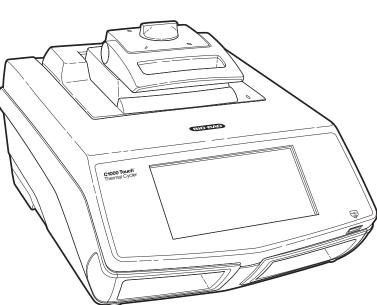
# C1000 Touch<sup>™</sup> Thermal Cycler

# **Instruction Manual**

Catalog #184-1100 #185-1138 #185-1148 #185-1196 #185-1197





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This base unit, Serial No. \_\_\_\_\_\_, in combination with its immediately attached Bio-Rad sample block module(s), constitutes a thermal cycler whose purchase conveys a limited non-transferable immunity from suit for the purchaser's own internal research and development and for use in human in vitro diagnostics and all other applied fields under U.S. Patent No. 5,475,610 (claims 1, 44, 158, 160-163 and 167 only), or corresponding claims in its non-U.S. counterpart, owned by Applera Corporation. No right is conveyed expressly, by implication, or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5<sup>′</sup> nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

This C1000 Touch<sup>™</sup> thermal cycler base unit, when combined with a CFX96<sup>™</sup> or CFX384<sup>™</sup> detection module, constitutes a real-time thermal cycler authorized for use in research, human in vitro diagnostics, and all applied fields except veterinary in vitro diagnostics. No rights are conveyed expressly, by implication or estoppel to any patents on real-time methods, including but not limited to 5<sup>°</sup> nuclease assays, or to any patent claiming a reagent or kit. For further information on purchasing license rights, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA.

These products are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: US Patent Nos. 6,767, 512 and 7,074,367.

#### **Bio-Rad Laboratories Resources**

Bio-Rad provides many resources for scientists. The Bio-Rad website contains useful information for running PCR and real-time PCR experiments:

#### • www.bio-rad.com

This site includes links to technical notes, manuals, product information, and technical support. This site also provides many technical resources on a wide variety of methods and applications related to PCR, real-time PCR, and gene expression.

Table 1 lists Bio-Rad resources and how to locate what you need.

Resource	How to Contact	
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad Laboratories web site ( <b>www.bio-rad.com</b> ). Find the nearest international office listed on the back of this manual.	
Technical notes and literature	Find technical information on the Bio-Rad Laboratories website ( <b>www.bio-rad.com</b> ). Type a term in the Search box and select Documents to find links to technical notes, manuals, and other literature.	
Technical specialists	To find local technical support on the phone, 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.	

#### Warranty

The C1000 Touch<sup>™</sup> 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.

## Writing Conventions Used in This Manual

This manual provides instructions on how to safely set up and operate the C1000 Touch thermal cycler and uses the writing conventions shown in Table 2 to quickly provide relevant information.

Convention	Meaning	
TIP:	Provides helpful instructions, including information explained in further detail elsewhere in this manual.	
NOTE:	Provides important information, including information explained in further detail elsewhere in this manual.	

Convention	Meaning	
WARNING!	Explains crucial information about a topic that may lead to injury to the user, instrument damage, or data loss.	
Touch X	Touch <b>X</b> using your finger. For example, touch the <b>NEW</b> button means use your finger to touch the <b>NEW</b> button on the screen.	
Touch X > Y	From menu <b>X</b> , touch <b>Y</b> . For example, touch <b>MAIN</b> > <b>RUN</b> means touch the <b>RUN</b> button in the <b>MAIN</b> menu.	

Table 2. Writing conventions. (Continued)

## **Safety and Regulatory Compliance**

The C1000 Touch thermal cycler heats and cools very quickly during operation. We strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

Use only Bio-Rad USB cable (catalog #184-8000) when using any 1000-series cycler.

#### **Safety Warning Labels**

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 3 to review the meaning of each safety warning label.

Table 3. Instrument safety warning labels

lcon	Meaning
!	<b>CAUTION: Risk of danger!</b> 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.
4	<b>CAUTION: Risk of electrical shock!</b> This symbol identifies components that pose a risk of electrical shock if improperly handled.
<u></u>	<b>CAUTION: Hot surface!</b> This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

#### **Instrument Safety Warnings**

The following warning labels display on the instrument, and refer directly to the safe use of this C1000 Touch thermal cycler (Table 4).

Table 4.	Instrument	safetv	warning	labels.
	moulamont	outory		laboloi

lcon	Meaning
!	Warning about risk of harm to body or equipment. Operating the C1000 Touch instrument before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel should operate this instrument.
4	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.
4	Never remove the outer case of a thermal cycler base. This may cause you to 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.
<u></u>	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 temperature before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns.
<u></u>	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 and Regulatory Compliance**

This instrument has been designed to be safely operated under the environmental conditions listed in Table 5.

Table 5. Conditions for safe use.

Usage Aspect	Conditions for Safe Use		
Rated input power	100-240 VAC, 50-60 Hz, 850 W Max		
Overvoltage category	II		
Fuses	10 A, 250 V, 5 x 20 mm, fast blow (qty. 2)		
Environment	Indoor use only		
Temperature	15-31°C		
Relative humidity	Up to 80% (noncondensing)		
Altitude	Up to 2,000 meters above sea level		
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:

- IEC 61010-1:2001 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- IEC 61010-2-010:2003 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials
- IEC 61010-2-081:2001+A1:2003 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-081: Particular Requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- CAN/CSA-C22.2 NO. 61010-1-04 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA-C22.2 NO. 61010-2-010-04 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials
- CAN/CSA-C22.2 NO. 61010-2-081-04 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-081: Particular Requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- EN 61010-1:2001 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- EN 61010-2-010:2003 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials
- EN 61010-2-081:2002+A1:2003 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2-081: Particular Requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- UL 61010-1:2004(R2008) Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements

#### **ELECTROMAGNETIC COMPATIBILITY (EMC)**

- IEC61326-1:2005 Electrical Equipment for measurement, control, and laboratory use
   EMC Requirements, Class A
- EN61326-1:2006 Electrical Equipment for measurement, control, and laboratory use - EMC Requirements, Class A
- FCC Part 15, Subpart B, Sections 15.107 and 15.109 as a Class A digital device

#### FCC WARNINGS AND NOTES

- **Warning.** Changes or modifications to this unit, not expressly approved by Bio-Rad Laboratories, 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 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

#### **Battery**

The C1000 Touch thermal cycler uses a 3 V lithium-metal coin cell battery and a 4.8 V nickelmetal hydride rechargeable battery pack to maintain time settings and run data in the event of AC power loss. If the time and/or run data do not remain set after the unit is turned off, it may be an indication that the batteries are getting weak. If this occurs, contact Bio-Rad Technical Support (See "Bio-Rad Laboratories Resources" on page ii.) for assistance.

**WARNING!** Do not attempt to change the batteries. Contact Bio-Rad technical support.

#### FOR THE STATE OF CALIFORNIA, USA ONLY

Perchlorate material – special handling may apply, see www.dtsc.ca.gov/hazardouswaste/ perchlorate.

Perchlorate material – lithium battery contains perchlorate.

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# 1 Introduction to the C1000 Touch™ Thermal Cycler

Read this chapter for information on setting up the C1000 Touch thermal cycler.

- System overview (page 1)
- Reaction modules (page 2)
- Setting up the C1000 Touch thermal cycler (page 3)
- Operating the reaction module lid (page 5)
- The home screen (page 6)
- Renaming the C1000 Touch thermal cycler (page 7)
- Setting up email notification (page 8)

#### **System Overview**

The C1000 Touch thermal cycler base (Figure 1) includes:

- Reaction module bay holds the inserted reaction module
- Reaction module locking bar locks reaction module in place
- **Touchscreen display** provides access to all the functions needed to create and run PCR protocols
- USB A port connects to a USB flash drive, mouse, or keyboard
- Air intake vents allow the thermal cycler to heat and cool quickly



Figure 1. Front view of the C1000 Touch thermal cycler.

The back panel of the C1000 Touch thermal cycler includes data ports (Figure 2).

- USB B port connects the C1000 Touch thermal cycler to a computer
- USB A ports connects up to three S1000 thermal cyclers to the C1000 Touch thermal cycler. These ports can also connect to any USB flash drive, mouse, or keyboard
- **Test port** for service testing only
- Ethernet port for connecting the C1000 Touch thermal cycler to your network

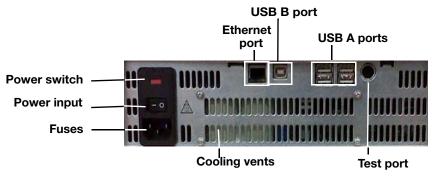


Figure 2. Back panel of the C1000 Touch thermal cycler.

## **Reaction Modules**

The C1000 Touch thermal cycler is compatible with any 1000-series reaction module. The reaction modules come in four module sizes: the 96-well fast module, 96-deep well module, dual 48/48-well fast module, or 384-well module. Each reaction module includes a fully adjustable heated lid that is capable of running reliably with a broad range of reaction vessels.

#### **Recommended Sample Volumes**

When using the C1000 Touch thermal cycler, the maximum sample volume is determined by the type of reaction module used. Table 6 lists the volumes to be used with different reaction modules.

Number of Wells	Number of Blocks	Recommended Sample Volume (Upper Limit)
96 fast	1	10–50 μl (50 μl limit)
96 deep	1	10–125 µl (125 µl limit)
Dual 48/48 fast	2	10–50 μl (50 μl limit)
384	1	3–30 µl (30 µl limit)

Table 6. Size and volume limit for	<sup>1</sup> 1000-series reaction modules.
------------------------------------	--

Each reaction module contains cooling fins for fast heating and cooling and a fully adjustable, heated lid.

- **Heated inner lid** maintains the lid temperature to prevent condensation and evaporation
- Sample/reaction block holds reaction vessels, including tubes and microplates

The top of a reaction module lid includes a lid lever, lid force knob, and status LED.

- Lid lever opens and closes the lid
- Lid force knob sets lid force and seals the reaction
- Status LED turns on to indicate that the block is in use

Figure 3 shows the components of a 96-well fast reaction module.

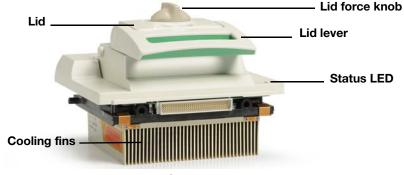


Figure 3. Components of a reaction module.

# **Setting Up the C1000 Touch Thermal Cycler**

The C1000 Touch thermal cycler package includes:

- C1000 Touch thermal cycler base
- Power cord
- 1 USB flash drive
- Instruction manual
- Quick guide for system installation

Reaction modules for use with the C1000 Touch thermal cycler are shipped in separate packaging. Remove all packaging materials and store them for future use. If any item is missing or damaged, contact your local Bio-Rad office.

Place the C1000 Touch thermal cycler base on a flat, dry surface with sufficient cool airflow to run properly.

The instrument can run in two modes: stand-alone or software-controlled by CFX Manager™ software version 2.1 or higher. When running the system in software-controlled mode, make sure there is sufficient space for a computer during setup.

# To insert a 1000-series reaction module into the reaction module bay of the thermal cycler base, follow these instructions:

1. With the locking bar in the down position and the lid lever of the reaction module pointing to the front, lift the reaction module into the reaction module bay (Figure 4). Leave 1–2 cm of space in front of the module.



Figure 4. Inserting the reaction module into the bay.

2. Pull the locking bar up to lock the reaction module in place (Figure 5). There should be no space at the front of the module when it is locked into the C1000 Touch thermal cycler base.



Figure 5. Locking the reaction module in place.

- 3. Plug the supplied power cord into the appropriate electrical outlet.
- 4. Turn on the thermal cycler using the power switch on the back panel of the thermal cycler base.

NOTE: Before operating the thermal cycler, be sure to read the safety specifications ("Safety and Regulatory Compliance" on page iii) and operating requirements.

5. When the C1000 Touch thermal cycler starts, it runs a self-test to verify proper functions, and then displays the home screen. Use the home screen to begin operating the thermal cycler.

#### To remove the reaction module from the thermal cycler base, follow these instructions:

- 1. Turn off the thermal cycler.
- 2. Unlock and release the reaction module by pushing the locking bar down.
- Carefully lift the reaction module out of the bay.
   WARNING! Cooling fins may be hot immediately after running a protocol or incubation. Before lifting the reaction module, make sure the fins are cool.
- 4. After removing the reaction module from the C1000 Touch thermal cycler, store it on a clean, flat surface where it cannot get bumped, scraped, or dropped.

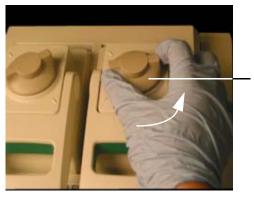
# **Operating the Reaction Module Lid**

The inner lid of the reaction module applies heat and force to the reaction vessel lids (caps or tape) to produce consistent and successful reactions. Heating the inner lid prevents condensation, while applying force seals the reaction to prevent evaporation.

**WARNING!** After a run, the heated inner lid can remain hot. Use caution when opening and closing the lid.

To open the lid, use the following steps:

1. Turn the lid force knob counterclockwise to release the inner lid (Figure 6).



Lid force knob (turn counterclockwise to release the lid)

#### Figure 6. Turn the lid force knob counterclockwise to release the inner lid.

- 2. To open the lid, lift the lid lever up.
- 3. Lift the lid lever completely until the reaction module stays open without assistance.

To close the lid, follow these steps:

- 1. Push the lid lever down, making sure that the front of the lid is secured beneath the housing.
- 2. Adjust the lid force by turning the lid force knob (Figure 7).
  - Turn the knob clockwise to increase the lid force
  - Turn the knob counterclockwise to decrease the lid force
- 3. Increase the lid force until the heated lid touches the reaction vessel. Continue to increase the lid force further based on the following recommendations.
  - If using tubes, increase the lid force by a quarter turn after the lid touches the tubes
  - If using plates, increase the lid force by a half turn after the lid touches the plate NOTE: The position marks on the lid indicate guarter turns

**WARNING!** If the lid is tightened past the recommended force, tightening the lid further will result in the knob slipping and causing a clicking sound. This is designed as a warning that the lid force is past the recommended setting and may result in damage to the reaction vessels. If this happens, decrease the lid force by

turning the lid force knob counterclockwise one full turn. Reapply the correct lid force by following the instructions in step 3.

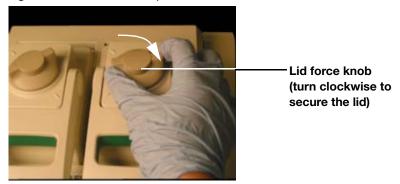


Figure 7. Adjust the lid force by turning the lid force knob.

#### **Loading Sample Vessels into the Reaction Block**

To ensure uniform heating and cooling of samples, vessels must be in complete contact with the reaction block. Adequate contact is achieved by:

- · Confirming that the block is clean before loading samples
- Firmly pressing the individual tubes or the microplate into the block wells

TIP: When using one or a few tubes, use the tube frame (catalog #184-9000 or 184-9001) or load at least one empty tube in each corner of the block to ensure that the lid exerts even pressure on individual tubes.

#### **The Home Screen**

The home screen provides access to all thermal cycler operations and displays the status of the reaction module and the name of the thermal cycler. In the example shown in Figure 8, the home screen displays the date and time, name of logged-in user (**Pete**), name of the instrument (**CC006622**), current status (**Idle**), and home screen buttons.

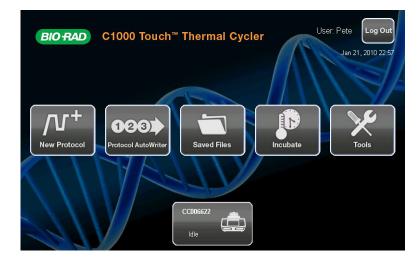


Figure 8. The home screen of the C1000 Touch thermal cycler.

To initiate the functions on the home screen, touch the associated button. Below are choices of functions:

- New Protocol Create a new protocol (page 11)
- **Protocol AutoWriter** Create a new protocol by entering amplicon length, annealing temperature and desired speed (page 27)
- Saved Files View the saved files and folders in the file library (page 33)
- Incubate Opens the incubate setup screen (page 25)
- **Tools** Opens the tools screen (page 40)
- Log in Log in to the C1000 Touch thermal cycler. Once you log in, the button name changes to Log Off (page 39)
- Status Open the status screen to view the current status of the reaction module (page 23)

#### **Renaming the C1000 Touch Thermal Cycler**

Each C1000 Touch thermal cycler is initially named using the serial number of the thermal cycler base. Users can rename a thermal cycler for easy identification.

To rename a C1000 touch thermal cycler, follow these steps:

- 1. From the home screen, touch the **Tools** button to open the tools menu. Touch **About**, then touch the **Name** button (Figure 9).
- 2. Enter a new name using the pop-up alphanumeric keypad.
- 3. Touch the **OK** button to accept the new cycler name.

User: Pete	About	Jan 27, 2010 23:06
	SC005016           Base SN:         \$C005016           Name:         \$C005016           Block SN:         RN015784           Block Type:         96 Well           PXA270:         1.101.260.128           HC12:         1.0158.0           FX2:         1.35.0.0	
	Total Hours: 23899	
Back		Generation System

Figure 9. Touch the Name button to rename the instrument.

#### **Setting Up Email Notification**

A run complete confirmation and run report can be emailed directly to any computer with Internet access. To configure the outgoing email from the C1000 Touch thermal cycler, use the following instructions:

1. Touch the **Log In** button on the home screen to log in to the thermal cycler as the Administrator.

NOTE: The logged in user name appears to the left of the log out button when you return to the home screen.

- 2. Touch the **Tools** button on the home screen to launch the tools menu.
- 3. Touch **Email Settings** in the admin menu.

#### Setting Up the Gmail server

NOTE: A Gmail account must be set up with user name and password prior to setting up the instrument Gmail server.

- 1. Select the Gmail server from the Mail Server dropdown list.
- 2. Enter the Gmail account user name and password.
- 3. Touch the Set as Default checkbox (Figure 10).

User: ADMIN	Outgoing Mail (SMTP) Server Settings Jan 27, 2010 23:09			
	Mail Servers:	smtp.gmail.co	m 🕑	
	Set As Default:	$\checkmark$		
	Mail Server Address:	smtp.gmail	.com	
	Mail Server Port:	587		
	Authentication Required:	$\checkmark$		
	Use SSL:	$\checkmark$		
	User Name:	Pete.Sm	th	
	Password:	****		
Back	- Server	+	Save	Test Email

# Figure 10. Gmail server is selected, user name and password have been entered, and server has been selected as the default server.

- 4. Touch the **Save** button to save the current server settings.
- 5. Touch the Test Email button.
- 6. Touch the **Test Email Address** button and enter an email address using the pop-up alphanumeric keypad.
- 7. Touch the Attachment Size in MB button and enter a test attachment size.

NOTE: The allowable limit on attachment size is dictated by your institute's server. We recommend testing an attachment size between 0.5 and 5 MB. Entering 0 will send a test email with no attachment.

8. Touch the Send Email button.

A test email will be sent to the test email address.

- 9. Touch the Cancel button to return to the server settings screen.
- 10. Touch the **Back** button to return to the tools menu.

#### Setting up a custom server

1. Touch the **New Server** button (Figure 11).

User: ADM	N OL	tgoing Mail (SM	TP) Server Setti	<b>ıgs</b> Ja	n 27, 2010 23:16
	N	Mail Servers: Set As Default: Server Address: Mail Server Port: ation Required: Use SSL: User Name: Password:			
Back		Remove Server	+ New Server	Save	Test Email

Figure 11. New Server button.

- 2. Touch the **Mail Server Address** button and enter an address using the pop-up alphanumeric keypad.
- 3. Touch the **Mail Server Port** button and enter a value using the pop-up numeric keypad.
- 4. Touch the Set As Default checkbox.
- Enter additional information (Authentication Required, Use SSL, User Name, and Password) only if required by your server.
   TIP: Contact your network administrator for server requirements.
- 6. Touch the Save button. The new server will appear in the Mail Servers dropdown list.
- 7. Repeat steps 5-10 in "Setting Up the Gmail server" (page 8).

#### **Removing a server**

- 1. Select the server to be removed in the Mail Servers dropdown list.
- 2. Touch the Remove Server button.
- 3. Touch the Yes button to confirm removal of selected server.

# **2** Creating, Editing, Naming, and Saving Protocols

Read this chapter for information on creating and editing protocols.

- Creating a new protocol or editing an existing protocol (page 11)
- Sample volume and lid temperature (page 17)
- Naming a protocol (page 19)
- Saving a protocol (page 19)

# **Creating a New Protocol or Editing an Existing Protocol**

To create a new protocol:

1. Touch the **New Protocol** button on the home screen to open the new protocol screen.

A template protocol opens (Figure 12).

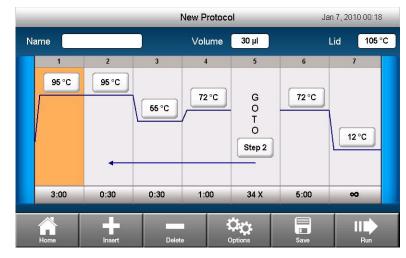


Figure 12. The template protocol.

To edit an existing protocol:

- 1. Touch the **Saved Files** button on the home screen to open the saved files screen.
- 2. Select a file to edit (Figure 13).

User: ADMIN	Save	Saved Files	
Locations	Folders	Files	Preview
Recent	<root></root>	PCR1	Method: Calc Lid: 105°C
📩 SC005016	Pete1		Volume: 30µl
Real-Time Data			1.95°C,3:00 2.95°C,0:30
			3. 55°C, 0:30 4. 72°C, 1:00
Pete			5. Goto 2, 34X 6. 72°C, 5:00
USB Drive			7.12°C,0:00
	+	⊿∿≎	
Home New I	Folder Folder Options	File Options	Edit Run

Figure 13. Selected file to edit.

- 3. Touch the **Edit** button to open the edit protocol screen. The protocol will display in a graphical format.
- 4. Select a desired step by touching anywhere in the step.
- 5. Change a time or temperature by touching the desired button and entering a value using the pop-up numeric keypad.
- 6. Change the reaction volume or lid temperature by touching the desired button and entering a value using the pop-up numeric keypad.
- 7. Change the protocol name by touching the **Name** button and entering a protocol name using the pop-up alphanumeric keypad.
- 8. Touch the **Run** button to run the protocol without saving it. Alternatively, touch the **Save** button to choose a file name, location, and folder in which to save the protocol.

#### **Protocol Parameters**

Table 7 lists the parameters for temperature and gradient steps with the limits of the parameters.

Step Name	Parameters and Ranges	Description	
Temperature	<b>Temperature in °C :</b> The target temperature between 0.0 and 100.0°C in tenths of a degree	Instructs the thermal cycler to ramp to the target temperature and hold that temperature for the specified amount	
	<b>Hold time :</b> The hold time between 1 sec and 18 hr in the format of hr:min:sec. To enter an infinite hold, touch the $\infty$ (infinite) button	of time	
Gradient range	<b>Lower</b> : The lower temperature in the gradient. Enter a number between 30.0 and 99.0°C in tenths of a degree	Instructs the thermal cycler to ramp to the target temperature gradient across the block and hold that temperature gradient for the specified amount of time	
	<b>Upper</b> : The upper temperature in the gradient. The maximum temperature is 100°C. Enter a temperature within 24.0°C of the lower temperature		
	<b>Time:</b> The hold time between 1 sec and 18 hr in the format of hr:min:sec. To enter an infinite hold, touch the $\infty$ (infinite) button		
Increment	A temperature from –10.0 to 10.0°C per cycle in tenths of a degree	Applies only to a temperature step. 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	
Ramp rate	A number from 0.1 to 5°C per sec	Applies only to a temperature step. Instructs the thermal cycler to ramp to the target temperature at the specified ramp rate in that step	
Extend	A time from –60 to 60 sec per cycle	Applies to both temperature and gradient steps. Instructs the thermal cycler to extend the hold time with each cycle. A positive number increases the hold time and a negative number decreases the hold time	
Веер	(No parameters)	Applies only to a temperature step. Instructs the thermal cycler to beep to signal that the thermal cycler has reached the target temperature for that step	

 Table 7. Protocol steps and parameters of the C1000 Touch thermal cycler.

#### **Changing Target Temperature and Hold Time**

To change the target temperature and hold time in a temperature step, follow these instructions:

- 1. Touch the desired time or temperature button of the step to edit.
- 2. Use the pop-up numeric keypad to enter a new value for the target temperature or hold time.

#### **Inserting a Protocol Step**

Insert a protocol step if a new temperature, GOTO, or gradient step is needed. Follow these instructions to insert a step to the right of a preexisting protocol step:

- 1. Touch a step to the left of where the new step will be inserted.
- 2. Touch the Insert button.
- 3. Choose the type of step to insert.

Touch the **Temperature** button to insert a temperature step, **Gradient** button to insert a gradient step, or **GOTO** button to insert a GOTO step.

4. A new temperature step has a default target temperature of **50°C** and a default time of **30 sec** (0:30). Touch the time or temperature buttons to edit the parameters in the new step.

#### **Deleting a Protocol Step**

To permanently remove a step from a protocol:

- 1. Touch the step to be deleted.
- 2. Touch the **Delete** button to delete the selected step.

#### Adding or Removing a Temperature Gradient

To add a temperature gradient to a temperature step:

1. Select a step in the protocol and touch the **Options** button.

2. In the **Step Options** window, touch the **Gradient** checkbox. A check mark will appear in the gradient checkbox and the gradient range will appear (Figure 14).

	-		Edit Pro	tocol		Jan 30, 2010 01:05
Name			Step Op	tions		105 °C
1	Temperature	55 °C	)	A	75 °C	7
	Time	0:30	)	В	73.9 °C	
95	Gradient	$\checkmark$		С	71.6 °C	
	Ramp Rate		) ±/	D	67.6 °C	
	Increment		<b>;</b> +	E	62.8 °C	2°C
	Extend		0	F	59 °C	2°C
				G	56.3 °C	
				Н	55 °C	
3:0			1			×
		Cancel		ок		
Back	Inser	t Del	ete	Options	Save	Run

Figure 14. The Step Options window displays the gradient.

3. Touch any gradient temperature to change the value by using the pop-up numeric keypad.

NOTE: A gradient range must be between 1 and 24°C.

NOTE: A gradient step can also include an **Extend** parameter but cannot include parameters such as **Increment**, **Beep**, or **Ramp Rate**.

- 4. Touch the **OK** button to continue.
- 5. Once a step has a gradient, the upper and lower temperatures can be edited by touching either button and using the gradient editor window (Figure 15).

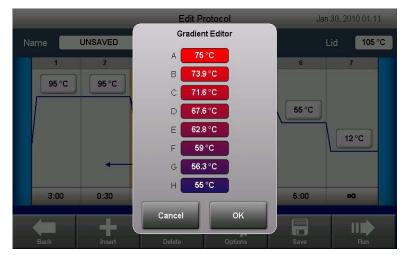


Figure 15. The gradient editor window.

To remove the temperature gradient:

- Select the gradient step and then touch the **Options** button. The **Step Options** window opens.
- 2. Touch the **Gradient** checkbox to remove the check mark.

Touch the **OK** button to confirm.

#### **Changing the Ramp Rate**

To emulate a thermal cycler that runs at a slower ramp rate than the C1000 Touch thermal cycler, change the ramp rate of the appropriate protocol step. To modify the ramp rate:

- 1. Select a step in the protocol and touch the **Options** button.
- 2. Touch the Ramp Rate button.
- 3. Enter a value using the pop-up numeric keypad.
- 4. Touch the **OK** button to confirm.
- 5. To remove a ramp rate, select a step in the protocol and then touch the **Options** button. The **Step Options** window opens. Touch the **Ramp Rate** button and use the back button on the pop-up numeric keypad to clear the entry.
- 6. Touch the **OK** button to confirm.

#### **Adding or Removing a Temperature Increment**

An increment raises or lowers the target temperature for a step with each cycle. To add or remove a temperature increment:

- 1. Select a step in the protocol and touch the **Options** button.
- 2. Touch the **Increment** button.
- 3. Enter a value using the pop-up numeric keypad.
- 4. Touch the **OK** button.
- 5. To remove an increment, select a step in the protocol and then touch the **Options** button.

The **Step Options** window opens. Touch the **Increment** button and use the back button on the pop-up numeric keypad to clear the entry.

6. Touch the **OK** button to confirm.

#### Adding or Removing an Extend Time

To increase or decrease the hold time with each cycle, use the extend time function:

- 1. Select a step in the protocol and touch the **Options** button.
- 2. Touch the Extend button.
- 3. Enter a value using the pop-up numeric keypad.
- 4. Touch the **OK** button to confirm.
- 5. To remove an extend time option, select a step in the protocol and then touch the **Options** button.

The **Step Options** window opens. Touch the **Extend** button and use the back button on the pop-up numeric keypad to clear the entry.

6. Touch the **OK** button to confirm.

#### Adding or Removing a Beep

A beep is a sound that the thermal cycler emits when it reaches the target temperature of a protocol step. To add or remove a beep:

- 1. Select a step in the protocol and touch the **Options** button.
- 2. Touch the **Beep** checkbox. A check mark will appear in the **Beep** checkbox.
- 3. Touch the **OK** button to confirm.
- 4. To remove the beep, select a step in the protocol and then touch the **Options** button. The **Step Options** window opens. Delete the beep by touching the **Beep** checkbox to remove the check mark.
- 5. Touch the **OK** button to confirm.

#### **Changing Parameters in a GOTO Step**

The **GOTO** step instructs the thermal cycler to repeat a set of steps in a loop. This step creates a cycle in the PCR experiment. Figure 16 shows an example where the step parameters include a **GOTO** step 1, and 34 additional repeats.

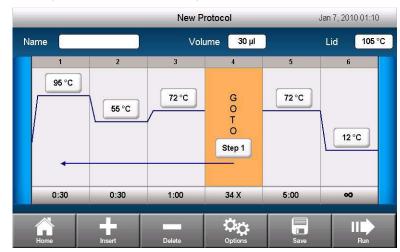


Figure 16. A protocol that includes a GOTO step 1 and 34 additional repeats.

To change parameters in a GOTO step, follow these instructions:

- 1. To change the **GOTO** step number, touch the **Step** number button.
- 2. Enter a value using the pop-up numeric keypad.
- 3. Touch the repeat times button to adjust the number of repeated sets of steps in a **GOTO** loop.
- 4. Enter a value using the pop-up numeric keypad.
- 5. Touch the **OK** button to confirm.

## **Sample Volume and Lid Temperature**

• Sample volume - determines the temperature control mode

• Lid temperature — determines the temperature of the heated lid. If the temperature is too high, the sample temperature might rise above the target temperature

The C1000 Touch thermal cycler provides two ways of managing and entering sample volume and lid temperature settings:

- Change the setting when creating and editing the protocol (page 18)
- Change the setting while initiating a run (page 21)

#### **Temperature Control Modes**

The C1000 Touch thermal cycler uses one of two temperature control modes to determine when the sample reaches the target temperature:

- **Calculated mode** When a sample volume of greater than zero is entered, the thermal cycler calculates the sample temperature based on the sample volume. The calculated mode is recommended, because it most accurately represents the actual sample temperature
- Block mode when a sample volume of zero (0) µl is entered, the thermal cycler assumes that the sample temperature is the same as the measured block temperature

#### **Choosing Lid Temperature**

The adjustable heated lid of the C1000 Touch thermal cycler allows the user to control the lid temperature and force. Heating the lid prevents condensation from forming inside the reaction wells. When the C1000 Touch thermal cycler is running, the heated lid maintains the 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 default lid temperature of the C1000 Touch thermal cycler is 105°C for 96- or dual 48-well reaction blocks and 95°C for 384-well blocks.

NOTE: When the block is running an infinite hold at a temperature below 30.0°C, the lid heater maintains 31.0°C.

#### **Changing Sample Volume**

To change the sample volume from the new protocol or protocol editor screen:

- 1. Touch the Volume button.
- 2. Enter a value using the pop-up numeric keypad.
- 3. Touch the **OK** button to confirm.

NOTE: Entering a value greater than zero in the **Volume** button selects **Calculated Temperature** control mode, which is the standard mode. Entering **zero (0)** in the **Volume** button selects **Block mode**. Calculated mode is the recommended mode because it most accurately represents the actual sample temperature.

#### **Changing Lid Temperature**

When using a 96- or dual 48-well reaction block, the recommended lid temperature is 105°C. When using a 384-well block, the recommended lid temperature is 95°C.

To change the lid temperature from the new protocol or protocol editor screen:

1. Touch the **Lid** button.

- 2. Enter a value using the pop-up numeric keypad.
- 3. Touch the **OK** button to confirm.

# **Naming a Protocol**

A new protocol requires a name, which can be changed later if desired. When editing an existing protocol, the user has the option of renaming the protocol. Renaming the protocol does not remove the original protocol.

To name a protocol from the new protocol or protocol editor screen:

- 1. Touch the Name button.
- 2. Enter a name using the pop-up alphanumeric keypad.
- 3. Touch the **OK** button to confirm.

NOTE: A protocol name on a C1000 Touch cycler can include up to twelve letters and numbers. It cannot be duplicated in the same folder.

#### **Saving a Protocol**

To save a new or modified protocol:

- 1. Touch the **Save** button once the protocol has been edited and is ready to be saved. Saving the protocol with the same name replaces the original protocol. Saving the protocol with a new name adds a new protocol, and does not remove the original protocol or its name.
- 2. To save the protocol with a new name, touch the **File Name** button and enter a new name.
- Select the destination and folder using the corresponding dropdown lists. In Figure 17, the Adam folder is selected. Touch New Folder to create a new folder, if desired.



Figure 17. The Adam folder is selected in this example.

4. Touch the **Save** button.

# **3 Running Protocols**

Read this chapter for instructions on running protocols.

- Preparing to run a protocol (page 21)
- Monitoring a run (page 23)
- Pausing and resuming a run (page 24)
- Skipping steps in a protocol (page 25)
- Canceling a run (page 25)
- Incubating samples (page 25)

#### **Preparing to Run a Protocol**

To start a run, use one of the following methods:

- Select a protocol from the saved files library, then touch the Run button
- Edit a protocol from the saved files library, then touch the Run button
- Create a new protocol, then touch the **Run** button
- 1. If more than one block is connected to the C1000 Touch<sup>™</sup> thermal cycler, the select blocks window opens.
- 2. Select the reaction module blocks on which to run the protocol by touching the checkbox next to the desired blocks. Alternatively, touch the **Select All** button to select all available blocks. A check mark appears in the checkbox to indicate that a block is selected.
- 3. When running a protocol on more than one block, determine whether you want to start blocks individually or simultaneously. Touch the **Confirm all blocks individually** checkbox when starting the runs at different times.
- 4. Touch the **OK** button to confirm.



In Figure 18, the protocol will start running on both blocks at the same time.

Figure 18. Multiple blocks running the same protocol at the same time.

5. Confirm or change the run parameters before starting the run.

TIP: The value entered in the **Sample Volume** field determines the temperature control mode. If a sample value greater than 0 (zero) is entered, the instrument uses Calculated Mode to calculate the target temperature. If 0 (zero) is entered in the **Sample Volume** field, the Block Mode is used, in which case the thermal cycler assumes that the temperature of the sample is the same as that of the block.

In Figure 19, the run parameters include the sample volume of 10ul and the lid temperature of 105°C. (Touch the **OK** button to bypass these fields and run the protocol without entering additional parameters).



Figure 19. The run parameters of the protocol.

6. To add optional additional settings, touch the **Additional Settings** button to enter a user name, email address (to send an email notification upon run completion), sample ID or to set a ramp rate for all steps of a protocol (Figure 20). Also, run a protocol on the C1000 Touch thermal cycler at the same ramp speed as that of the DNA Engine® thermal cycler by touching the **DNA Engine Ramp** checkbox.

	Sav	ed Files	Jan 30, 2010 02:53
Locations	Additio	nal Settings	<i>i</i> ew /
Recent	User Name	PETE	METHOD CALC HOTLID 105,30
LP	Email Address	@BIO-RAD.C	OM VOLUME 30 TEMP 95.0,180
Real-Time Dat	Sample ID	TEST	TEMP 95.0,30 TEMP 55.0,30
	Ramp Rate	1.5 °C/s	TEMP 72.0,60 GOTO 2,34
S JOHN		DNA Engine Ramp	TEMP 72.0,300           TEMP 12.0,∞
8 Pete			END
Į. T	Cancel	ок	
Home			

Figure 20. Add additional settings to your protocol including running in DNA Engine Ramp mode and sending an email notification upon run completion.

- 7. Touch the **OK** button to confirm.
- 8. Start the run by touching the **OK** button on the run confirm window.

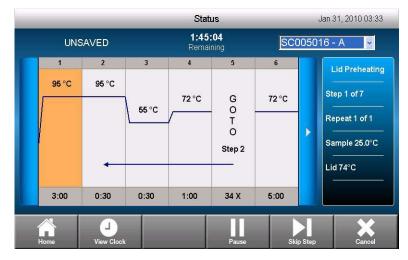
#### **Monitoring a Run**

During a run, the status buttons on the home screen (buttons contains an icon of the instrument and time remaining) provide a quick way to monitor all blocks.

To see the detailed run status for a particular thermal cycler, touch the status button for the cycler of interest.

NOTE: If a thermal cycler has a dual 48/48 reaction module, you may touch the individual reaction blocks labeled A and B to see the detailed run status of each block. Once on the status screen, choose other reaction blocks to view by selecting them from the dropdown list.

9. For an actively running block, touch **View Clock** to switch between screens showing status graphically (Figure 21) and status by time remaining (Figure 22).



Touch View Status to return to the status screen.

Figure 21. The status screen.



Figure 22. Screen shows status by time remaining.

#### **Pausing and Resuming a Run**

A running protocol may be temporarily paused from the protocol status screen. During a pause, the thermal cycler continues heating or cooling to the target temperature, while the lid temperature is maintained.

TIP: After a power failure, the C1000 Touch thermal cycler automatically resumes running a protocol but also displays a warning message.

To pause and then resume a running protocol:

1. Touch the **Pause** button on the status screen.

**WARNING!** Pausing a step can adversely change the outcome of the PCR reaction. If the protocol is paused during a temperature step, the PCR reaction stays at the target temperature for a longer hold time than the protocol step requires.

2. To resume the protocol, touch the **Resume** button.

#### **Skipping Steps in a Protocol**

Skip a step if it is necessary to shorten a protocol while it is still running. By repeatedly skipping steps, it is possible to bypass several cycles of a GOTO loop and shorten a protocol.

- 1. To skip the current protocol step in the status screen, touch the Skip button.
- 2. Touch the Skip button multiple times to skip more than one step.

#### **Canceling a Run**

A protocol may be canceled while it is running. When the protocol is canceled, the block immediately stops changing temperature.

NOTE: Do not turn the thermal cycler off immediately after canceling a run. The fans should be allowed to run long enough to cool the block.

To cancel a protocol running on a single block:

- 1. Open the status screen.
- 2. Touch the **Cancel** button to stop the protocol running on a single block.

NOTE: To cancel a protocol running on multiple blocks, select each block individually from the dropdown list and touch the **Cancel** button. Continue until the protocol is canceled on all blocks.

#### **Incubating Samples**

You may keep samples at a constant temperature for any amount of time.

**WARNING!** Incubating samples for extended periods of time at 4–10°C, particularly in areas of high humidity, can cause excessive moisture condensation around the block.

To start incubating samples at a single temperature:

1. Touch the **INCUBATE** button on the home screen.

The Incubate screen opens (Figure 23).

	Jan 31, 2010 03:35	
	Block temperature	
	50 °C	
	Hold time	
	∞ Noid arrie	
	Lid temperature 105 °C	
	105°C	
Home		Run

Figure 23. The Incubate screen.

NOTE: The default block temperature, hold time, and lid temperature will be the same as the most recently run incubation.

NOTE: When incubation is occurring at temperatures below 30°C, the lid will maintain a temperature of 31°C to prevent excessive condensation.

- 2. Enter the **Block temperature**, **Hold time**, and **Lid temperature** by touching the corresponding buttons and entering values using the pop-up numeric keypad.
- Touch the **Run** button to start the incubation.
   NOTE: If multiple blocks are connected, the user can select the appropriate blocks

to run the incubation.

4. To end the incubation and view the final status screen, touch the **Cancel** button.

# **4 Protocol AutoWriter**

Read this chapter for information on using the Protocol AutoWriter.

- Creating a protocol with the Protocol AutoWriter (page 27)
- Overview of the Protocol AutoWriter (page 29)
- The T<sub>a</sub> calculator (page 30)

## **Creating a Protocol with the Protocol AutoWriter**

Follow these steps to use the Protocol AutoWriter for creating a new protocol:

- 1. Touch the Protocol AutoWriter button on the home screen.
- 2. Select the enzyme to be used for the reaction.

The default selection for the polymerase is **iTaq**<sup>™</sup> DNA polymerase. If iProof<sup>™</sup> DNA polymerase is used, touch the **iProof** radio button to select iProof polymerase.

NOTE: If iProof polymerase is selected, the protocol automatically adds  $3^{\circ}$ C to the  $T_a$  value. If the **Other** radio button is selected, additional options are presented, such as Hot Start Activation (Figure 24).

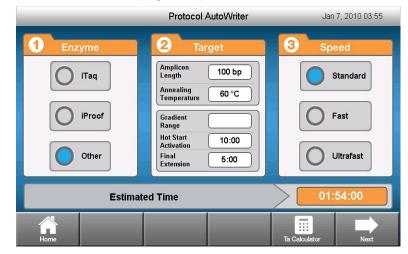


Figure 24. If Other is selected for the enzyme, additional options are presented.

3. Touch the Amplicon Length button and enter the amplicon length in base pairs (bp).

- 4. Touch the **Annealing Temperature** button and enter the  $T_a$  in °C.
- 5. Touch the radio button corresponding to the desired reaction speed.

The annealing temperature and estimated total run time will change as the speed is changed. The annealing temperature used in the program will be 4°C below the primer average  $T_m$  for the standard speed and 2°C below the primer  $T_m$  average for fast speed. The unadjusted primer  $T_m$  average will be used for the ultrafast speed.

- Touch the Next button to review the new protocol in the edit protocol screen.
   To edit the protocol, see "Creating, Editing, Naming, and Saving Protocols" on page 11.
- 7. Touch the **Back** button to return to the Protocol Autowriter screen.
- 8. Touch the Save button to save the protocol or the Run button to run the protocol.

#### Creating a Protocol when the T<sub>a</sub> is Not Known

When the T<sub>a</sub> is unknown, use the instructions to create a protocol with the Protocol AutoWriter.

- 1. Touch the Protocol AutoWriter button on the home screen.
- 2. Touch the T<sub>a</sub> Calculator button.
- 3. Touch the **Forward Primer** button and enter the primer sequence using the **A**, **T**, **G**, **C** buttons. Touch the **OK** button to confirm.
- 4. Touch the **Reverse Primer** button and enter the primer sequence using the **A**, **T**, **G**, **C** buttons. Touch the **OK** button to confirm.

To correct a wrong base entry in the sequence entry pop-up window, press the **BACK** button and erase the previously added base.

To erase an entire primer sequence in the T<sub>a</sub> calculator pop-up window, touch the corresponding clear button.

The Protocol AutoWriter calculates and displays the  $T_m$  of each primer and the average  $T_m$  and  $T_a$  values (Figure 25).



Figure 25. The  $T_m$  of each primer and the average  $T_m$  and  $T_a$  values are calculated.

NOTE: If the primer  $T_m$  values are greater than 4°C apart, the Protocol AutoWriter uses the lower primer  $T_m$  value + 2°C as a basis for calculating the  $T_a$  value, which can be further modified by the enzyme and reaction speed selections. NOTE: The  $T_a$  calculator generates an annealing temperature for standard speed

with iTaq DNA polymerase. When using a different enzyme, the speed settings automatically adjust the  $T_a$ .

5. Touch the **OK** button to return to the Protocol AutoWriter screen.

#### **Overview of the Protocol AutoWriter**

The Protocol AutoWriter uses standard PCR guidelines that automatically generate cycling protocols with initial template denaturation and enzyme activation, followed by cycles of denaturation, annealing, and extension, and then final extension steps. Protocols are based on user input parameters of target amplicon length, enzyme type, and annealing temperature or primer sequences.

Protocols generated by the Protocol AutoWriter at various speed settings (**standard**, **fast**, and **ultrafast**) may result in different product yields, because the software may adjust the annealing temperature, reduce the total number of protocol steps or GOTO repeats, shorten hold times, or reduce the temperature differentials between steps to generate these protocols.

The Protocol AutoWriter uses established PCR standards that reference data tables to produce the final suggested protocols: These are either a standard two-step protocol or a three-step protocol with a final extension step.

The steps and range of values generated by the Protocol AutoWriter are as follows:

- 1. **Initial hot start activation/denaturation step** this step should be 95–98°C for 30 or 180 sec, depending on the enzyme type and speed setting used.
- 2. **Denaturation step** this step should be either 95 or 98°C for 5–30 sec, depending on the enzyme type and speed setting used.
- 3. **Annealing step** for this step, the Protocol AutoWriter uses either the primer annealing temperature (T<sub>a</sub>) at **standard** speed that is calculated by the T<sub>a</sub> calculator or a value that has been entered by the user.
  - Length of annealing time this range is 10–30 sec depending on the speed setting. Two-step protocols combine annealing and extension steps
  - The annealing temperature  $(T_a)$  this temperature is calculated based on primer characteristics and selected reaction speed. If the iProof enzyme is selected, 3°C are added to the  $T_a$  value
- 4. Extension step (for three-step protocols only) all extension steps are performed at 72°C with the duration based on calculating the times from set times per kbp (for example, 60 sec/kbp) of the largest amplicon in a size bin, often with some modifications at the smaller amplicon sizes.
- 5. **Number of repeats** the steps are repeated 25–40 times depending on the speed selected.
- Final extension step this step is performed at 72°C for 1–5 min depending on speed settings.

The Protocol AutoWriter uses information about the reaction to automatically write a protocol. This feature enables the user to enter the following information about the PCR experiment:

• **Primers** — enter the reaction  $T_a$  for the primers being used. If the  $T_a$  is not known, enter the primer sequence in the  $T_a$  calculator so that the Protocol AutoWriter calculates this value

NOTE: The  $T_a$  is adjusted from the primer melting temperature ( $T_m$ ) information that is based on the selected enzyme and the protocol speed selected.

- Amplicon length enter the expected length of the PCR product
- Enzyme type enter the DNA polymerase enzyme (iTaq, iProof, or Other)
   If an enzyme other than iTaq or iProof DNA polymerase is to be used, enter additional information including the hot-start activation time and the final extension time
- Desired run speed select a reaction speed (standard, fast, or ultrafast). The Protocol AutoWriter optimizes the protocol depending on the selected speed setting

The total run time is determined by the number of steps and cycles, the incubation time at each step, and the time it takes to reach uniformity at the target temperature.

To reduce the overall run time, the Protocol AutoWriter reduces one or more of the following:

- The total number of protocol steps (for primers with high T<sub>m</sub> values, the annealing and extension steps can be combined into one step)
- The number of cycles
- The hold time in each temperature step
- The ramp time between steps (by reducing the temperature change from one step to the next)

For example, the Protocol AutoWriter may shorten a protocol by:

- Changing the initial template denaturation and enzyme activation step from 95°C for 3 min to 98°C for 30 sec hold time in each temperature step
- Changing the denaturation step in each cycle from 95°C for 30 sec to 92°C for 1 sec
- Combining the annealing and extension steps into a single step at 70°C for 20 sec

#### The T<sub>a</sub> Calculator

The  $T_a$  calculator calculates the  $T_m$  value for each primer as well as the  $T_a$  value for the protocol at **standard** speed.

The  $T_a$  for the protocol is based on the average primer  $T_m$  values with the following applied:

- If the difference between the primer  $T_m$  values is > 4°C, the  $T_a$  = (lower of the two primer  $T_m$  values + 2) 4°C
- If the difference between the T<sub>m</sub> values is < or = 4°C, the T<sub>a</sub> = (average of the primer T<sub>m</sub> values) 4°C

For each primer, the  $T_a$  calculator uses the base pair counting method for sequences of 14 pairs or less.

•  $T_m = ((w^*A + x^*T) * 2) + ((y^*G + z^*C) * 4)$ 

where **w**, **x**, **y**, and **z** are the number of the bases **A**, **T**, **G**, and **C** in the sequence, respectively.

For sequences longer than 14 bp, the nearest neighbor method is used:

In the nearest neighbor method, the melting temperature calculations are based on the thermodynamic relationship between entropy (order or a measure of the randomness of the oligonucleotide), enthalpy (heat released or absorbed by the oligonucleotide), free energy, and temperature, where:

 $\Delta H = \Delta G + T^* \Delta S$  where

△H = Enthalpy value, Cal/Mole\*K

T = temperature, Kelvin.

 $\Delta S$  = Entropy value, Cal/Mole\*K

 $\Delta G$  = Gibbs free energy in Cal/Mole\*K

The change in entropy and enthalpy is directly calculated by summing the values for nucleotide pairs shown in Figure 26 (Breslauer et al. 1986).

The relationship between the free energy and the concentration of reactants and products at equilibrium is given by:

 $\Delta G = R^T \ln ((DNA * Primer)/(DNA + Primer))$ 

where R is the gas constant (1.986 Cal/Mole\*K)

Substituting G in the two equations and solving for T gives

 $T = \underline{\land} H / (\underline{\land} S + R^* Ln((DNA * Primer) / (DNA + Primer)))$ 

Assumes the concentration of DNA and the concentration of the DNA-primer complex are equal.

It has been determined empirically that there is a 5 kcal free energy (3.4 kcal (Sugimoto et al. 1996) change during the transition from single-stranded to B-form DNA.

This is presumably a helix initiation energy. Finally, adding an adjustment for salt gives the equation that the  $T_a$  calculator uses:

 $T = (\triangle H - 5(KCal/K*Mole))/(\triangle S + (R * ln(1/(primer)))) + 16.6 \log_{10}(SaltMolarity)$ 

No adjustment constant for salt concentration is needed, since the various parameters were determined at 1 M NaCl, and the log<sub>10</sub> of 1 is zero.

The thermodynamic calculations assume that the annealing occurs at pH 7.0. The  $T_m$  calculations assume that the sequences are not symmetric and contain at least one **G** or **C**.

INTER	ACTION	Delta (H)	Delta (S)	Delta (G)
AA	TT	9.1	24	1.9
AT	ТА	8.6	23.9	1.5
AC	TG	6.5	17.3	1.3
AG	TC	7.8	20.8	1.6
TA	AT	6	16.9	0.9
TT	AA	9.1	24	1.9
TC	AG	5.6	13.5	1.6
TG	AC	5.8	12.9	1.9
CA	GT	5.8	12.9	1.9
СТ	GA	7.8	20.8	1.6
CC	GG	11	26.6	3.1
CG	GC	11.9	27.8	3.6
GA	CT	5.6	13.5	1.6
GT	CA	6.5	17.3	1.3
GC	CG	11.1	26.7	3.1
GG	CC	11	26.6	3.1

The oligonucleotide sequence should be at least 14 bases long to give reasonable  $T_m$  values. Less than 14 bases uses the base pair counting method (Figure 26).

Figure 26. Breslauer interaction constants.

## **5** Managing Files and Folders

Read this chapter for information on managing protocol files and folders.

- The saved files screen (page 33)
- Managing files and folders (page 34)

#### **The Saved Files Screen**

Many functions begin with choosing a protocol from the saved files screen. For example, before running or editing a protocol, select the desired protocol file:

- Touch Run to run the selected protocol
- Touch Edit to edit the selected protocol

To open the saved files screen, touch the **Saved Files** button on the home screen. The saved files screen provides access to multiple locations and folders (Figure 27).

	Save	d Files	Jan 31, 2010 03:40	
Locations	Folders	Files	Preview	
Recent	<root></root>	IPRF15KB	Method: Calc Lid: 105°C	
C005016	Bio-Rad PCR	IPRF1KB	Volume: 10µl	
Real-Time Data	Bio-Rad qPCR	IPRF8KB	1.98°C,0:30 2.98°C,0:05	
		ISCRIPT	3. 60°C, 0:10 4. 72°C, 7:30	
S Pete		ITAQEST	5. Goto 2, X29 6. 72°C, 5:00	
USB Drive		NESTPR2	7.12°C,0:00	
Home New F	Folder Folder Options	File Options		

Figure 27. The saved files screen of the C1000 Touch™ thermal cycler.

• Thermal cycler location — contains protocols stored on the thermal cycler, including the **Bio-Rad PCR** folder that stores the preinstalled PCR protocols. If a CFX96<sup>™</sup> or CFX384<sup>™</sup> optical reaction module has been installed on the base, this location will also have a **Bio-Rad qPCR** folder that contains preinstalled qPCR protocols.

- Recent location contains a list of all previously run protocols
- Real-time data location contains the last 100 real-time PCR data files
- User locations contains folders created by users for personal protocol storage. The user folders can be accessed by everyone, but only a user who is logged in can edit, delete, or save into user folders
- Attached locations (optional) exist when external devices such as USB flash drives or S1000<sup>™</sup> cyclers are attached to the C1000 Touch thermal cycler

#### **Managing Files and Folders**

To manage files and folders, touch the **Saved Files** button on the home screen to open the saved files screen. The menu of functions in the saved files screen provides options for managing files and folders; the menu changes based on what is selected. Table 8 lists all the folder and file functions available in the saved files screen. Folder and file names can have a maximum of 12 characters when stored on a C1000 Touch instrument. Folder and file names have a maximum of 8 characters when stored on an S1000 instrument.

Function	Description	
Folder Options		
Сору	Copies an existing folder to another cycler or USB flash drive	
Delete	Deletes the folder and all of its contents	
Rename	Renames a folder	
File Options		
Сору	Copies an existing file to another folder location	
Delete	Deletes a file	
Rename	Renames a file	
New Folder	Creates a new folder in the C1000 Touch thermal cycler, an attached S1000 thermal cycler, or a USB flash drive	
Home	Returns to the home screen	

Table 8. List of folder and file functions in the saved files screen.

#### **Creating a New Folder**

To create a new folder:

- 1. Touch the Saved Files button on the home screen.
- 2. Select a Location.

	d Files	Jan	31, 2010 03:42		
Locations	Folders	Files	Preview	v	
Recent	<root></root>	PCR2	Method: Calc Lid: 105°C		
SC005016	Bio-Rad PCR		Volume:	30µl	
Real-Time Data	Bio-Rad qPCR		1.95°C, 2.95°C,	0:30	
C ADMIN				3. 55°C, 0:30 4. 72°C, 1:00	
S Pete				5. Goto 2, 34X 6. 72°C, 5:00	
VSB Drive			7.12°C,	0:00	
Home New F	Folder Folder Options	File Options	<b>L</b> í	Run	

In Figure 28, the new folder will be created in the C1000 Touch thermal cycler location (named SC005016).

Figure 28. Select the location where the folder will be created.

3. Touch the **New Folder** button and enter a folder name using the pop-up alphanumeric keypad. Then touch the **OK** button to confirm.

#### **Copying a Folder**

To copy a folder to another location:

- 1. Touch the **Saved Files** button on the home screen.
- 2. Select the folder to be copied.
- 3. Touch the Folder Options button.
- 4. Touch the **Copy** button.
- 5. Touch the **Folder Name** button if you wish to change the copied folder name.
- 6. Select a new destination from the Location dropdown list as shown in Figure 29.



Figure 29. New location for copied folder.

7. Touch the **Save** button.

#### **Deleting a Folder**

To delete a folder and all of its contents:

- 1. Touch the Saved Files button on the home screen.
- 2. Select the folder to be deleted.
- 3. Touch the Folder Options button.
- 4. Touch the **Delete** button.
- 5. Touch the Yes button to confirm.

#### **Renaming a Folder**

To rename a folder:

- 1. Touch the Saved Files button on the home screen.
- 2. Select the folder to be renamed.
- 3. Touch the Folder Options button.
- 4. Touch the **Rename** button.
- 5. Enter a new folder name by using the pop-up alphanumeric keypad.
- 6. Touch the **OK** button and confirm that the folder was successfully renamed.

#### **Copying a File**

To copy a file:

- 1. Touch the Saved Files button on the home screen.
- 2. Select the file to be copied.
- 3. Touch the File Options button.
- 4. Touch the **Copy** button.
- 5. Touch the File Name button if you wish to change the copied file name.
- 6. Use the corresponding dropdown lists to select a **Location** and **Folder** to copy the file into as shown in Figure 30.

		Saved Files	Jan 7, 1	2010 04:35
Locations				
Recent		Save As		DD CALC D 105,30
CC006622	File Name	test12		UME 30 95.0,180
Real-Time I	Location	CC006622	100000	95.0,30 55.0,30
C ADMIN	Folder	Adam	TO:	72.0,60 0 2,34
名 вво		J. Malori	EMP EMP	72.0,300 P 12.0,∞
S JOHN	Cancel	New Folder	Save	END
~				
Â			1	
Home				

Figure 30. Select the new location and folder.

NOTE: If desired, touch the **New Folder** button to create a new folder to copy the file into.

7. Touch the Save button and confirm that the file was successfully copied.

#### **Deleting a File**

To delete a file:

- 1. Touch the Saved Files button on the home screen.
- 2. Select the protocol to be deleted.
- 3. Touch the File Options button.
- 4. Touch the **Delete** button.
- 5. Touch Yes to confirm.

#### **Renaming a File**

To rename a file:

- 1. Touch the Saved Files button on the home screen.
- 2. Select the protocol to be renamed.
- 3. Touch the File Options button.
- 4. Touch the **Rename** button.
- 5. Enter a new name using the pop-up alphanumeric keypad.
- 6. Touch the **OK** button to confirm.

## **6** Advanced Functions

Read this chapter for information on advanced functions on the C1000 Touch™ thermal cycler.

- Logging in to or logging off the C1000 Touch thermal cycler (page 39)
- Tools screen (page 40)
- Using communication ports on the C1000 Touch thermal cycler (page 41)
- Controlling S1000™ thermal cyclers with the C1000 Touch thermal cycler (page 42)

# Logging In To or Logging Off the C1000 Touch Thermal Cycler

Logging in to the C1000 Touch thermal cycler is optional. A user can run, edit protocol templates, and create folders and protocols without logging in. When a user selects the **New User** option, a corresponding user account is created. Only a user who is logged in can save, edit, or delete protocols in their folders.

Once logged in, a user has privileges to:

- Run a protocol on the main instrument or USERS folder
- · Create and edit protocols in the corresponding USERS folder
- Copy any protocol and save it to a USERS folder
- · Copy a protocol and store it in a USERS folder with a new name

Use the following steps to create a new user:

- 1. Touch the Log In button on the home screen to open the user login screen.
- 2. Touch the New User button.
- 3. Touch the **User Name** button and enter a user name using the pop-up alphanumeric keypad.
- 4. Touch the **Password** button to enter a user password (this is optional).
- 5. Touch the **Email Address** button to enter a user email address (this is optional). If an email address is entered, an email message will be sent to the address upon run completion if the user is logged in when a run is started.
- 6. Touch the Create User button.

Use the following steps to log in to or log off the C1000 Touch thermal cycler:

- 1. Touch the Log In button on the home screen and select a user.
- 2. Enter the password using the pop-up alphanumeric keypad, and then touch the **OK** button.

NOTE: A password can contain a combination of letters and numbers.

NOTE: The alphanumeric keypad will not be displayed if the user account does not have a password.

3. To log off the thermal cycler, touch the **Log Out** button on the home screen.

#### **Tools Screen**

The **User** menu provides a list of functions available to an operator that is either not logged in or logged in as a user. These functions control the C1000 Touch thermal cycler as shown in Figure 31.



1. Touch the **Tools** button on the home screen.

Figure 31. The User menu.

The functions in the User menu offer the following options:

- About for viewing the current firmware versions and serial numbers
- System Logs for viewing:
  - System Messages Log a list of messages that occurred during each run
  - System Usage Log a list of all events that occurred during each run
- **Run Reports** viewing the run report for each time a protocol is run. Reports can also be exported to a USB flash drive (for use when servicing the thermal cycler)
- Gradient Calculator for calculating a temperature gradient across a block

The **Administrator** menu provides a list of functions available to an operator that is logged in as the administrator. There are two ways to log in as the administrator:

- 1. Touch the **Log In** button on the home screen. Select **ADMIN**. Enter the administrator password if one has been set up.
- 2. Touch the **Tools** button on the home screen. Touch the **Admin** button. Enter the administrator password if one has been set up.

Once a user is logged in as the administrator, the **User** and **Admin** menus are visible. The administrator has access to all User and Admin functions.



Figure 32 shows some of the Admin menu functions.

Figure 32. The Admin menu.

NOTE: Only a logged in Administrator may access the Admin menu.

The functions in the Admin menu offer the following options:

- System Settings for setting the user access levels to Open or Login required. Also for setting the date and time on the thermal cycler
- User Management for adding or removing users and clearing passwords
- Email Settings for setting email server information.
- Firmware Update for updating the firmware on the thermal cycler
- USB Thumb Key Test for testing USB thumb key compatibility
- Touchscreen Calibration for calibrating the touchscreen (for use when servicing the cycler)
- System Backup and Restore for backing up or restoring system settings from a USB flash drive
- **Diagnostic Test** for performing a series or subset of diagnostic self tests on the cycler (for use when servicing the cycler)

# Using Communication Ports on the C1000 Touch Thermal Cycler

The C1000 Touch thermal cycler has several data ports that can be used to transfer data to and from the thermal cycler:

- USB A port below the front panel to transfer data to and from a USB flash drive or to attach a USB mouse or keyboard
- USB B computer port on the back panel for connecting a C1000 Touch thermal cycler to a computer
- Four USB A ports located on the back panel for connecting up to three S1000 thermal cyclers; can also be used to connect a USB flash drive, mouse, or keyboard
- Serial test port on the back panel for testing the thermal cycler by service personnel

• Ethernet port — for connecting the thermal cycler to a network

### **Controlling S1000 Thermal Cyclers with the C1000 Touch Thermal Cycler**

Up to **three** S1000 thermal cyclers can run under the control of a C1000 Touch thermal cycler. Each S1000 thermal cycler can be connected to the C1000 Touch thermal cycler through the USB A ports of the C1000 Touch thermal cycler.

When connected to a C1000 Touch thermal cycler, the S1000 thermal cyclers can be controlled by either the touchscreen on the C1000 Touch thermal cycler or by a PC connected to the C1000 Touch thermal cycler and running CFX Manager™ 2.1 software (or higher).

For detailed instructions on running the S1000 thermal cycler when it is connected to a C1000 Touch thermal cycler, refer to the instruction manual for the S1000 thermal cycler.

#### **Connecting S1000 Cyclers Directly to a C1000 Touch Thermal Cycler**

To connect up to three S1000 thermal cyclers directly to a C1000 Touch Thermal Cycler, follow these instructions:

- 1. Plug a high quality, shielded USB cable (such as the Bio-Rad USB cable catalog # 184-8000) into the USB B port on the back of the S1000 thermal cycler.
- 2. Plug the other side of the USB cable into a USB A port on the back of the C1000 Touch thermal cycler.

The C1000 Touch Thermal Cycler instrument detects the attached S1000 thermal cycler and displays the current status on the C1000 Touch thermal cycler home screen.

NOTE: If the S1000 thermal cycler instrument has a name, then the name is displayed on the status button instead of the serial number.

3. Repeat steps 1 and 2 to connect up to three S1000 cyclers directly to the same C1000 Touch thermal cycler.

#### **Operating S1000 Thermal Cyclers Under the Control of the C1000 Touch Thermal Cycler**

When an S1000 thermal cycler is under the control of the C1000 Touch Thermal Cycler cycler, it is in semi-lockdown mode. In this mode, the S1000 thermal cycler does not respond when control panel keys are pressed. However, the following keys function on the control panel:

- SCREEN to access the running, graphical, and time remaining screens
- PAUSE to temporarily stop a protocol that is currently running on the S1000 thermal cycler. This function is active when an individual protocol screen is being displayed
- CANCEL to cancel a protocol that is currently running on the S1000 thermal cycler. This function is active when an individual protocol screen is being displayed
- **ENTER** to begin a run that has been sent remotely from the CFX Manager software
- ENTER to skip a step. This function is active when an individual protocol screen is being displayed

## 7 Maintenance and Troubleshooting

Read this chapter for information on maintaining the C1000 Touch™ thermal cycler and troubleshooting problems.

- Cleaning and maintaining the C1000 Touch thermal cycler (page 43)
- Maintaining sufficient airflow (page 45)

#### **Cleaning and Maintaining the C1000 Touch Thermal Cycler**

The C1000 Touch thermal cycler requires little maintenance for proper operation and precise thermal control. However, with long and constant use, the thermal cycler requires some cleaning and other maintenance. Information on cleaning the thermal cycler base and reaction module is included in this chapter. In addition, instructions on replacing the fuses are provided.

#### **Cleaning the C1000 Touch Thermal Cycler**

The C1000 Touch 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.

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 thermal cycler base, follow the instructions below; pay careful attention to the warnings:

**WARNING!** To 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 airflow for precise thermal control during a run
- **Clean the touchscreen.** Clean the touchscreen with any commercially available touchscreen cleaning agent. Use a soft cloth to clean the touchscreen

WARNING! Do not spray or pour cleaning agent directly onto the touchscreen

**WARNING!** Do not use abrasive detergents or rough material; they can scratch the control panel.

• Clean the reaction module bay. Clean with a damp soft cloth to remove debris and spilled liquids. Cleaning the 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; surface scratches can 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 case of the thermal cycler base. Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and remove the residue completely. Cleaning the outside case prevents corrosion

#### **Cleaning the Reaction Modules**

Clean the reaction modules of the C1000 Touch thermal cycler on a regular schedule to prevent reagents from accumulating and interfering with the ability of the reaction block to change temperature quickly.

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

**WARNING!** To prevent electrical shock, always remove the reaction module from the thermal cycler base before cleaning it.

- **Clean the cooling fins.** Remove dust from the cooling fins with a soft brush or damp cloth. Remove any heavy dust that is deep in the fins 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 the residue completely. Cleaning the fins improves precise sample heating and cooling
- Clean the outside cover of the reaction block. Use a soft cloth and water to remove debris from the outer block

**WARNING!** Never clean the block with strong alkaline solutions (strong soap, ammonia, or highly concentrated 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 inside wells. Use disposable plastic pipets 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!** If left in the block wells, bleach, ethanol, or soap 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.

• If oil is used, the wells must be cleaned thoroughly and often. Use of oil in the wells is not recommended. 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 damages the block, lid, and thermal cycler base.

• Clean the inner lid of the reaction module. Use a soft cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material that scratch the surface. Cleaning the inner lid improves precise sample heating and cooling

• Clean the outer lid surface of the reaction module. Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and rinse the surface with a damp cloth. Cleaning the cover prevents corrosion

### **Maintaining Sufficient Airflow**

The C1000 Touch thermal cycler requires sufficient airflow to heat and cool precisely to the correct target temperature. If the flow of air is blocked, the thermal cycler cannot ramp to the correct temperature in the specified time. This section includes instructions for testing the airflow and provides suggestions for fixing low or warm airflow.

#### **Testing for Sufficient Airflow**

The airflow is sufficient when the thermal cycler heats and cools to the correct target temperatures promptly. When the C1000 Touch thermal cycler is first set up in a new location, use the following steps to determine the presence of sufficient airflow:

- 1. Set up the instrument in the location where it is going to be used, then turn the power on.
- 2. Adjust the local environment for typical conditions.

Turn on any nearby equipment, such as fans. Also open any window blinds to reproduce typical 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 min.

To run a protocol, samples are not required; however, an empty microplate or tubes should be included. The lid does not heat correctly if it touches the hot block of the reaction module.

4. Measure the air temperature at the air intake vents of all the thermal cyclers.

If the air intake temperature increases above 31°C, see "Fixing Insufficient Airflow" 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 the flow of cooler air around the thermal cycler:

- Adjust air conditioning to lower the ambient air temperature
- Move the thermal cycler to another location
- Provide more space around the C1000 Touch thermal cycler and between adjacent instruments. Arrange instruments so that the warm exhaust air from one instrument does not enter the air intake vents of another
- Shield the thermal cycler from heat sources such as radiators, heat-producing instruments, and bright sunlight

### **Replacing Fuses**

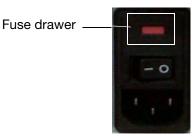
Fuses on the C1000 Touch thermal cycler are designed to blow in case of severe power surges or other causes of electrical short. This process protects both the user and the instrument from excessive electric charge. Fuses on the C1000 Touch thermal cycler rarely need to be replaced. However, some institutions prefer to replace fuses on a regular basis to maintain uninterrupted operation.

If the thermal cycler does not turn on, first check that the power cord is plugged in to a functioning power source. Also, check that the power cord and power source are within the specifications for this instrument. To replace a power cord, contact Bio-Rad Technical Support (for more details, see "Bio-Rad resources." on page ii).

Finally, check that the fuses are intact. The C1000 Touch thermal cycler runs with two fuses (Figure 33). To remove and check the fuses, follow these steps:

**WARNING!** To prevent electrical shock, always turn off and unplug the instrument from an electrical outlet before checking the fuses.

1. Use a small coin to open the fuse drawer.



#### Figure 33. The fuse drawer on the back of the C1000 Touch thermal cycler.

- 2. Pull out the fuse drawer and remove each fuse.
- 3. If a fuse is damaged, replace it with a new fuse, and close the drawer.

A bad fuse shows a break or burned spot in the metal. A good fuse has intact metal.

## **Appendix A: References**

- 1. Breslauer KJ, et al. (1986). Predicting DNA duplex stability from the base sequence, Proc Nat Acad Sci 83, 3746–3750.
- 2. Sugimoto N, et al. (1996). Improved thermodynamic parameters and helix initiation factor to predict stability of DNA duplexes, Nucleic Acids Res 24, 4501–4505.



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