this chapter assume you know how to use the control panel to operate the S1000 thermal cycler (page 13).

Running a Protocol With the `RUN` Option

The `RUN` option provides a quick way to choose and run an existing protocol. Choose a protocol from the preinstalled protocols from the `MAIN` folder or any user folder in the file tree (page 16). The following instructions assume you know how to operate the main menu and control panel (page 13).

To run a protocol, follow these instructions:

1. Select `RUN` in the main menu, and load the samples in the block

Check that the main menu status shows `Block is idle`, then press the arrow keys to select `RUN` if it is not already selected. Choose and select the protocol you want to run. Finally, press `ENTER` to confirm the selection and continue to the next screen.

Load samples into the block. Then close the lid and set the lid force (page 13). Use sufficient force on the lids to achieve the following:

- Push the tubes or microplate down into the block for complete contact with the block
- Seal the sample lid to prevent evaporation of the sample

In this example, the `RUN` option is selected and the block is idle.



NOTE: With a dual 48 reaction module, the status message is `Blocks are idle` when the blocks are both available to run a protocol.

2. Select a protocol to run

Press the arrow keys to select a folder, and then press the right arrow key to begin selecting a file in that folder. Choose the `MAIN` folder to select a preinstalled protocol. Finally, press `ENTER` to confirm the selection and continue to the next screen.

NOTE: A protocol that is running cannot be edited. Changes made in a protocol that is running apply to the next time the protocol runs.

In this example, the preinstalled protocol `ITAQFAST` is selected in the `MAIN` folder. This protocol will run a fast PCR with iTaq polymerase:

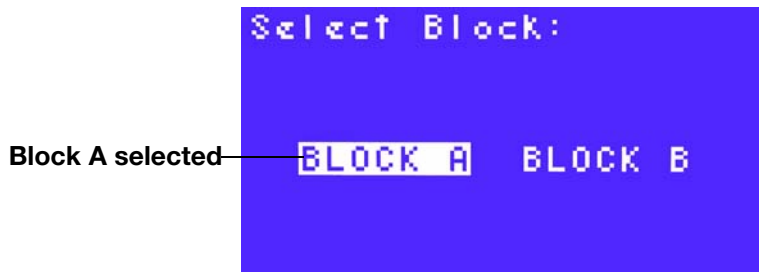


TIP: You can run a protocol in a secure folder without the password. See “Protecting Files in a Folder With the `SECURE` Function” on page 71 for more information about the files in secure folders.

3. Select block (Dual 48 module only)

Press the right and left arrow keys to select `BLOCK A` or `BLOCK B`. Then press `ENTER` to continue to the next screen. In a dual reaction module, `Block A` is on the left side.

In this example, `BLOCK A` is selected:



TIP: When a block is selected the LED on the reaction module lights up.

4. Enter the sample volume

The sample volume you enter determines the temperature control mode (page 33). To use the Calculated mode (standard), enter the volume of your samples (between 1 and 50 uL). To use Block mode, enter 0 (zero).

NOTE: Calculated mode is the recommended temperature control mode because it most accurately represents the actual sample temperature. For more information, see page 33.

In this example, the default sample volume for the thermal cycler is 10 uL, and the mode is Calculated. The thermal cycler will use the sample temperature to determine when it reaches the target temperature for a step:

Enter sample volume



TIP: When running a dual block, the message is Run ITAQFST on A, where ITAQFST is the protocol and A is the block.

Press the numeric keys to enter a new sample volume, then press ENTER to continue to the next screen. In this example the sample volume is changed to 25 uL:

Change sample volume



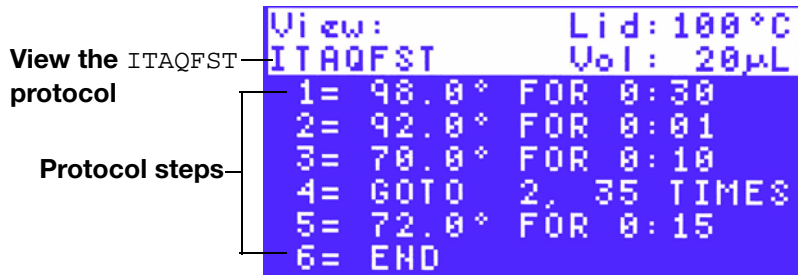
5. Select VIEW to review the protocol before beginning the run (optional)

Press the right arrow key to select VIEW, then press ENTER to confirm the selection and review the protocol:

Select VIEW



While reviewing the protocol, press **ENTER** to scroll down through the steps in the protocol. When you reach the last step in the protocol, press **ENTER** again to exit. In this example, the screen shows the steps in the **ITAQFST** protocol:



6. Select **RUN to begin the run**

Press the arrow keys to select **RUN** (if it is not already selected). Then press **ENTER** to start running the protocol.

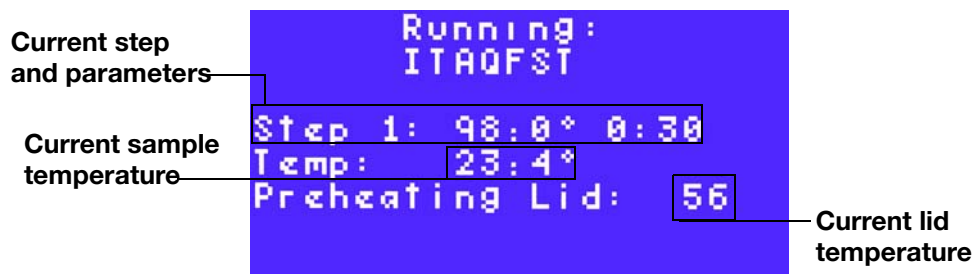


NOTE: To run a PCR successfully, a thermal cycler must be able to heat and cool quickly. Do not block the air vents. Keep other instruments and clutter away from the thermal cycler (page 99).

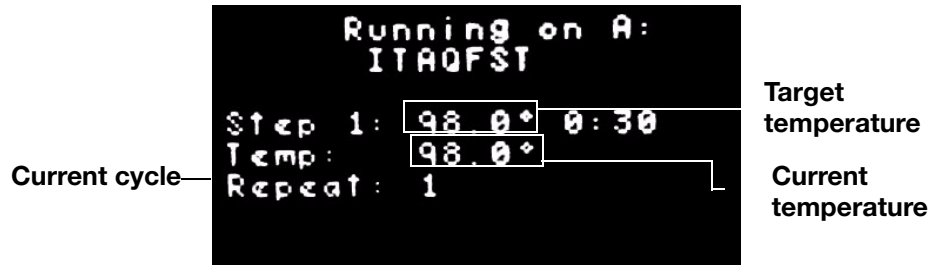
7. Monitor the protocol (optional)

Once you start a run, the **Main Menu** is displayed and the status line at the bottom of the screen will indicate that the protocol is running. Pressing the **<Screen>** key displays the **Running** screen, which shows the step parameters at the top of the list, and the current status of the step below it.

In this example, the lid is preheating before the first step of the protocol begins, and the current lid temperature is **56°C**:



Once the lid is heated to within 10°C of target temperature, the S1000 thermal cycler begins the first step and first cycle of the protocol. In this example, the S1000 thermal cycler is heating to 98°C for Step 1 in the first cycle:



TIP: To have the instrument beep when the S1000 thermal cycler reaches the target temperature, add the BEEP parameter to the step (page 28).

When the sample reaches the target temperature, the S1000 thermal cycler begins to count up to the total hold time for that step.

TIP: Once the run starts, you can view the progress of the run in several screens. To view these screens, press the SCREEN key. Each time you press the SCREEN key, the thermal cycler displays the next screen in a series that displays the status of the run (page 46).

After running the first cycle, the screen shows the GOTO repeats. In this example, the S1000 thermal cycler is running Cycle 2, and the total number of cycles is 31:



NOTE: The number of GOTO repeats does not include the first cycle; the total number of cycles is the number of repeats, plus one.

When the protocol runs the final temperature step, it completes the protocol. In this example the final temperature step is Step 4:



8. View the Protocol complete screen

When the protocol is finished, the S1000 thermal cycler displays the Protocol complete screen. After viewing this screen, press ENTER to return to the main menu. In this example the ITAQFST protocol ran for 10 minutes and 53 seconds.

**9. View the LAST RUN screen (optional)**

Once the S1000 thermal cycler returns to the main menu, press the SCREEN key to view the LAST RUN screen. This screen displays a synopsis of the last protocol that was run. To return to the main menu, press the SCREEN key again.

NOTE: For Dual 48 blocks, press the SCREEN key again to view the LAST RUN screen for each block.

The LAST RUN screen provides useful information to use for troubleshooting PCR experiments. For information about optimizing your PCR runs with the S1000 thermal cycler, see page 53. In this example, the ITAQFST protocol was the last run:



TIP: The LAST RUN screen is also available in the TOOLS option (page 80).

10. Press the SCREEN key to return to the main menu

Monitoring a Run with the SCREEN Key

Once a protocol is running, you can monitor the progress of the run with any one of three screens. Press the SCREEN key to toggle through the three screens.

TIP: For the Dual 48 reaction module, the thermal cycler displays these screens for Block A and then for Block B. Pressing the SCREEN key toggles the display through each screen and both blocks.

Each time you press the SCREEN key, the instrument shows the following three screens in this order starting from the main menu:

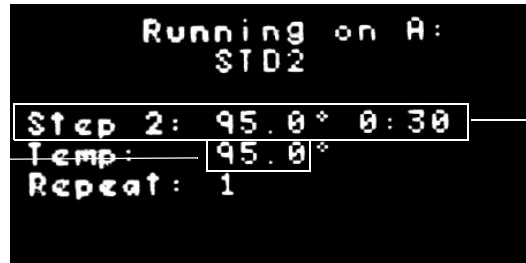
1. The Running screen

This screen displays the current step parameters, temperature, hold time, and cycle. In this example, the STD2 protocol is running Step 2 and the current cycle

is 2. The current temperature is 95.0°C and the hold time is currently 30 seconds:

Current step and parameters

Current temperature

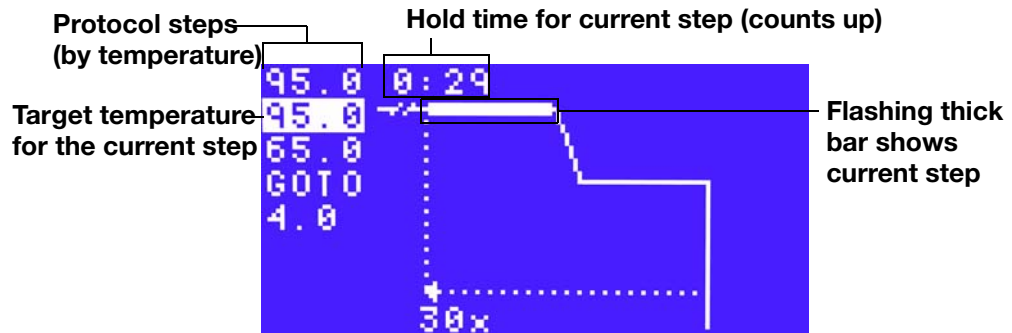


Current hold time

2. The graphical screen

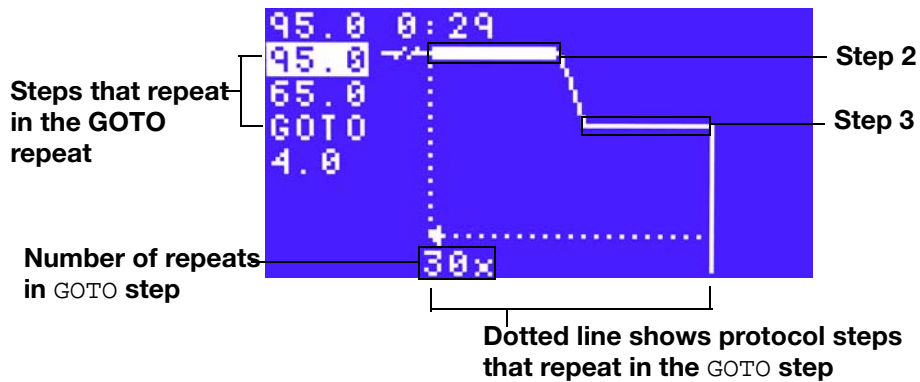
This screen displays a graph that approximates the relationship of the target temperatures in each step in the protocol. Each step is listed by temperature only.

To display the current step, a highlighted temperature and flashing bar display the temperature in the graph. The current hold time blinks above the graph, while counting up to the total hold time. In this example, the protocol has five steps including one GOTO step. Step 2 is currently running and the temperature is 95.0°C:



To display the GOTO step, a dotted line in the graph links the steps within the GOTO repeat. The total number of repeats in the GOTO step is displayed below the dotted line.

In this example, the GOTO repeat begins with step 2 and repeats 30x. The GOTO includes steps 2 and 3:



3. The Time Remaining screen

This screen displays the amount of time remaining until the end of the protocol. In this example, the protocol has 33 minutes and 24 seconds remaining in the run:



NOTE: The calculation for the time remaining includes the hold time for each protocol step and the time it takes to ramp to each target temperature. Additionally, infinite holds are ignored in the calculation.

NOTE: Dual blocks have the time remaining and other information for each block.

Pausing and Resuming a Run with the PAUSE Key

When you want to temporarily stop a protocol, use the **PAUSE** key. During a pause, the thermal cycler maintains the block temperature at the current target temperature. If the block temperature has not reached the target temperature, then the thermal cycler will continue to heat or cool until it reaches that target temperature.

WARNING! Pausing a protocol can alter the results of your PCR experiment. When you pause a protocol during a temperature step, you create a longer hold time for that step.

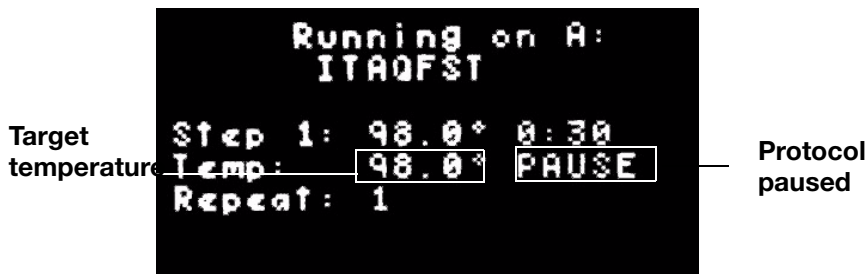
To pause and resume a protocol, follow these instructions:

1. Press **PAUSE** to stop the protocol

Press the **PAUSE** key. Notice that the time display shows "PAUSE" to indicate that the protocol is paused. In this example, step 1 in cycle 1 is paused and the temperature is currently ramping up to the target temperature.

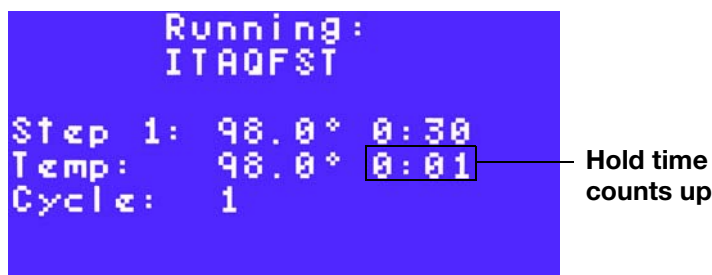


Once the protocol reaches the target temperature, it maintains that temperature throughout the pause:



2. To resume the protocol, press **PAUSE** again

Press the **PAUSE** key again, while on the Running Screen or Graphical Screen, to instruct the thermal cycler to continue running the protocol. In this example, step 1 continues, and the hold time begins counting up from 0 to 30 seconds:



Skipping a Step

If you need to skip a step in a protocol that is running, press the **ENTER** key when the step you want to skip is running. The thermal cycler will skip to the next step in the protocol.

TIP: The S100 cannot skip a GOTO repeat, unless the S1000 is controlled by a C1000 thermal cycler.

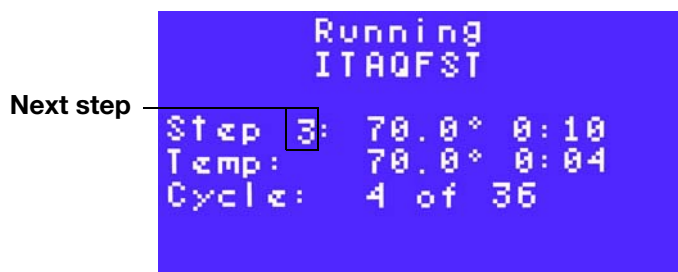
To skip a protocol step, follow these instructions:

1. Press **ENTER** while the step you want to skip is running

Press the **ENTER** key to instruct the thermal cycler to skip the current step in the protocol. Then press the right and left arrow key to select **Yes** or **NO**. Finally, press **ENTER** again to confirm your selection.

2. Notice that the next step is now running

In this example, step 3 is running in Cycle 4:



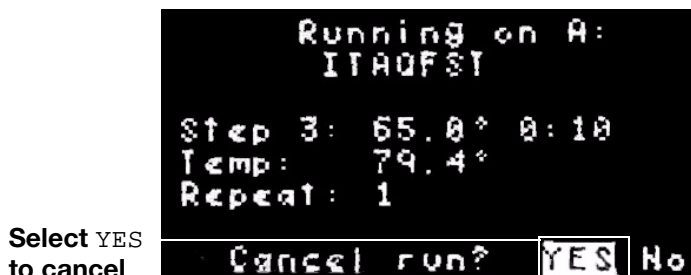
Stopping a Run With the CANCEL Key

To stop a protocol, press the **CANCEL** key. You can stop a protocol at any point during the run. When a protocol is cancelled, the block immediately stops changing temperature.

To stop a protocol, follow these instructions:

1. Press **CANCEL** and select **YES**

Press the **CANCEL** key, then press the right and left arrow key to select **YES** or **No**. Finally, press the **ENTER** key to confirm the selection, and continue to the next screen. In this example, **YES** is selected:

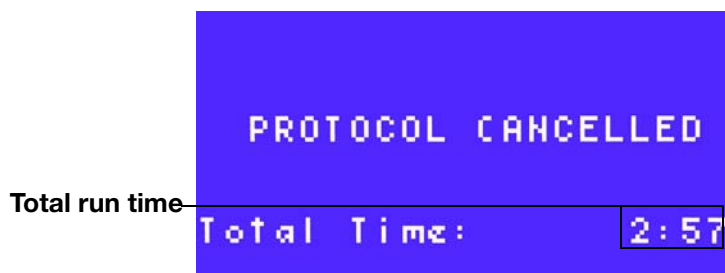


Select **YES**
to cancel

TIP: For the Dual 48 reaction module, if you press **CANCEL** while viewing the main menu, then you need to select the block that is running the protocol you want to cancel before proceeding.

2. Press **ENTER** to stop the protocol and view the **PROTOCOL CANCELLED** screen

Press the **ENTER** key to confirm that you want to stop the protocol. The S1000 thermal cycler displays the **PROTOCOL CANCELLED** screen. This screen displays the total time that the protocol ran. In this example, the protocol ran 2 minutes and 57 seconds:



Total run time

3. Press **ENTER** again to return to the main menu

Incubating Samples With the INCUBATE Key

To incubate samples at a single temperature, press the **INCUBATE (0)** key. Once the incubation is started, it continues until stopped. To stop the incubation, press the **CANCEL** key.

WARNING! Do not incubate samples overnight at a high temperature. Heating tubes or microplates indefinitely at a high temperature can evaporate all the liquid in your samples.

To incubate samples, follow these instructions:

1. Load your samples into the thermal cycler block and press **INCUBATE**. Press the **INCUBATE** key to begin an incubation after loading the samples. **NOTE:** For the Dual 48 reaction module, you must select the block that contains your samples before continuing to the next screen.

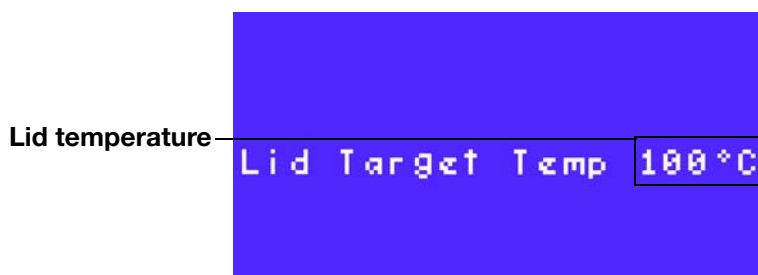
2. Enter a lid temperature

Select **YES** or **No**. To heat the lid during the incubation, select **YES** and press **ENTER**. To turn off the lid during incubation, select **No** and press **ENTER**. In this example, **YES** is selected:



Enter the lid temperature if you want to use the heated lid. Press **ENTER** to accept the default lid temperature. Press the number keys to type a new lid temperature.

In this example, the lid temperature is 100°C:



3. Enter an incubation temperature

Press **ENTER** to accept the default incubation temperature of 75°C. Press the number keys to type a new incubation temperature between 0.0 and 100.0°C. Press the **PAUSE (.)** key to enter a decimal point. To delete a number, press the **CANCEL** key.

In this example, the incubation temperature is 95°C:



4. Start the incubation

Press the **ENTER** key to start the incubation. In this example the block is incubating at 95.0°C:



TIP: During incubation, use any options in the main menu except **RUN**. You cannot start a run on a block that is running an incubation.

5. Press **CANCEL** to stop the incubation

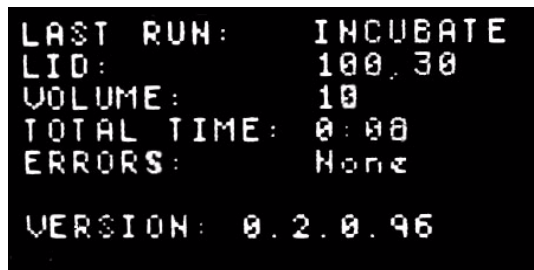
Press the **CANCEL** key to stop the incubation, then select **YES** or **NO**. Select **YES** to stop the incubation or select **NO** to continue the incubation. Finally, press **ENTER** to confirm the selection and return to the main menu. In this example, **YES** is selected:



6. View the incubation parameters

To see the parameters of the incubation, press the **SCREEN** key three times to view the **LAST RUN** screen.

To view the parameters of the run, press the **SCREEN** key twice to view the **LAST RUN** screen. In this example, the lid temperature was 100°C, the incubation ran for 8 seconds, and the version of the firmware at the time of the incubation was 0.2.0.96:



5 Optimizing PCR With the S1000 Thermal Cycler

Optimizing a reaction is critical for successful and repeatable PCR. Optimizing involves choosing the best reagents, as well as choosing the most effective temperatures and hold times for each step of the protocol. This chapter describes how to use the features of the S1000 thermal cycler to optimize PCR protocols and recommended PCR supplies:

- Optimize for a faster PCR protocol (below)
- Optimize for efficient reactions by including a temperature gradient (page 55)
- Optimize runs with small sample volumes (page 56)
- Transfer protocols that were run on another thermal cycler (page 57)
- Troubleshooting PCR (page 57)
- Select compatible PCR supplies, including microplates, sealers, tubes, and caps (page 58)

Complete optimization of a protocol involves selecting appropriate reagents and enzymes, as well as testing the parameters of the PCR protocol. For more detailed information about optimizing protocols for fast PCR, search the sites listed here. These articles include tips for optimizing the reagents, in addition to the tips for optimizing a PCR protocol presented in this section:

- **Gene Expression Gateway (bio-rad.com/genomics):** Go to the web site and select *Application | Techniques > Quantification > Fast PCR*.
- *BioRadiations Magazine* volume 118 in Adobe PDF format: Go to discover.bio-rad.com and search the *Literature* (page 3) for “Fast PCR”.

Optimizing for Faster Protocols

Optimizing a protocol for faster PCR can reduce the total run time by a third. In contrast, running the same protocol on a thermal cycler with a faster ramp rate only cuts minutes from the total run time. Furthermore, optimizing the protocol for speed can also result in better PCR results.

TIP: Automatically create a protocol that is optimized for speed by using the Protocol Autowriter on the C1000 thermal cycler or in 1000 series software. Run the S1000 thermal cycler under the control of a C1000 thermal cycler and use the Protocol Autowriter to write protocols to run on both thermal cyclers (page 88).

To optimize a PCR protocol to run faster by using the features of the S1000 thermal cycler, follow these guidelines:

- **Shorten the denaturing step**

The initial denaturation step (page 61) requires a longer hold time than other denaturation steps during the cycle. This difference is due to the activation of the polymerase and the longer initial DNA template. Once the PCR target is amplified, the amplicons then serve as shorter templates that are easier to denature during cycling.

To shorten the denaturation step, enter a hold time of 1 second for PCR products that are less than 500 base pairs (bp). Then test this shorter hold time to verify that 1 second denaturation is sufficient to produce amplicons. Alternatively, add an increment (page 28) to the denaturation step to test for the best hold time.

- **Create a two-step protocol by combining annealing and extension into a single step**

Most polymerases remain active throughout the typical range of annealing temperatures (55 to 70°C). Reduce the total run time by creating a two-step protocol that combines the annealing and extension steps in a single step. A two-step protocol can produce a yield that is similar to a three-step protocol for target sequence up to 200 bp.

To create a two-step protocol, keep the annealing temperature step and omit the extension step. Then adjust the hold time for the annealing step based on the length of your amplicon. Start with a hold time that is 10 seconds per 100 base pairs of the target.

Alternatively, optimize the annealing temperature using a temperature gradient across the block (Step 6 on page 23), and pick the final annealing temperature from the best results of the gradient experiment. See “Optimizing Temperature Steps With a Gradient” (page 55) for an example. Optimization of the annealing step is critical because it determines the specificity of the reaction. If the annealing temperature is too high the primers do not anneal easily, and if the annealing temperature is too low, the reaction will result in lower PCR yield, primer mismatches, and nonspecific amplification.

- **Optimize temperature steps to minimize the ramping time**

The larger the temperature difference between two successive steps in a protocol, the longer the time required to reach the next target temperature. Shorten the run time by minimizing the difference between target temperatures in successive steps.

To minimize target temperature differences, add a temperature gradient (Step 6 on page 23). Begin by optimizing the difference between the annealing and extension temperatures. Use the results of this gradient experiment to determine the highest possible annealing temperature that does not sacrifice the PCR yield. See “Optimizing Temperature Steps With a Gradient” (page 55) for an example. Finally, choose an annealing temperature with the smallest temperature difference from the extension temperature.

- **Minimize the final extension step**

The final extension step completes the synthesis of amplicons. Optimize this step when you need a high percentage of full-length amplicons at the end of the PCR. During each cycle, the extension step is typically 30 seconds. If amplification for 30 seconds is sufficient during cycling, then a longer final extension step is unnecessary.

To minimize the final extension step (Step 9 on page 26), choose a hold-time between 30 seconds and 2 minutes for targets between 100 and 1,000 bp. Then test the hold time you choose for sufficient amplification. Alternatively, add an increment (page 28) to the extension step to test for the best hold time.

- Minimize the number of repeats in a GOTO step.

Minimize the repeats in the GOTO step to minimize the number of cycles in the protocol. Before you adjust the number of cycles you must know the approximate concentration of PCR template.

To approximate the concentration of an unknown template, start with 30 to 45 cycles the first time you run the protocol. Then, detect the PCR product in a gel stained with Ethidium Bromide, and estimate the starting concentration. If the concentration is sufficient, then shorten the number of cycles by 5 and run the protocol again. Once the concentration of the target sequence is known, minimize the number of cycles in the protocol until the concentration of the PCR product is too low. Choose the best number of GOTO repeats from the results of these reactions.

Optimizing Temperature Steps With a Gradient

For an efficient reaction and clean product, optimizing the annealing step is critical. Use a gradient to optimize the temperature of the annealing step. In addition, use a gradient step to optimize other temperature steps.

Follow these instructions as a suggested approach to optimizing the annealing temperature:

1. Calculate the predicted annealing temperature based on the melting temperature (T_m) of the primers and template.

Use a T_m calculator to find the T_m of any combination of primers and template. When you run the S1000 thermal cycler under the control of a C1000 thermal cycler, use the T_a Calculator available onboard the C1000 thermal cycler.

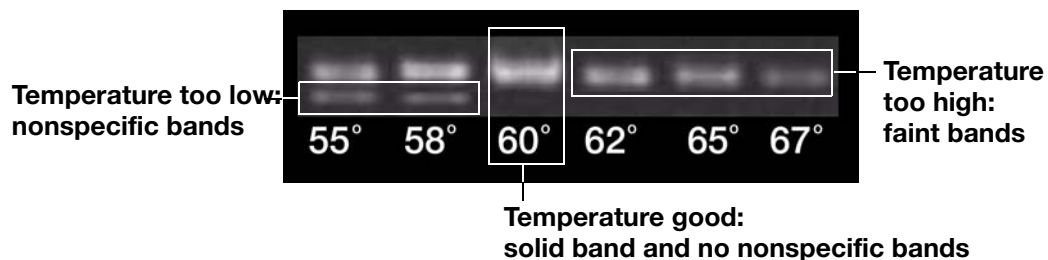
2. Create a PCR protocol with a gradient step in the annealing step.

Choose a gradient range that is as wide as possible to test for the optimal annealing temperature, and bracket the calculated T_a by 5 to 12°C. For example, if the calculated T_a is 55°C, then use a 20°C gradient to bracket that T_a . In this example, the gradient temperature range is from 45 to 65°C.

NOTE: The widest possible gradient on the S1000 thermal cycler is 24°C.

3. Choose a high annealing temperature from the results of the gradient PCR run. By choosing a high annealing temperature, you reduce the chance of nonspecific primer binding. Select a high annealing temperature from the results of a PCR experiment with a gradient in the run. See Step 2 on page 55 for details

about creating a gradient. In this example, the gradient ran from 55 to 67°C. The best annealing is found at 60°C.



As a starting place, use the highest annealing temperature and subtract 1 or 2°C. Don't use the absolute highest temperature. At higher temperatures, the yield decreases, resulting in faint bands. Furthermore, at high temperatures, the primers might behave inconsistently from PCR run to PCR run.

4. Narrow the temperature gradient in the annealing step (optional).

Run a narrower annealing temperature gradient using the results from the first, wider temperature gradient. Bracket the annealing temperature that you choose in Step 3 by 5°C. Choose the final annealing temperature from the highest successful temperature in the results for this gradient.

For example, if the highest successful annealing temperature in Step 3 is 60°C, then bracket that temperature with a narrower gradient ($\pm 5^\circ\text{C}$). In this example, the narrow gradient is from 55 to 65°C. Choose the final annealing temperature from a successful high temperature in the gradient experiment (as described in Step 3 on page 55).

Optimizing Runs With Small Sample Volumes

Running a protocol with a small volume requires optimization to prevent evaporation and condensation. Follow these suggestions for optimizing PCR with a sample volume below 10 μL . Read the following list for instructions about optimizing reactions with small sample volume:

- Control evaporation with a wax seal.
A wax such as Chill-out™ Liquid Wax, is the best seal for reducing evaporation. Further reduction is possible by adding a second seal, such as a cap or film in addition to the wax.
- Reoptimize the annealing temperature to prevent nonspecific priming, and increase target amplification.
When running an established protocol with a smaller sample volume (<10 μL) you might need to reoptimize the annealing temperature. If you observe mispriming or low amplification that is not a result of reagent problems, then adjust the protocol by optimizing the annealing temperature step by using a gradient (page 55).
- Lower the lid temperature to reduce of sample loss due to evaporation.
The heated lid prevents condensation from forming in the microplate or tube, which is critical when the sample volume is small. However, using the same lid temperature with a smaller sample volume can increase evaporation when the lid heat is the same. To prevent evaporation, lower the lid temperature and test the reaction for evaporation.

- Run the reaction in a 384-well reaction module.
The 384-well block is optimized for small sample volume, and therefore this is the best block for this application.

Transferring Protocols From Another Thermal Cycler

To transfer a PCR protocol from another thermal cycler to the S1000 thermal cycler with the same results, you might need to lower the ramp rate. If the results of identical reactions run on a thermal cycler with a slower ramp rate do not result in the same data, then change the ramp rate.

Follow these suggestions when transferring a protocol:

- Match the ramp rate of the other thermal cycler by changing the rate in each relevant step (page 31). In general, the ramp rate for the annealing step could be lowered when you move a protocol from a thermal cycler with a slower ramp rate to the S1000 thermal cycler.
- Increase the amount of Mg⁺⁺ in the reagents to help the primers anneal when the conditions have changed. This increase could cause some secondary PCR products, but should also increase the primary product.
- Adjust the temperature in each step to reoptimize the protocol. Most importantly, reoptimize the annealing temperature (page 55).

Troubleshooting PCR

This section is a quick guide for PCR troubleshooting options. For more detailed and extensive troubleshooting, go to the *PCR Doctor*. To find the PCR Doctor, open the Gene Expression Gateway (bio-rad.com/genomics) in a web browser window, and select *Support > Amplification Central > PCR Doctor*.

Follow these suggestions to re-optimize a reaction that fails by adjusting the protocol:

- Nonspecific PCR products, in addition to the target product.
Nonspecific products result from mispriming. When mispriming is not a result of a reagent problem, then adjust the annealing temperature.
Increase the annealing temperature to increase specificity of primer binding. To find the optimal annealing temperature, use a gradient (page 23).
- Nonspecific PCR products without the target product.
Nonspecific product with no target production is a result of complete mispriming. When the mispriming is not a result of a reagent problem, then adjust the hold time.
Increase the hold time in the annealing and extension steps to increase specificity of primer binding provide more time for complete extension.
- Low yield of the target PCR product.
Low PCR yield is a result of mispriming, an overly short extension hold time, or too high an annealing temperature. When the low yield is not a result of reagent problems, adjust the protocol.
Run a touchdown protocol (page 102) to increase amplification of the target product. Alternatively, decrease the annealing temperature, or run a gradient to optimize the annealing temperature (page 55).

Microplate, Tube, Sealer and Cap Supplies

The 1000 series thermal cyclers can run using a large variety of standard supplies, such as microplates, tubes, sealers and caps. The supplies listed in this section are a subset of supplies that are verified by testing. They function within the specifications of the S1000 thermal cycler thermal cycler. However, the S1000 thermal cycler is designed to work well with a broad range of standard supplies.

TIP: The composition and thickness of these supplies influences the outcome of a reaction. Whenever you significantly change the source or composition of supplies, it is good practice to reoptimize the protocol before running an important experiment.

Microplates, tubes, sealers, and caps come in a variety of compositions and colors. Bio-Rad recommends the standard supplies listed in Table 10 for compatibility with the 1000 series thermal cyclers. For a full list of available reagents and supplies, refer to the Life Sciences catalog, which is available in print by contacting your local Bio-Rad Laboratories office (page 3), or online (discover.bio-rad.com).

NOTE: Sealing wax, such as Chill-out™ is recommended to seal small sample volumes of less than 10 µL. Wax solidifies at room temperature. Pierce the solid wax with a micropipet tip to remove the sample. For more information about optimizing protocols for small sample volumes, see “Optimizing Runs With Small Sample Volumes” (page 56).

Table 10 lists the tested sealing options for microplates and tubes offered by Bio-Rad. However, if you have a verified source of these supplies, the S1000 thermal cycler is compatible with a wide variety of PCR supplies. Find a full list of supplies for PCR in any of these locations:

- Life Science Research Product catalog : See “Bio-Rad Resources and References” on page 3) for information about how to order a copy.
- Bio-Rad Life Science website (discover.bio-rad.com): Go to the web site and select *Products > Amplification*.
- Gene Expression Gateway (bio-rad.com/genomics): Open the website and select *Catalog Index > Amplification*.

In Table 10, each series of supplies is listed with the prefix of the catalog numbers:

Table 10: Compatible sealing options for PCR microplates and tubes

TUBES AND MICROPLATES		CAPS AND SEALERS	
Description (Catalog Prefix*)	Volume and Number	Description (Catalog Prefix*)	
Tubes			Caps (TCS*)
Individual tubes with caps (TFI* and TWI*)	0.2 ml		(Included)
Strip tubes (TBS*)	0.2 ml, 8- and 12-tube		Strip
Strip tubes, low profile (TLS*)	0.2 ml, 8-tube		Strip
<i>Microplates</i>		Film (MS*)	Caps (TCS*)

Table 10: Compatible sealing options for PCR microplates and tubes

TUBES AND MICROPLATES		CAPS AND SEALERS	
Unskirted and full height (MLP*)	24-, 48-, and 96-well	A, B, F	Strip
Unskirted and low profile (MLL*)	48- and 96-well	A, B, F, P	Strip
Skirted and full height (MSP*)	96- and 384-well	A, B, F	Strip
Skirted, full height, and hard-shell (HSP*)	96- and 384-well	A, B, F	Strip

* The prefix of the catalog number for each series of supplies.

6 Protocol Files and Folders

This chapter describes the content and management of protocol files. The **VIEW** and **FILES** options in the main menu provides quick access to the functions you need to view protocol file content and manage protocol files.

Read this chapter to learn about the following topics:

- Overview of the content of protocol files, including step options and instrument settings (page 61)
- Overview of the management of protocol files and folders with the **FILES** option (page 65)
- Manage protocol files, including how to copy, move, delete, and rename files (page 65)
- Secure protocol files in a folder by entering a password (page 71)
- Manage folders, including how to create, delete, secure, and rename folders (page 72)
- View protocol files with the **VIEW** option (page 75)

Protocol File Contents

The protocol file contains both temperature steps and instrument settings that can be used to optimize a PCR reaction. In the S1000 thermal cycler, the temperature (**TEMP**) and gradient (**GRAD**) protocol steps create the temperature steps found in a typical PCR protocol. However, the **GOTO** and **END** protocol steps differ. Read this section for detailed information about the content and structure of S1000 thermal cycler protocol files:

- Overview of the four types of protocol steps (page 61)
- Overview of instrument options that can be added to a protocol step (page 64)

Protocol Steps

A PCR protocol includes temperature steps, where the sample is kept at a target temperature for a specific amount of time (called the hold time). In the S1000 thermal cycler protocol file, these temperature steps are called temperature (**TEMP**) and gradient (**GRAD**) steps. A gradient step is a specialized temperature step that includes a temperature gradient across the block.

In general, a PCR protocol includes these types of steps:

1. An initial temperature step

This initial temperature step denatures the template, and also activates the polymerase.

To add an initial step in an S1000 thermal cycler protocol file, add a temperature step (page 22).

2. Cycle of several temperature steps that amplify the PCR product

These steps repeat in a cycle that amplifies the PCR product. Typically the cycle includes three temperature steps called denaturing, annealing, and extension steps. These temperature steps denature the DNA template, anneal the primer to the template, and allow the polymerase to extend the complementary strand of DNA. However, under certain conditions the annealing and extension steps are combined in a shorter two-step cycle.

To add the denaturing, annealing, or extension steps in the S1000 thermal cycler, add a temperature step (page 22) or gradient step (page 23). To cycle these temperature steps, add a GOTO step immediately after the last step that you want to repeat in the cycle (page 25).

3. A final temperature step to extend the PCR product

This final extension temperature step serves to finish extending any PCR product that did not fully elongate during the cycles.

To add a final step in an S1000 thermal cycler protocol file, add a temperature step (page 26).

Protocol steps on the S1000 thermal cycler vary from the typical PCR protocol steps. When you transfer a typical PCR protocol to the S1000 thermal cycler, you translate them into the four S1000 thermal cycler protocol steps:

- Temperature (TEMP), which is the same as a typical PCR protocol step.
- Gradient (GRAD), which is a temperature step that includes a gradient.
- Goto (GOTO), which is used to add repeats and create cycle in PCR.
- End (END), which instructs the thermal cycler to end the protocol.

The following is a composite screen showing an S1000 thermal cycler protocol with all four types of protocol steps:

Protocol name	New: STD3GRAD	Lid: 100°C	Vol: 20µL	
	1= 95.0° FOR 4:00			Temperature step (includes a gradient step)
Gradient step	2= 95.0° FOR 0:30			
	3= 55.0/75.0, 0:30			
GOTO step	4= 72.0° FOR 0:30			
	5= GOTO 2, 30 TIMES			
END step	6= 72.0° FOR 7:00			Temperature step
	7= END			

Table 11 describes the four S1000 thermal cycler protocol steps with the name, parameters, limits, and function:

Table 11: S1000 thermal cycler protocol steps and parameters.

Step Name	Parameters and Ranges	Description
TEMP (temperature)	TEMP °C: The target temperature between 0.0 and 100.0 °C in tenths of a degree.	A temperature step that instructs the thermal cycler to ramp to the specified target temperature and hold that temperature for the specified amount of time (see Step 5 on page 22).
	TIME: The hold time between 1 second and 18 hours in the format of hours:minutes:seconds. To enter an infinite hold (Forever), press the ∞ (Infinite, 0) key.	
GRAD (gradient)	Lower: The lower temperature in the gradient. Enter a number between 30.0 and 99.0 °C in tenths of a degree. Upper: The upper temperature in the gradient. The maximum temperature is 100°C. Enter a temperature within 24.0 °C of the Lower temperature.	A temperature step that instructs the thermal cycler to ramp to the specified target temperature gradient across the block and hold that temperature gradient for the specified amount of time (see Step 6 on page 23).
	TIME: The hold time between 1 second and 18 hours in the format of hours:minutes:seconds. To enter an infinite hold (Forever), press the ∞ key (zero, 0) key.	
GOTO	GOTO STEP: The step number of the first step in the repeat.	A protocol step that instructs the thermal cycler to repeat a set of steps for the specified number of times (see Step 8 on page 25). NOTE: The total number of cycles in the protocol is the number of GOTO repeats, plus the first cycle.
	ADDNL REPEATS: The number of additional times that the steps repeat.	
END	(No parameters)	A protocol step that instructs the thermal cycler to finish the protocol (see Step 10 on page 27).

Options for Temperature and Gradient Steps

Each S1000 thermal cycler temperature or gradient step can include options. Most of the options add parameters to the protocol step while creating or editing the protocol steps. The following shows the four options that apply to temperature steps:

INC EXT RATE BEEP

The following shows the two options that apply to gradient steps. Only the **EXT** option modifies the parameters in a gradient step:

PREVIEW EXT

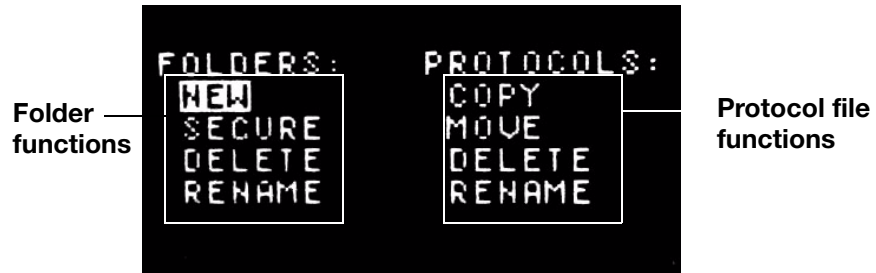
Table 12 describes all five options for temperature and gradient steps, and includes the limits and range of the parameters:

Table 12: Options for temperature and gradient steps

Option	Parameter and Range	Description
INC (increment)	A temperature from -10.0 to 10.0°C per cycle in tenth of a degree	Applies only to a temperature step (page 28). Instructs the thermal cycler to increment (change) the target temperature of a step with each cycle, where a positive number increases the temperature, and a negative number decreases the temperature.
EXT (extend)	A time from -60 to 60 seconds per cycle	Applies to both temperature and gradient steps (page 30). Instructs the thermal cycler to extend the hold time with each cycle, where a positive number increases the hold time, and a negative number decreases the hold time.
RATE (thermal ramp rate)	A number from 0.1 to 5°C/second	Applies only to a temperature step (page 31). Instructs the thermal cycler to ramp to the target temperature at the specified ramp rate in that step. Applies only to the Dual 48 and 96 Fast reaction modules.
BEEP	(No parameters)	Applies only to a temperature step (page 32). Instructs the thermal cycler to beep as a signal that the thermal cycler reached the target temperature of that step.
PREVIEW	(No parameters)	Applies only to a gradient step (Step 6 on page 23). Instructs the thermal cycler to displays the temperature gradient on the block.

Managing protocols With the FILES Option

The FILES option contains all the functions you need to manage protocol files and the folders that contain them. Select the FILES option from the main menu to see these functions:



Notice that there are two lists of options. Use the options listed below **FOLDERS** to manage folders (page 72). Use the options listed below **PROTOCOLS** to manage protocol files (page 65). Table 13 describes all functions in the **FOLDERS** and **PROTOCOLS** lists.

Table 13: List of functions in the FILES option.

Function	Description
PROTOCOLS:	
COPY	Copy an existing protocol file and save it with a new name. For instructions, see page 66.
MOVE	Move an existing protocol file to another folder. For instructions, see page 67.
DELETE	Delete an existing protocol file. For instructions, see page 68.
RENAME	Rename an existing protocol file. For instructions, see page 69.
FOLDERS:	
NEW	Create a new folder. For instructions, see page 72.
SECURE	Protected files can be deleted and edited but only after the password protecting them is used. For instructions, see page 71.
DELETE	Delete an empty folder. For instructions, see page 73. NOTE: A folder cannot be deleted if it contains protocol files.
RENAME	Rename an existing folder. For instructions, see page 74.

Copying, Moving, Deleting, and Renaming Protocol Files

The FILES option lists all the functions available for managing protocol files. This section provides step-by-step instructions for using those functions:

- Select **COPY** to copy a protocol file (next section, page 66)
- Select **MOVE** to move a protocol file to another folder (page 67)

- Select **DELETE** to delete a protocol file (page 68)
- Select **RENAME** to rename a protocol file (page 69)

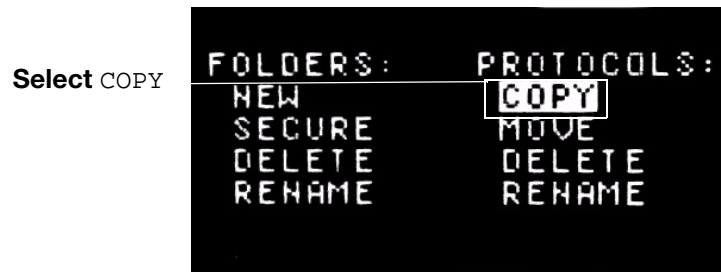
Copying a File With the **COPY** Function

To begin copying a protocol file, select **FILES > COPY**. The **COPY** function makes a copy of the existing file and requires you to enter a new file name for the copy. You can also move the protocol to a new folder. For example, move secured protocols or preinstalled protocols to your folder by copying them.

To copy a protocol file, follow these instructions:

1. Select **COPY**

To select **COPY**, press the arrow keys, then press **ENTER** to confirm the selection and continue to the next screen.



2. Select the protocol file to copy

Press the arrow keys to select the folder that contains the protocol file, then press the right arrow key to select a file. Press **ENTER** to continue to the next screen.

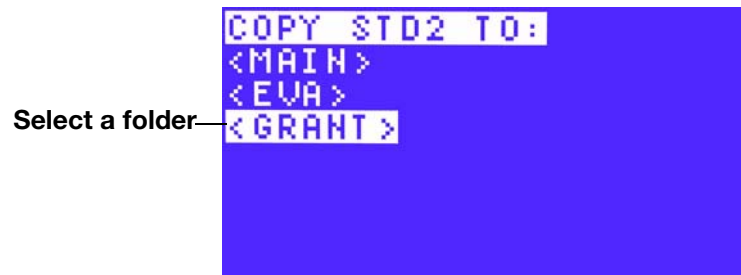
In this example, the **STD2** file is selected in the **MAIN** folder:



3. Select the folder where you want to store the copy

Press the arrow keys to select a folder where you want to store the copied protocol file. Press **ENTER** to confirm the selection.

In this example, the copy will be put into the `GRANT` folder:



TIP: If the folder you select is secure, enter the password to copy a file into that folder.

4. Enter a name for the copied file

Type a new name for the protocol copied file. Press the up and down arrows to select letters, and press the number keys to type numbers. Then press `ENTER` to accept the selection.

NOTE: A protocol file name can contain from one to eight characters. The characters are numbers or capital letters. Each protocol name must be unique.

In this example, the name of the copied protocol is `STD2GR`:



TIP: After copying the protocol file, make changes to the file by selecting `EDIT` in the main menu (page 34).

Moving a File With the `MOVE` function

To move a protocol file to another folder, select `FILES > MOVE`. Moving a file is useful when you want to move protocol files stored in the main menu to a folder you created.

TIP: To secure a protocol file that was saved in the `MAIN` folder, move the file to a folder that you created (page 72) and secured (page 71).

To move a protocol file, follow these instructions:

1. Select **MOVE**

To select **MOVE**, press the arrow keys. Then press **ENTER** to confirm the selection and continue to the next screen.



2. Select the file that you want to move

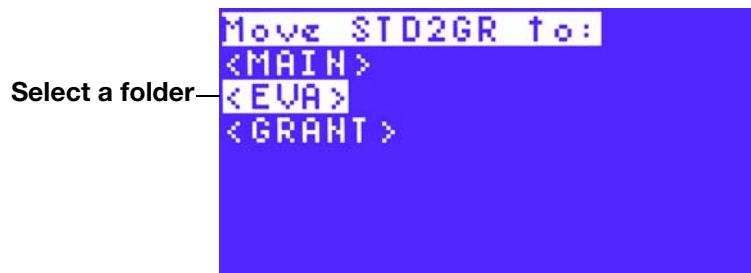
Press the arrow keys to select the folder that contains the protocol file. Then press the right arrow key to begin selecting the file within that folder. Finally, press **ENTER** to confirm the file selection. In this example, the **STD2G** protocol is selected:



NOTE: If the folder is secure, then you must enter a password to move the file.

3. Select the folder where you want to move the file

Press the up and down arrow keys to select the folder where you want to move the file. Then press **ENTER** to confirm the move. In this example, the file is moved to the **EVA** folder:



NOTE: If the folder is secure, then you must enter a password to move the file.

Deleting a File With the **DELETE** Function

Select **FILES > DELETE** to delete a protocol file when you want to empty a folder or remove extra copies of files.

To delete a protocol file, follow these instructions:

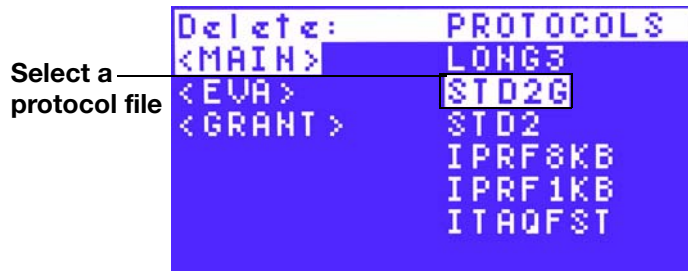
1. Select DELETE

To select DELETE, press the arrow keys. Then press ENTER to confirm the selection and continue to the next screen.



2. Select the file

Press the up and down arrow keys to select a folder, and then press the right arrow key to select the files in that folder. Finally, press ENTER to continue to the next screen. In this example, the STD2G file is selected in the MAIN folder:



NOTE: If the protocol file is in a secure folder, you must enter a password to delete a file.

3. Confirm the deletion

Select YES or NO. Select YES and press the ENTER key to delete the file and return to the main menu. Select NO to cancel the deletion.

WARNING! Deleting a file permanently removes all records of the file. Deleted protocol files cannot be retrieved. Protocol names must be unique, but once deleted a file name can be reused.

In this example, DELETE is selected:



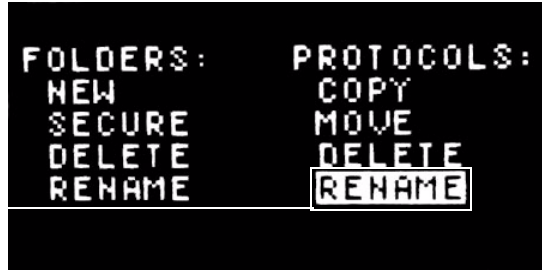
Renaming a File With the RENAME Function

Select FILES > RENAME to rename an existing protocol file when you have edited the protocol steps and you want the name to reflect the change. To rename a protocol file, follow these instructions:

1. Select **RENAME**.

To select **RENAME**, press the arrow keys. Then press **ENTER** to confirm the selection and continue to the next screen.

Select **RENAME**

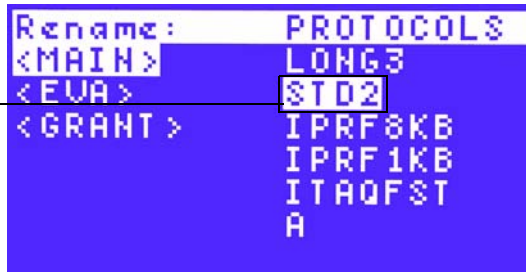


2. Select a file to rename.

Press the up and down arrow keys to select a folder. Then press the right arrow key to begin selecting a file. Finally, press the **ENTER** key to confirm the selection.

In this example, the **STD2** file is selected:

Select a protocol file



TIP: If the folder that contains the file is secure, then you must enter the password to rename the file.

3. Enter a new protocol file name.

Press the up and down arrows to select letters, and press the number keys to type numbers. Then press **ENTER** to accept the new name and return to the main menu.

NOTE: A protocol file name can contain from one to eight characters. The characters are numbers or capital letters. Each protocol name must be unique to all folders on the S1000 thermal cycler.

In this example, the new protocol name is **STD222**.

Enter a new file name



Protecting Files in a Folder With the *SECURE* Function

To protect all the files in a folder, select `FILES > SECURE` to create a new password or change an existing password. Securing a folder protects protocol files stored in that folder. To edit, move, or delete files stored in a secure folder a password must be entered. However, viewing, copying, and running protocol files that are located in a secure folder does not require a password.

To secure a folder, follow these instructions:

1. Select *SECURE*

To select *SECURE*, press the arrow keys. Then press `ENTER` to confirm the selection and continue to the next screen.



2. Select a folder to secure

Press the up and down arrow keys to select the folder you want to secure with a password.

In this example, the `GRANT` folder is selected:



TIP: When you want to change a password, follow the same instructions you used to create the password. When you change a password, you will be required to enter the original password before you can change it.

NOTE: If the password is lost, then protocols in the secured folder cannot be deleted, moved, or edited. Furthermore, the folder cannot be deleted. However, the protocols in a secure folder can be copied to another folder.

3. Enter a new password

Press the number keys to type a number. Then press `ENTER` to confirm the password and return to the main menu.

In this example the password is 222:



NOTE: A password can contain from one to four numbers, and cannot contain letters.

TIP: To disable security for a folder, repeat steps 1-3, and specify a blank, new password.

Creating, Deleting, and Renaming Folders

The S1000 thermal cycler stores protocol files in the `MAIN` folder by default. If you want to store the files in another location a folder must be created. This section describes how to manage folders by creating, deleting, and renaming them.

In a folder, the protocol files are listed in the order that they were created. Therefore, storing all files in the `MAIN` folder can result in a long, unordered list of files, making it difficult to find a file. Alternatively, storing protocol files in a user-created folder has these advantages:

- Locate your protocol files easily, without sorting through a long list of protocol files.
- Password-protect your protocol files by storing them in a secure folder (page 71). Secure folders prevent other instrument users from editing, deleting, or moving your files (page 71). Securing protocol files in a folder does not prevent other instrument users from viewing, running, or copying the files.

TIP: If you have not yet created a folder, then you can temporarily store a new protocol file `MAIN` folder. After you create your folder, select `MOVE` (page 67) to transfer your protocol files to that folder.

This section provides step-by-step instructions about managing folders:

- Select `CREATE` to make a new folder (next section, page 72)
- Select `DELETE` to delete a folder (page 73)
- Select `RENAME` to change the name of an existing folder (page 74)

Creating a New Folder With the `NEW` Function

Select `FILES > NEW` to create a new folder when you want to store a set of protocol files in an easy-to-find location.

NOTE: The S1000 thermal cycler can contain 11 folders in addition to the `MAIN` folder.

To create a new folder, follow these instructions:

1. Select NEW

NEW is the default selection when you first open the FILES menu. If NEW is not selected, press the arrow keys to select it. Then press ENTER to confirm the selection and continue to the next screen.



NOTE: The S1000 thermal cycler can contain up to 12 folders including the MAIN folder.

2. Type the folder name

Press the up and down arrows on the keypad to select a letter, and press the number keys to type a number. Press ENTER to accept a selection and move to the next space. When you complete the name, press ENTER to accept the name and return to the main menu.

NOTE: A folder name can contain from one to eight characters. The characters are numbers or capital letters. Each folder name must be unique to the S1000 thermal cycler.

In this the folder name of the new folder is ANNA:



TIP: To secure the new folder and all files in that folder, follow the instructions on page 71.

Deleting a Folder with the DELETE Function

Select FILES > DELETE to completely remove an existing folder when it is no longer needed. Alternatively, rename the folder to continue using it for a different purpose (page 74).

WARNING! Deleting a folder permanently removes it.

NOTE: A folder that contains protocols will not be deleted. Before you delete a folder, select VIEW in the main menu to view the contents of the folder (page 61). To delete a folder, first empty the folder by moving or deleting all protocol files (page 65).

To delete a folder, follow these instructions:

1. Select **DELETE**

To select **DELETE**, press the arrow keys. Then press **ENTER** to confirm the selection.



2. Select a folder to delete

Press the up and down arrow keys to select a folder. Then press **ENTER** to continue to the next screen. In this example the **ANNA** folder is selected:



TIP: If the folder is secure, you must enter the password to delete it.

3. Confirm the deletion

Press the arrow keys to select **YES** or **NO**. Select **YES** to delete the folder or select **NO** to cancel the deletion. Then press **ENTER** to confirm your selection and return to the main menu.

NOTE: A folder that contains protocol files will not be deleted.

Renaming a Folder With the **RENAME** Function

Select **FILES > RENAME** to rename an existing folder. Renaming a folder does not change the protocol files stored in that folder. To rename a folder, follow these instructions:

1. Select **RENAME**

To select **RENAME**, press the arrow keys. Then press **ENTER** to confirm the selection and move to the next screen.



2. Select a folder to rename

Press the up and down arrow keys to select the folder that you want to rename. In this example, the *ANNA* folder is selected:



3. Type the new name of the folder

Press the up and down arrows to select letters, and press the number keys to type numbers. Then press *ENTER* to confirm a selection and move to the next space in the name. When the name is complete, press *ENTER* to confirm the name and return to the main menu.

NOTE: A protocol file name can contain from one to eight characters. The characters are numbers or capital letters. Each protocol name must be unique.

NOTE: To rename a folder that is secure, enter the password before typing the name.

Viewing Protocol Files With the *VIEW* Option

To view the content of protocol files and folders, select the *VIEW* option in the main menu. Follow these instructions to view files and folders:

1. Select *VIEW*

To select *VIEW* in the main menu, press the arrow keys. Then press *ENTER* to confirm the selection and continue to the next screen.



2. Select a folder to view

Press the up and down arrow keys to select a folder. Once the folder is selected, the right side of the screen lists all the protocol files in that folder.

In this example, the MAIN folder is selected:



3. Select a protocol file to view

Press the right arrow keys to begin selecting a protocol file within the folder. Then press the up and down arrows to select a protocol file. Finally, press ENTER to continue to the next screen.

NOTE: If the folder lists more than 6 files, view the full list of files by pressing the down arrow key.

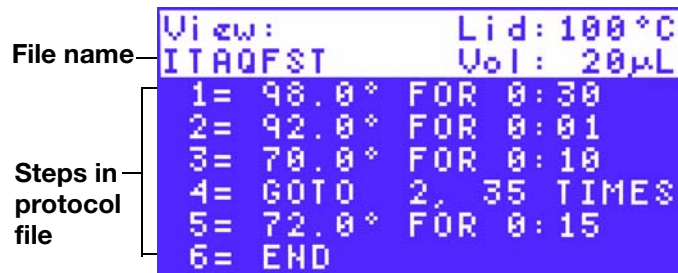
In this example, the ITAQFST file is selected:



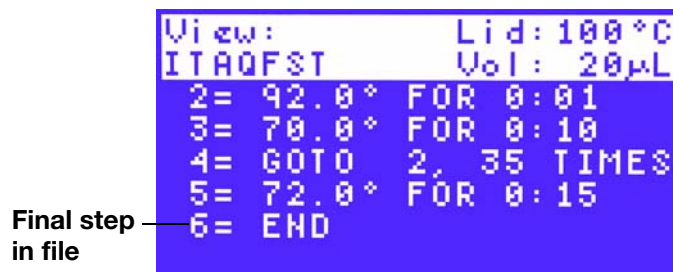
4. View the protocol file

Press the ENTER key or the up and down arrow keys to scroll through the protocol file until the END step.

In this example, all six steps in the protocol fit on one screen:



View the end of the protocol:



- 5. Press ENTER or CANCEL again to finish viewing the file**
Press the ENTER key to finish viewing the protocol and return to the main menu.

7 Advanced Tools and Functions

The S1000 thermal cycler includes several advanced tools for stand alone and remote control. The `TOOLS` option provides a list of tools, including default settings and port settings. Through USB or serial (RS232) connections, the S1000 thermal cycler can also be controlled externally by the C1000 thermal cycler or by a robotic system.

This chapter describes the tools listed in the `TOOLS` option and introduces the options for controlling the S1000 thermal cycler with another instrument:

- Select the `TOOLS` option to view or change instrument settings (below)
- Control the S1000 thermal cycler with the C1000 thermal cycler and software, through a USB port (page 88)
- Run the S1000 thermal cycler under automated robotic control through a USB or serial port (page 90)

The `TOOLS` Options

The `TOOLS` option in the main menu provides a list of instrument settings and tools. Select the `TOOLS` option in the main menu to see the list of tools:



The `TOOLS` include the following functions:

- `LAST RUN`: View the last protocol that was run (page 80)
- `SELFTEST`: Run a self test on the thermal cycler (page 81)
- `VERSION`: View the current instrument firmware version (page 82)
- `NAME`: Enter a name for the thermal cycler (page 82)
- `DEFAULTS`: Change default lid temperature, “turn off below” feature, and sample volume (page 83)

- GRADCALC: **View a temperature gradient based on user defined parameters (page 84)**
- CONTRAST: **Change the instrument's LCD contrast (page 86)**
- PORT: **[Change the port used to control the S1000 thermal cycler remotely (page 87)**

View the LAST RUN Tool

When you want to see a synopsis of parameters in the last protocol that was run on the S1000 thermal cycler, select `TOOLS > LAST RUN`.

NOTE: For Dual 48 reaction modules, the last run tool toggles to show the last protocol that was run on each block.

Follow these instructions to view the last protocol that was run:

1. Select LAST RUN

The `LAST RUN` option is the default selection when you open the `TOOLS` option. If this option is not selected, press the arrow keys to select it. Then press `ENTER` to confirm the selection and continue to the next screen.



2. View the LAST RUN screen

The `LAST RUN` screen shows a synopsis of the parameters in the last protocol that was run. Press `ENTER` to return to the `TOOLS` option.

Notice that the parameters listed identify the protocol and any settings that could influence the data from that run. The `LAST RUN` screen displays the following parameters:

- Name of the protocol
- Lid temperature (page 33) during the run
- Turn off below temperature (page 33) for the lid during the run
- Sample volume which determines the temperature control mode during (page 33)
- Total run time
- Number of errors detected during the run
- Firmware version on the S1000 thermal cycler during the run

In this example, the last protocol run is *STD2* and the firmware version is 2.0.96:

	Protocol name	
Lid temperature	LAST RUN: <i>STD2</i>	Turn of below temperature
Sample volume	LID: <i>100.30</i>	
Total run time	VOLUME: <i>10</i>	
	TOTAL TIME: <i>0:23</i>	
	ERRORS: <i>None</i>	
Firmware version	VERSION: <i>0.2.0.96</i>	

TIP: If you want to run this protocol again, note the name of the protocol, then select the *MAIN > RUN* and select the protocol by name.

Test the Instrument With the *SELFTEST* Tool

Select the *TOOLS > SELFTEST* tool to instruct the thermal cycler to run a test to check that it is running within specifications. The S1000 thermal cycler automatically runs a self test every time it starts. During the *SELFTEST* protocol, the instrument checks that it is working correctly before a run. Follow these instructions to manually run a self test:

1. Select *SELFTEST*

To select *SELFTEST*, press the arrow keys. Then press *ENTER* to continue to the next screen.

Select <i>SELFTEST</i>	<pre> TOOLS: LAST RUN DEFAULTS SELFTEST GRADCALC VERSION CONTRAST NAME PORT </pre>
------------------------	---

2. View the self testing screen

The thermal cycler displays the following self testing screen while it runs the tests. You will also hear the instrument fans turn on and off during the test. When the test has successfully completed, the main menu displays.

```

SELF-TEST IN PROGRESS
>***          ***<
        
```

Check the Firmware Version With the VERSION Tool

When you want to check the firmware version that is currently on the thermal cycler, select the `TOOLS > VERSION` tool. Follow these instructions to view the version:

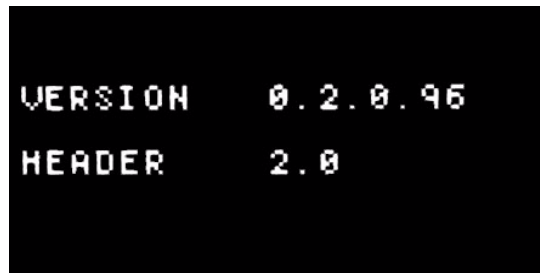
1. Select `VERSION`

To select `VERSION`, press the arrow keys. Then press `ENTER` to continue to the next screen.



2. View the firmware version

View the firmware version on this screen. Additionally, the header version of the alpha is also shown, which means the firmware expects at least the specified version. Press `ENTER` to return to the main menu.



Name the Thermal Cycler with the NAME Tool

To name or rename the thermal cycler, select the `TOOLS > NAME` tool. The S1000 thermal cycler name is displayed on the main menu. Follow these instructions to name the thermal cycler or change the existing name:

1. Select `NAME`

To select the `NAME`, press the arrow keys, then press `ENTER`.



2. Enter a name

Enter a name by pressing the UP & DOWN ARROWS to select a letter, and pressing the number keys to type a number. Then press ENTER to accept the selection and continue to the next character in the name.

NOTE: A cyclor name can contain from one to eight characters. The characters can be numbers or capital letters.

In this example, the letter M is entered by pressing the UP ARROW 12 times:



The name or serial number identifies the S1000 thermal cyclor when it is controlled by the C1000 thermal cyclor (page 88). The thermal cyclor name appears in the C1000 thermal cyclor instrument tree or in the 1000 series software instrument tree. If the S1000 thermal cyclor is not named, then the S1000 thermal cyclor serial number will be used.

3. Confirm the name

Press the ENTER key to confirm the thermal cyclor name and to return to the main menu.

**Change Default Parameters With the DEFAULTS Tool**

When you want to change the default parameters on the thermal cyclor, select *TOOLS* > *DEFAULTS*. These three parameters apply for a new protocol or instant incubation:

- **Lid Target:** Sets the default lid temperature for new protocols (page 33)
- **Turn off below:** Sets the temperature at which the lid heats to 31°C during a protocol run (page 33)
- **Sample Vol:** Sets the default sample volume (Vol) in a new protocol (Step 4 on page 21)

Follow these instructions to view and change these default parameters:

1. Select DEFAULTS

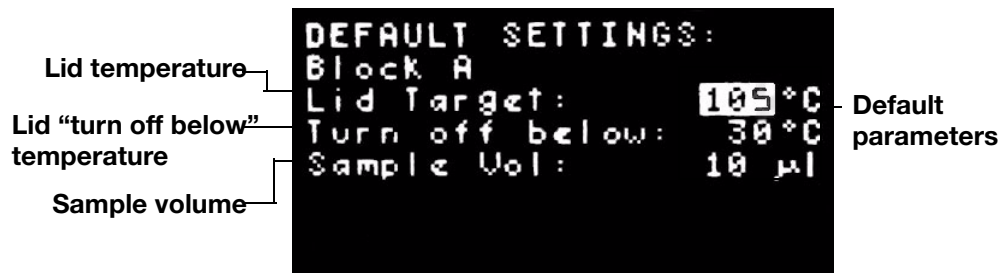
Press the arrow keys to select DEFAULTS. Then press ENTER to continue to the next screen.



2. Change the default settings (optional)

Press the up and down arrow keys to select a setting.

This example shows the system default parameters for the S1000 thermal cycler:



NOTE: The DEFAULT SETTINGS have the following ranges that apply to new protocols.

- Lid Target temperature setting can range from 0 to 110°C. The default is set to 105°C.
- When the block is running an infinite hold at a temperature below the Turn off below parameter, the lid heater maintains 31°C.
- Sample Vol setting can range from 0 to 50 uL (or 30 uL for 384-well reaction modules) and the default system parameter is 10 uL.
- Press the number keys to enter changes to the parameters. Press ENTER to confirm the number, and move to the next parameter.

3. Save the changes

Select YES or No. Select YES and press ENTER to save the parameters.

Alternatively, press the right arrow key to select No, and press ENTER to reject changes and return to the main menu.

View a Temperature Gradient with the GRADCALC Tool

If you want to review a temperature gradient to determine the temperature in each row, select the TOOLS > GRADCALC tool. Select this tool to recreate the temperature in each row of wells across a block. In the S1000 thermal cycler, the gradient is distributed from the front (Row H; coolest temperatures) to the back (Row A; hottest temperatures) of the block.

Follow these instructions to view a gradient:

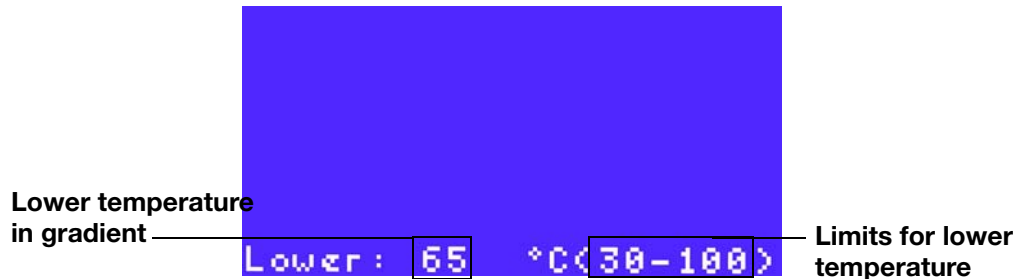
1. Select GRADCALC

To select GRADCALC, press the arrow keys. Then press ENTER to continue to the next screen.



2. Enter the lower temperature in the gradient

Press the number keys to type the lower gradient for the back row of the block. Press the CANCEL (.) key to type a decimal point. The limit is 30.0 to 99.0 degrees. In this example, the lower temperature is 65°C:



3. Enter the upper temperature in the gradient

Press the number keys to type the upper gradient for the back row of the block. Press the CANCEL (.) key to type a decimal point. The upper temperature must be greater than the lower temperature, and be within 24.0°C of the lower temperature.

In this example the upper temperature is 75°C:

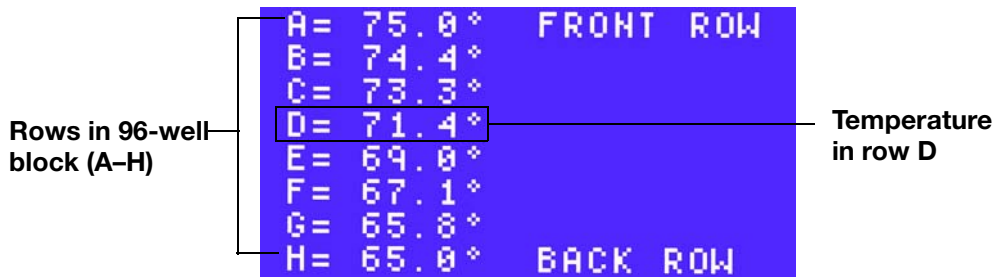


NOTE: The upper temperature is limited to a temperature that is within the widest possible gradient. The widest possible gradient is 24°C.

4. View the temperature gradient.

The temperature gradient is displayed as the temperature in each row of wells in the block.

In this example, the temperature in row D of this 96-well block is 71.4°C:



NOTE: The temperature in the middle rows is estimated. The estimate is based on the temperatures in the front and back rows and on the number of rows in the block.

Changing Screen View with the CONTRAST Tool

Select the **TOOLS > CONTRAST** tool to change the contrast of the LCD when you want to improve the visibility of characters displayed on the screens.

To change the **CONTRAST**, follow these instructions:

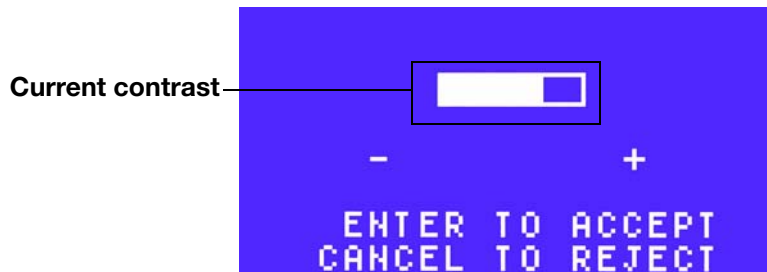
1. Select **CONTRAST**

To select **CONTRAST** press the arrow keys. Then press **ENTER** to continue to the next screen.



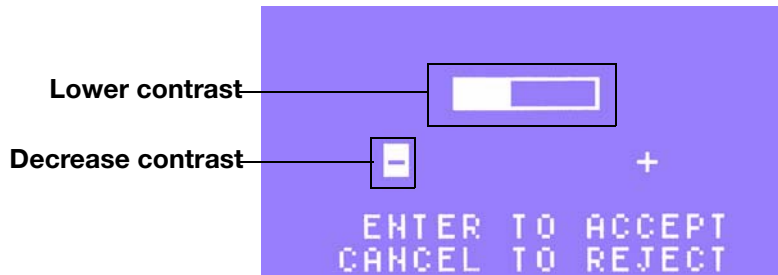
2. Adjust the contrast

Press the right and left arrow keys to adjust the contrast.

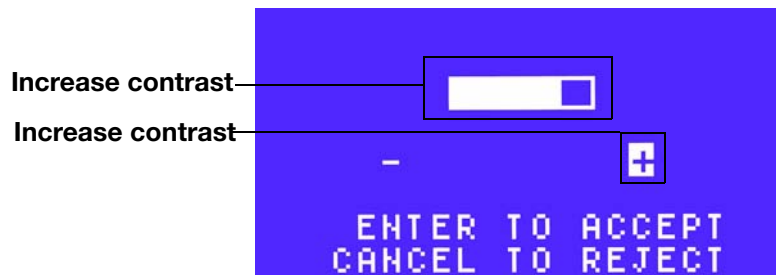


Press the left arrow key to decrease (-) the contrast.

In this example, the contrast is decreased:



Press the right arrow key to increase (+) the contrast. In this example, the contrast is increased:



3. Accept or reject the contrast.

Press the `ENTER` key to accept the changes and return to the `TOOLS` options. If you want to return to the original contrast, press the `CANCEL` key to reject the changes.

Change Settings With the `PORT` Tool

When you set the S1000 thermal cycler for remote control, select the port that you want to use for data transfer with the `PORT` tool. Select `TOOLS > PORT` tool to instruct the thermal cycler to list the data transfer ports (page 8). To select a port, follow these instructions:

1. Select `PORT` tool.

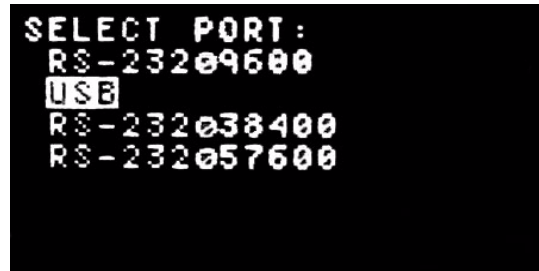
To select `PORT`, press the arrow keys. Then press `ENTER` to continue to the next screen.



2. Select a port.

Press the up and down arrow keys to select a port from the list of all ports on the thermal cycler. Then press ENTER to return to the `TOOLS` option.

Select a port



Control an S1000 Thermal Cycler With the C1000 Thermal Cycler

Up to three S1000 thermal cyclers can run under the control of a C1000 thermal cycler. The S1000 thermal cycler(s) can connect directly to the C1000, or in a “daisy chain” configuration, where the S1000 thermal cycler is connected, in series, to a C1000 thermal cycler.

When connected to a C1000 thermal cycler, the S1000 thermal cycler can be controlled by:

- The C1000 thermal cycler control panel
Connect the S1000 thermal cycler directly or indirectly to the C1000 thermal cycler through a USB cable, then program and run protocols using the C1000 thermal cycler user interface.
- The 1000 series Manager software
Connect the C1000 thermal cycler to a computer through a USB cable, then control the C1000 thermal cycler and any connected S1000 thermal cyclers with the 1000 series software.

The following three sections describe how to connect and operate an S1000 thermal cycler using a C1000 cycler:

- Connect directly to a C1000 thermal cycler via the S1000 USB B port (below)
- Connect indirectly to a C1000 thermal cycler via the S1000 USB A port (page 89)
- Operating the S1000 thermal cycler while under the control of the C1000 thermal cycler (page 90)

For detailed instructions about running the S1000 thermal cycler when it is connected to a C1000 thermal cycler see the instructions in the C1000 thermal cycler manual, or the 1000 series software online help or manual.

Connecting Directly to a C1000 via the S1000 USB B Port

To connect the S1000 thermal cycler directly to a C1000 thermal cycler follow these instructions:

1. **Plug a USB cable into the S1000 thermal cycler**
Plug a high quality, shielded USB cable into the USB B port on the back of the S1000 thermal cycler (page 8). For USB cable part numbers, see page 111.
2. **Plug the USB cable into the C1000 thermal cycler**
Plug the other side of the USB cable into a USB A port on the back of the C1000 thermal cycler. The C1000 thermal cycler will detect the S1000 thermal cycler.
3. **Connect additional S1000s directly to the C1000**
Repeat steps 1 and 2, to connect up to three S1000s directly to the same C1000.
4. **Open the MAIN screen or instrument tree on the C1000 thermal cycler**
Open the MAIN screen or instrument tree on the C1000 thermal cycler, or in the 1000 series software if the C1000 thermal cycler is connected to a computer.
5. **Select the S1000 thermal cycler**
Select the S1000 thermal cycler by serial number or name. If the S1000 thermal cycler has a name (page 82), then the name will be displayed instead of the serial number.

Connecting Indirectly to a C1000 Thermal Cycler via the S1000 USB A Port

The S1000 thermal cycler can function under the control of the C1000 thermal cycler even when it is connected indirectly. To accomplish this follow these instructions:

1. **Plug a shielded USB cable into the S1000 thermal cycler**
Plug a high quality, shielded USB cable into the USB B port on the back of a S1000 thermal cycler (page 8). A shielded USB cable can be ordered directly from Bio-Rad.
2. **Plug the USB cable into the C1000 thermal cycler**
Plug the other side of the USB cable into a USB A port on the back of the C1000. The C1000 thermal cycler will detect the first S1000 thermal cycler.
3. **Connect another S1000 thermal cycler indirectly to the previous S1000**
Plug a high quality, shielded USB cable into the USB B port on the back of another S1000 thermal cycler. Plug the other end of the cable into a USB A port on the previous S1000 thermal cycler (the S1000 connected directly to the C1000).
4. **Connect an additional S1000 indirectly to the C1000**
By repeating steps 1-3 you may connect up to three S1000s indirectly to the same C1000.
5. **Open the instrument tree**
Open the instrument tree on the C1000 thermal cycler or in the 1000 series software.
6. **Select the S1000 thermal cycler**
Select the S1000 thermal cycler by serial number or name. If the S1000 thermal cycler has a name (page 82), then the name will display instead of the serial number.

Operating the S1000 Thermal Cycler While Under C1000 Thermal Cycler Control

When the S1000 thermal cycler is under the control of the C1000 thermal cycler, it is in “Semi-lock down mode”.

In Semi-lock down mode, the S1000 thermal cycler will not respond when control panel keys are pressed. However, the following keys function on the control panel:

- **SCREEN key:** Press this key to access the Running Screen, Graphical Screen, and Time Remaining Screen.
- **PAUSE key:** Press this key to temporarily stop a protocol that is currently running on the S1000 thermal cycler
- **ENTER key:** Press this key to skip a step
- **CANCEL key:** Press this key to cancel a protocol that is currently running on the S1000 thermal cycler

Automated Control for Robotic Systems

The S1000 thermal cycler is capable of responding to robotic control commands in a high-throughput workflow. To connect the S1000 thermal cycler to a robotic system, use either a USB or serial (RS232) port. Choose a port by selecting the **PORT** tool (page 87) and then selecting the port.

Once connected, robotic commands automate basic operations of the S1000 thermal cycler. For information about these commands, download the *Automation Guide* from the Gene Expression Gateway (bio-rad.com/genomics). This guide includes instructions and commands for running S1000 thermal cyclers under the control of a robotic system.

For additional information about running the thermal cyclers in the *Bio-Rad 1000 series™* within an automated environment, refer to the C1000 thermal cycler manual.

8 Maintenance & Troubleshooting

The following chapter contains important suggestions for maintaining and troubleshooting the S1000 thermal cycler:

- Cleaning and maintaining the S1000 thermal cycler for optimal safety and performance (below)
- Maintaining sufficient air flow around the thermal cycler to optimize thermal control while running PCR (page 93)
- Troubleshooting options, including how to interpret and respond to error messages (page 95)

Cleaning and Maintaining the S1000 Thermal Cycler

The S1000 thermal cycler requires little maintenance in order to maintain proper operation and precise thermal control. However, with long and constant use the thermal cycler will require some cleaning and upkeep:

- Clean the base (page 91).
- Clean the reaction module (page 92).
- Replace the fuses (page 94)

TIP: For robotic installations where many instruments run constantly, use a regular program checking for dust, spills, and debris that could interfere with optimal instrument performance.

Cleaning the S1000 thermal cycler Base

The S1000 thermal cycler should be cleaned on a regular schedule to remove any debris or dirt that might interfere with proper function. Clean the base to prevent damage to the air intake or reaction module bay. In general, always use a soft cloth and water to wipe off spilled solutions and debris immediately.

NOTE: For instructions on handling and cleaning radioactive or biohazardous materials, consult the guidelines for radiation safety and biosafety provided by your institution. These guidelines include cleaning, monitoring, and disposal methods for hazardous materials.

To clean the base, follow these instructions, paying careful attention to the warnings:

WARNING! Prevent electrical shock. Always turn off and unplug the instrument before cleaning it.

- **Clean the air vents:** Remove dust with a soft brush, damp cloth, or vacuum cleaner. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Cleaning the vents allows sufficient air flow for precise thermal control during a run.
- **Clean the control panel:** Remove debris on the control panel with a soft cloth and mild soap solution. Cleaning this panel prevents damage that will obscure the display.
NOTE: Use of abrasive detergents or rough material will scratch the control panel.
- **Clean the reaction module bay:** Clean with a damp soft cloth to remove debris and spilled liquids. Cleaning this bay allows precise heating and cooling of the reaction block.
WARNING! Never use cleaning solutions that are corrosive to aluminium. Avoid scratching the surface of the bay. Scratches and damage to this surface interfere with precise thermal control.
WARNING! Never pour water or other solutions in the reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.
- **Clean the outside base cover:** Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution. Cleaning the cover prevents corrosion.

Cleaning the Reaction Modules

Clean the S1000 thermal cycler reaction modules on a regular schedule to prevent reagents from accumulating and interfering with the ability of the block to change temperature quickly.

To clean the reaction module, follow these instructions, paying careful attention to the warnings:

WARNING! Prevent electrical shock. Always remove the reaction module from the thermal cycler base before cleaning it.

- **Clean the cooling fins:** Remove dust with a soft brush or damp cloth. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Use water and a soft cloth to remove debris that is stuck to the fins. Avoid scratching the surface. Never use cleaning solutions that are corrosive to aluminum, such as bleach or abrasive cleansers. If needed, use a mild soap solution and rinse well to remove residue completely. Cleaning the fins improves precise sample heating and cooling.
WARNING! Clean the block: Use a soft cloth and water to remove debris from the outer block. Never use abrasive detergents, caustic solutions, or rough material that will scratch the block.
WARNING! Never clean the block with strong alkaline solutions (strong soap, ammonia, or high-concentration bleach). Never use corrosive or abrasive cleaning solutions. These cleaning agents can damage the block and prevent precise thermal control.
- **Clean the block wells:** Clean spills immediately to prevent them from drying. Use disposable plastic pipettes with water (recommended), 95% ethanol, or a 1:100 dilution of bleach in water. Always rinse the wells with water several times to remove all traces of ethanol, bleach, or soap.

WARNING! Bleach, ethanol, or soap that is left in the blocks could corrode the block and/or destroy tubes and microplates during a run. Always rinse the block well after cleaning it with any solution other than water.

Use of oil in the wells is not recommended. However, if oil is used, the wells must be cleaned thoroughly and often. Clean the oil when it is discolored or contains dirt. Use a solution of 95% ethanol to clean oil. Do not allow oil to build up in the block.

WARNING! Never heat the block after adding a cleaning solution. Heating the block with cleaning solution will damage the block, lid, and thermal cycler base.

- **Clean the inner lid:** Use a soft cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material that will scratch the surface. Cleaning the inner lid improves precise sample heating and cooling.
- **Clean the outer lid surface:** Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and then rinse the surface with a damp cloth. Cleaning the cover will prevent corrosion.

Maintaining Sufficient Air Flow

The S1000 thermal cycler requires sufficient airflow to heat and cool precisely. If the flow of air is blocked or too warm the thermal cycler cannot efficiently ramp to the correct temperature. This section includes instructions for testing the air flow and suggestions for fixing low or warm air flow.

Testing for Sufficient Airflow

Airflow is sufficient when the thermal cycler heats and cools to the correct target temperatures efficiently. When you first set up the S1000 thermal cycler in a new location, you can test for sufficient local airflow by following these instructions:

1. **Set up the instrument where you plan to use it and turn on the power**
For instructions about setting up the thermal cycler, see page 5.
2. **Adjust the local environment for typical conditions**
Turn on any nearby equipment, such as fans. Also open any window blinds to achieve optimal conditions during a run. If more than one thermal cycler is in the area, run a protocol on all the thermal cyclers at the same time.
3. **Run a typical PCR protocol for 30 minutes**
You do not need to include a sample, but must include an empty microplate or tubes. The lid will not heat correctly if it touches the hot block of the reaction module.
4. **Measure the air temperature at the air intake vents**
If the air intake temperature increases above 31°C, use the following information to ensure sufficient airflow.

Fixing Insufficient Airflow

If the air temperature near the thermal cycler is above 31°C, make one or more of the following changes to increase and optimize the airflow around a thermal cycler:

- Increase circulation of the air in the room by adding fans or adjusting the air conditioning system.
- Adjust air conditioning to lower the ambient air temperature.
- Move the thermal cycler to another location.
- Provide more space around the S1000 thermal cycler and between adjacent instruments. Arrange instruments so that the warm exhaust of one instrument does not enter the air intake vents of another.
- Shield the thermal cycler from heat sources, such as radiators, other heat-producing instruments, and bright sunlight.

Replacing Fuses

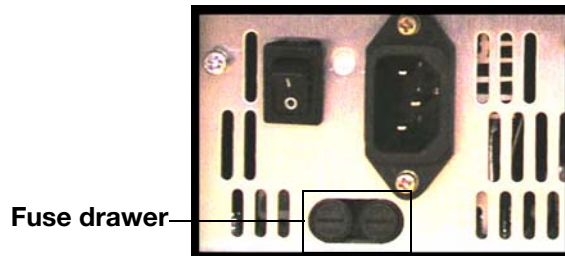
The S1000 thermal cycler fuses are designed to blow in case of severe power surges or other sources of electrical short. This process protects the user and instrument from excessive electric charge. These fuses rarely need to be replaced. However, some institutions prefer to replace fuses on a regular basis to maintain full operability.

If the thermal cycler does not turn on, first check that the power cord is plugged in to a functioning power source. Additionally, check that the power cord and power source are within the specifications for this instrument (Table 21 on page 107). To replace a power cord, contact Bio-Rad Technical Support (page 3).

Finally, check that the fuses are intact. The S1000 thermal cycler runs with two fuses that have specifications listed in Table 21 on page 107. To remove and check the fuses, follow these instructions:

WARNING! Prevent electrical shock. Always turn off and unplug the instrument from an electrical outlet before cleaning it.

1. Use a small coin to unscrew the fuse drawer



2. Pull out the fuse drawer and remove the fuses
3. If a fuse is damaged, replace it with a compatible fuse, and close the drawer. A bad fuse shows a break or burned spot in the metal. A good fuse has intact metal.

Troubleshooting

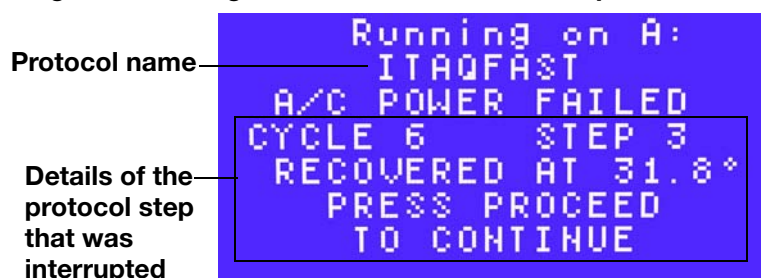
The S1000 thermal cycler is designed to run without errors. In general, when the thermal cycler displays a warning or error message, the message includes instructions for fixing the problem.

These sections discuss warnings and error messages:

- Restarting after a power failure (below)
- S1000 thermal cycler error messages (page 95)

Restarting After a Power Failure

If the external power is interrupted while the S1000 thermal cycler is running a protocol, it resumes running the protocol as soon as power is restored. In this case, the following error message will alert the user that the protocol was interrupted:



WARNING! A power failure can change the outcome of a PCR run. The hold time for the step that was running when the power failed is lengthened, causing the sample to deviate from the target temperature until the power resumes.

Warning and Error Messages

The S1000 thermal cycler tracks errors that occur during a run. After a run, all message display in sequence. For example, when a run is cancelled, the following PROTOCOL CANCELLED screen displays:



Press the **ENTER** key to clear a message or error, and continue to the next screen. When all messages are cleared, the S1000 thermal cycler returns to the main menu.

WARNING! Once the message is cleared, it cannot be displayed again.

The total number of error messages that occur during a run is displayed on the **LAST RUN** screen (page 80). However, there is no process in the S1000 thermal cycler to open these error messages after they are closed at the end of a run. If you want to track and log error messages, run the S1000 thermal cycler under the control of a

C1000 thermal cycler or a robotic system that can retrieve a list of errors in the system and run logs.

Several error messages indicate problems that can be resolved or that might not change the results of your PCR. The table below contains a list of error messages and possible solutions:]

Table 14: Warning and error message solutions

Message	Cause
A/C POWER FAILED POWER OUTAGE DURING CYCLE X STEP Y RESTARTED AT ZZ.Z TO CONTINUE PRESS ENTER	Displayed when a machine running a protocol has been turned off, either intentionally or due to a power outage, and then turned on again.
PLEASE RESTART CYCLER PLEASE CALL BIO-RAD FOR SERVICE BLOCK OVERHEATED	Reaction module has exceeded maximum temperature of 107.5°C or sensor has a malfunction and is not measuring temperature accurately. Protocol terminated.
PLEASE RESTART CYCLER HEATSINK OVERHEATED PLEASE CALL BIO-RAD FOR SERVICE	Heatsink temperature has exceeded 75°C. Protocol terminated.
PLEASE RESTART CYCLER SYSTEM OVERHEATED PLEASE CALL BIO-RAD FOR SERVICE	Amplifier 1 temperature has exceeded 85°C. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE ALL BLOCK SENSORS FAILED	All block sensors have failed (see below for failure criteria). Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE POWER SUPPLY OVERHEATED	Power Supply temperature has exceeded 85°C. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE HEATED LID FAILED PROTOCOL CANCELLED	Lid sensor has failed during lid preheat. Protocol terminated.
PLEASE CHECK AIRFLOW HTSINK OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Heatsink has exceeded 70°C. System beeps and displays error.
PLEASE CHECK AIRFLOW SYSTEM OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Amp temp has exceeded 80°C. System beeps and displays error.
PLEASE CHECK AIRFLOW PS OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Power Supply temperature has exceeded 80°C. System beeps and displays error.

Table 14: Warning and error message solutions

Message	Cause
PLEASE CALL BIO-RAD FOR SERVICE SLOW BLOCK CYCLING	Block failed to achieve target in the estimated time.
PLEASE CALL BIO-RAD FOR SERVICE SLOW LID CYCLING	Lid failed to achieve target in the estimated time.
PLEASE CALL BIO-RAD FOR SERVICE SLOW GRADIENT	Block failed to achieve gradient in the estimated time.
PLEASE CALL BIO-RAD FOR SERVICE HEATED LID FAILED	(singles only) If the right and left lid heater channels deviate from each other by more than 5°C the lid is shut off.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 0 FAILED	Block sensor 0 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 1 FAILED	Block sensor 1 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 2 FAILED	Block sensor 2 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 3 FAILED	Block sensor 3 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 4 FAILED	Block sensor 4 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 5 FAILED	Block sensor 5 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE LEFT LID SENSOR FAILED	Left lid sensor has failed*. If a dual, protocol terminated. If a single and BOTH lid sensors failed, protocol terminated and block sent to 4°C.
PLEASE CALL BIO-RAD FOR SERVICE RIGHT LID SENSOR FAILED	Right lid sensor has failed*. If a dual, protocol terminated. If a single and BOTH lid sensors failed, protocol terminated and block sent to 4°C.
PLEASE CALL BIO-RAD FOR SERVICE LEFT HEATSINK SENSOR FAILED	Left heatsink sensor has failed*, system using average of amplifier temperatures to continue.
PLEASE CALL BIO-RAD FOR SERVICE RIGHT HEATSINK SENSOR FAILED	Right heatsink sensor has failed*, system using average of amplifier temperatures to continue.

Table 14: Warning and error message solutions

Message	Cause
PLEASE CALL BIO-RAD FOR SERVICE LID OVERHEATED AND WAS SHUT OFF	(duals only) Lid has overheated and has been shut off and protocol has been terminated.
PLEASE CALL BIO-RAD FOR SERVICE AMP1 TEMP SENSOR FAILED	Amplifier temperature sensor 1 has failed*.
PLEASE CALL BIO-RAD FOR SERVICE POWER SUPPLY SENSOR FAILED	Power Supply sensor has failed*.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK POWER FAILURE PROTOCOL CANCELLED	Power to block is out of range.
PLEASE CALL BIO-RAD FOR SERVICE LOGIC POWER FAILURE	Logic power sensor is out of bounds.
PLEASE CALL BIO-RAD FOR SERVICE BASE POWER FAILURE PROTOCOL CANCELLED	Base power sensor is out of bounds. Protocol cancelled.
PLEASE CALL BIO-RAD FOR SERVICE AMP2 TEMP SENSOR FAILED	Amplifier temperature sensor 2 has failed*.
BLOCK MISSING PROTOCOL CANCELLED PLEASE CALL BIO-RAD FOR SERVICE	Reaction module has been removed. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE MEMORY CORRUPT PROTOCOLS MAY BE LOST	Protocol storage memory has been corrupted.
PLEASE RESTART CYCLER BAD REACTION MODULE PLEASE CALL BIO-RAD FOR SERVICE	Unable to read information from reaction module properly.
PLEASE RESTART CYCLER INCORRECT CHECKSUM PLEASE CALL BIO-RAD FOR SERVICE	Information read from reaction module appears incorrect.
PLEASE RESTART CYCLER BLOCK POWER SHUT OFF PLEASE CALL BIO-RAD FOR SERVICE	There was a problem with the block and power was shut off
PROTOCOL CANCELLED SAMPLES COOLED AT 4C BLOCK SENSOR FAILED AT CYCLE X, STEP Y PLEASE RESTART CYCLER PLEASE CALL BIO-RAD	One of the block sensors in a single block has failed. The system has cancelled the protocol at step x, cycle y and sent the block to 4°C to preserve the samples.

The error message may instruct the user to contact Bio-Rad. In this event, call the nearest Bio-Rad Laboratories Technical Support team (page 3).

***Sensor Failure means that the sensor was deemed short, open or had changed more than 3°C in a 50ms period and that this condition was present for more than 2 seconds.**

If two or more block sensors fail (or both lid sensors fail) the protocol is terminated and the block is sent to 4°C to preserve samples.

Appendix A: Preinstalled Protocols

The S1000 comes with preinstalled protocols that provide a template for running new PCR experiments. Use these protocols as templates. The following sections describe the preinstalled protocols, and include tips about when to use them:

- Standard 2 and 3-step protocols (below).
- Touchdown PCR protocol to avoid non-specific primer binding (page 102).
- iTaq protocols optimized to run with iTaq™ hot-start polymerase (page 102).
- iProof protocols optimized to run with iProof™ high-fidelity polymerase (page 103).
- Reverse transcription protocols to amplify DNA from an RNA template (page 104)
- Nested primer protocols to amplify a specific DNA sequence from a large, complex DNA template (page 104).

Standard Protocols

Two standard protocols that run two-step and three-step PCR with a standard DNA polymerase. Choose these protocols to begin running PCR with new primers and DNA template, or copy them to begin writing a new protocol. Table 15 lists the parameters in each protocol.

Table 15. Standard Protocol*

	STD2		STD3	
	To Run a Standard Two-Step Protocol		To Run a Standard Three-Step Protocol	
Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	95	3:00	95	3:00
2	95*	0:30	95*	0:30
3	65	0:30	55	0:30
4	GOTO 2	29x	72	0:30
5	72	7:00	GOTO 2	29x

Table 15. Standard Protocol*

6	4	Infinite hold ∞	72	1:00
	END		4	Infinite hold ∞
7			END	

Touchdown Protocol

A touchdown protocol tests for the best annealing temperature for a specific primer-template pair. Choose this protocol to test a new set of primers and DNA template for the optimal annealing temperature. Table 16 lists the parameters in each protocol

Table 16. Touchdown Protocol*

TCHDOWN		
To run a protocol with an increment temperature step that automatically finds the most stringent conditions for primer binding		
Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	95	3:00
2	95*	0:30
3	60 (increment at -0.5°C/ cycle)	0:30
4	72	30
5	GOTO 2	29x
6	95	0:30
7	45	0:30
8	72	0:30
9	GOTO 6	29x
10	72	7:00
11	4	Infinite hold ∞
12	END	

iTaq Polymerase Protocols

iTaq DNA polymerase is an antibody-mediated hot-start polymerase that is suitable for both PCR and real-time. The iTaq protocols run PCR using the optimal parameters

for this polymerase and associated buffers. iTaq polymerase is designed to be activated during the first step at 98°C and to amplify small to medium-size templates. Table 17. iTaq Protocol*

iTAQ-FST		
To Run Fast PCR		
Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	98	30
2	92*	01
3	70	10
4	GOTO 2	29x
5	72	30
6	END	

iProof Polymerase Protocols

iProof DNA polymerase is a high-fidelity polymerase that is designed to quickly and precisely amplify long targets using a proofreading enzyme combined with a DNA binding protein. These iProof protocols include optimal parameters for this enzyme and associated buffers, including an initial 95°C step to activate the enzyme and a final long extension step. Each of these protocols is adjusted for a distinct range of target sizes. Table 18 lists the parameters in each protocol.

Table 18. iProof Protocol*

	IPRF1KB		IPRF8KB		IPRF15KB	
	To amplify 1 kb or smaller targets fast		To amplify 8 kb or smaller targets		To amplify 15 kb or smaller targets	
Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	98	0:30	98	0:30	98	0:30
2	98*	0:05	98*	0:05	98*	0:05
3	60	0:10	60	0:10	60	0:10
4	72	0:30	72	4:00	72	7:30
5	GOTO 2	29x	GOTO 2	29x	GOTO 2	29x
6	72	5:00	72	5:00	72	5:00
	4	Infinite hold ∞	4	Infinite hold ∞	4	Infinite hold ∞

Table 18. iProof Protocol*

	END		END		END	
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Iscript Polymerase Protocol

Table 19. Iscript Protocol

Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	25	5:00
2	42	30:00
3	85	5:00
4	4	Infinite hold ∞
5	END	

Nested Primer Protocols

Nested primers amplify a specific DNA sequence from a large, complex DNA template such as genomic DNA. Table 20 lists the parameters in each protocol.

Table 20. Nested Primer Protocol*

Step	NESTPR2		NESTPR3	
	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
	To Run Two-step Protocol Using Nested Primers		To Run a Three-step Protocol Using Nested Primers	
1	95	4:00	95	0:30
2	95*	0:30	95*	0:30
3	65	0:30	55	0:30
4	GOTO 2	39x	72	0:30
5	72	7:00	GOTO 2	39x
6	4	Infinite hold ∞	72	7:00
7	95*	0:30	4	Infinite hold ∞
8	65	0:30	95	0:30
9	GOTO 7	39x	55	0:30
10	72	7:00	72	0:30
11	4	Infinite hold ∞	GOTO 8	39x

Table 20. Nested Primer Protocol*

12	END		72	7:00
13			4	Infinite hold ∞
14			END	

Appendix B: Operating Specifications

The S1000 thermal cycler is designed for repeatable, consistent results. It heats and cools very fast during operation. Users should follow the operating specifications listed in this section to achieve reliable results:

- Instrument operating specifications and recommendations
- Gradient specification

Instrument Operating Specifications

This thermal cycler is designed to operate safely in a standard research laboratory for research applications.

NOTE: This instrument is for research use only.

For safe and reliable results, the operation of this instrument must follow the specifications listed in this manual. To ensure safe operation, follow these recommendations:

- Place the thermal cycler base on a flat surface
- Do not block fans during operation; keep the area around the instrument clear of objects or debris that could block airflow
- Read this manual for best usage practices
- Never remove the instrument's outer casing
- Do not operate in extreme humidity where condensation can impede proper operation
- Do not operate at extreme altitude
- Use only the provided power cord

Table 21. Operating specifications

Requirement	Specification
Thermal Block Uniformity	$\pm 0.4^{\circ}\text{C}$ well-to-well uniformity at 90°C within 10 seconds
Thermal Accuracy	$\pm 0.2^{\circ}\text{C}$ of programmed target temperature
Maximum Temperature	100°C
Minimum Temperature	0°C

Table 21. Operating specifications

Requirement	Specification
Max Ramp Rate	5°C/second
Heated Lid Range	0-110°C
Dimensions	13" x 18" x 8"
Weight	23 lbs
Line Voltage	100-240 VAC rms (no adjustment needed among voltages within these ranges)
Frequency	50-60 Hz single phase
Input Power	400 W max
Fuses	Two 6.3 A, 250 V, 5 x 20 mm
Display	LCD, semi-graphical
Ports	5 USB-A, 1 USB-B

Gradient Specifications

This thermal cycler can generate a temperature gradient across a 96-well or dual 48-well block. To view the estimated temperature in each well, use the [GRADCALC](#) tool.

Table 22. Gradient specifications

Requirement	Specification
Gradient Direction	Back (upper temperature) to front (lower temperature) of block
Maximum Gradient	30°C to 100°C
Gradient Temperature Differential	1-24°C
Gradient Uniformity	±0.4°C well to well uniformity within 10 seconds of step initiation
Gradient Accuracy	±0.2°C of programmed target temperature
Gradient Calculator Accuracy	±0.4°C of the actual well temperature

Appendix C: Catalog Numbers

This appendix lists useful catalog numbers and descriptions for the following components and accessories in the 1000-series:

- 1000-series components and descriptions (below)
- Accessories for the 1000-series with descriptions

Components of the 1000-series

Table 23 lists the components of the 1000-series instruments and software with the descriptions and catalog numbers:

Table 23: Catalog numbers for the 1000-series instruments and software.

Product	Description	Catalog number
Thermal cycler bases and reaction modules		
C1000 thermal cycler base	Thermal cycler base for PCR and real time PCR	184-1000
S1000 thermal cycler base	Thermal cycler base for PCR	184-2000
96 Fast reaction module	96-well block for PCR with adjustable, heated lid	184-0096
Dual 48 Fast reaction module	Two 48-well blocks for PCR with adjustable, heated lid	184-0048
384 reaction module	384-well block for conventional PCR with adjustable, heated lid	184-0384
C1000 96-well Fast System	C1000 chassis and 96 Fast reaction module system	185-1096
C1000 48/48 Fast System	C1000 chassis and 48/48 Dual Fast reaction module system	185-1048
C1000 384-well System	C1000 chassis and 384-well reaction module system	185-1384
S1000 96-well Fast System	S1000 chassis and 96 Fast reaction module system	185-2096
S1000 48/48 Fast System	S1000 chassis and 48/48 Dual Fast reaction module system	185-2048
S1000 384-well System	S1000 chassis and 384-well reaction module system	185-2384
Real time detection modules		
CFX96 Real Time Detection module	Real time detection module with 96-well block	184-5096
Software		
C1000 Manager software	Software package to run PCR with one or more thermal cyclers in a single window	184-4000
CFX Manager software	Software package to run real-time PCR and PCR with one or more thermal cyclers in a single window	184-5000

Table 23: Catalog numbers for the 1000-series instruments and software.

Product	Description	Catalog number
CFX Manager Security Edition software	Software package to run real-time PCR and PCR with one or more thermal cyclers in a single window. Includes security features for CFR part 11 compliance	184-5001

Accessories for the 1000-series

The Table 24 lists accessory parts for the 1000-series instruments. It includes descriptions and catalog numbers:

Table 24: Catalog numbers of accessories to the 1000-series.

Product	Description	Catalog number
USB cable	Shielded USB cable to connect the S1000 thermal cycler directly to C1000 thermal cycler.	184-8000
Tube Tray	Provides structural support for one or a few tubes	184-7000



**Bio-Rad
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