User's Guide

CE

Exicycler™ 96

Real-Time Quantitative Thermal Block



Cat. No.: A-2060





Exicycler[™] 96

Real-Time Quantitative Thermal Block

(Installation and Calibration Guide)

User's Guide

Version No.: 1.2 (2013-12)

Please read all the information in booklet before using the instrument



Bioneer

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Warranty and Liability

All Bioneer products are tested under extensive Quality Control procedures. Bioneer guarantees the quality under the warranty period. Any problems should be reported immediately. Liability is conditional upon the customer providing full details of the problem to Bioneer. Once the problem occurrs, customers must report to Bioneer within 30 days.

QC Testing

Each lot of Bioneer's product is carefully tested by the quality control team.

Notice

Patent Pending

Exicycler is a trademark of Bioneer Corporation.

Certain applications of this product are covered by pending or issued patents in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used. For more information, please contact Legal manager, Bioneer Corporation, 49-3, Munpyeong-dong, Daedeok-gu, Daejeon 306-220, Republic of Korea.

Appearance and specification are subjected to change without notice.

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Before Starting

How to Use This Guide

Who This Guide is Written for

This guide is written for researchers and laboratory staff responsible for installing and maintaining the Exicycler[™] 96 Real-Time Quantitative Thermal Block.

General Requirements for Installation

This guide assumes that you:

- Are familiar with the Microsoft[®] Windows[®] XP operating system.
- Have basic techniques for handling DNA and RNA samples for PCR.
- Have basic skills of data storage, copying, and pasting in hard drives.
- Have experience in setting up a network if you want to use any data generated by Exicycler[™] 96 through a network.

Word Conventions

- Bold signifies user action such as typing a text or clicking a button. For example:
 Type **Test** and click **OK** to move to the next step.
- *Italic* represents important words or sentences and is also used for emphasis. For example: *After analysis, you must save data using Save As.*
- A right arrow in bold (>) separates consecutive commands you select from a main menu or shortcut menu. For example:

File> Config> Scan

Safety and Warning

The following safety alert words are used in this manual and require a particular level of observation. Each alert word is defined as below:

Note: Indicates relevant or helpful information about an instrument, however doesn't affect instrument operation.

Important: Indicates information that is necessary for proper instrument operation.

Danger: Indicates an imminently hazardous situation which, if not avoided, could result in serious injury. This safety word is used to alert improper instrument operation.

Warning: Indicates a potentially hazardous situation which, if not avoided, will result in death or serious injury if not avoided.

How to Use an Instrument Safely

Refer to this guide when installing or maintaining the Exicycler[™] 96 Real-Time Quantitative Thermal Block.

How to Obtain More Information

For more information about Exicycler[™] 96 , please visit us online at <u>www.bioneer.co.kr</u> or <u>www.bioneer.com</u>.

How to Reach Customer Support

To obtain prompt customer support, please call us at 1588-9788. You can also obtain technical support through <u>www.bioneer.co.kr</u> or <u>www.bioneer.com</u>.

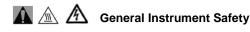
Safety Instructions

Safety Warnings and Cautions

Safety instructions in this guide book are to cover possible dangerous conditions and situations that may occur. It is your responsibility to read this guide thoroughly when installing, maintaining, or operating Exicycler[™] 96. The following safety alert words will be used in this section:

Warning: Hazards or unsafe actions that may result in severe injury.
 Caution: Hazards or unsafe actions that may result in minor injury or damage.
 Warnings: Hazards or unsafe actions that may result in High temperature and Skin burn.
 Warnings: Hazards or unsafe actions that may result in Electronic risk.

Location Consideration and Installation



- Check the power voltage rating before connecting the instrument to an electrical outlet. Exicycler[™] 96 system is configured for either 110V or 220V (±10%). Using AVR (Automatic Voltage Regulator) or UPS (Uninterrupted Power Supply) is recommended for uninterruptible power supply.
- The Exicycler[™] 96 must be grounded for protection against electric shock. If not, it may cause serious injury and system damage.
- Do not use a loose power cable or connector. An overheated power cable may break and lead to a fire or electric shock.
- 4) If you use a power extension cable, do not connect too many devices to it and operate them at the same time. Overload may occur and cause a fire.
- 5) Dry your hands completely when handling a power cord for protection against electric shock.
- 6) Do not place any objects in front of the main door of the Exicycler[™] 96 that can interrupt door operation.
- 7) Leave 30 cm space between the Exicycler[™] 96 and the wall for proper ventilation.
- B) Do not install the Exicycler[™] 96 in a dusty environment to help prevent false operation or technical damage.
- 9) Keep the Exicycler[™] 96 away from heat sources.
- 10)The Exicycler[™] 96 must not be installed in an area where it is exposed to water or is humid.It may cause electric shock, a fire, or system damage.
- 11)The Exicycler[™] 96 must not be installed in an area where it is exposed to combustible or flammable vapor. In the case of a gas leak, open the windows and let fresh air in. Do not

operate any electrical switch during a gas leak. It may cause explosion or fire.

12)Do not disassemble or repair the Exicycler[™] 96 yourself. It may cause a fire, electric shock, and system damage. A limited warranty does not cover unauthorized alterations or damage by abuse.

🛕 🖄 Installation Safety

- 1) Place and install the Exicycler[™] 96 away from direct sunlight.
- It is recommended that you turn off the computer as well as unplug the power cable before connecting it to the Exicycler[™] 96. When the computer power is on while connecting, the communication port connector in either the computer or the Exicycler[™] 96 may get damaged.
- Make sure that the USB cable is firmly connected between the Exicycler[™] 96 and the computer. Unstable connection may cause damage of the communication port connector or data transfer errors.
- The built-in camera in the Exicycler[™] 96 is a static-sensitive device. Pay particular attention to any cables connected to the Exicycler[™] 96 to avoid static damage.

Cautions

- 1) Ensure the power cable is clean and connect it firmly to the Exicycler[™] 96.
- Operate the Exicycler[™] 96 in a place where the temperature is always between 15[°]C and 30[°]C. Poor performance is influenced by extreme temperatures. High temperatures can cause mis-operation and poor performance.
- 3) Operate the system in a place where the humidity is always between 20 % and 80% with no condensation. High humidity conditions can cause corrosion of internal components and low humidity can lead to errors.
- Do not place any objects behind or by the side of the Exicycler[™] 96 that can interrupt ventilization and cause errors.
- 5) The internal optical components of the Exicycler[™] 96 may get damaged if the instrument falls or is exposes to excessive physical shock.
- Unplug the power cable from the Exicycler[™] 96 when not in operation for a long period of time to prevent the possibility of fire by overheating.

Operation and Maintenance



1) The system can be hazardous if misused.

- 2) Keep the 96-well thermal block area clean to prevent damage and to generate accurate experimental data.
- After any Real-Time PCR runs including 'Scan' or 'Melting', allow the Exicycler[™] 96 at least 10 minutes to cool down the light source lamp. Continuous operation without a break will reduce the lamp life time and cause errors.
- 4) Do not place a piece of paper or a plastic cover under the Exicycler[™] 96. It could cause fire.
- 5) Do not turn off the Exicycler[™] 96 right after a Real-Time PCR run is done. Wait until the cooling fan stops running completely. The cooling fan still runs for about 2 minutes to cool down the lamp after every Real-Time PCR run.
- 6) Do not cover the Exicycler[™] 96 with a piece of paper or a plastic cover. It may cause a fire or failure.
- 7) Set 'Turn off monitor', 'Turn off hard disks', 'System standby', and 'System hibernates' to 'Never' using 'Power Options Properties' in the Control Panel. Otherwise, data transfer between the Exicycler[™] 96 and the computer will be interrupted.

IMPORTANT:

Exicycler[™] 96 should be operated in clean condition. Contaminants such as dust can cause troubles and reduce the life time of Exicycler[™] 96. Please prevent dust from coming into Exicycler[™] 96. Through removing dust periodically from the Exicycler[™] 96 system, the life time can be extended.

European Safety and EMC Standards (CE)

This instrument meets the requirements of the European Directive for in vitro diagnostic medical devices 98/79/EC. This instrument has been tested according to the standards listed below and complies with them.

- 1) Council Directive 98/79/EC of 27 October 1998 concerning in-vitro diagnostic medical devices
- EN 61010-1 [2001] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1: General requirements for safety.
- 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.
- 4) EN 61010-2-081 [2001] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes.
- EN 61010-2-101 [2002] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
- EN 61326 [1997+A1] [1998+A2] [2001+A3] [2003 Class A] Electrical equipment for measurement, control and laboratory use - EMC requirements.
- EN 60601-1-2 [2001] Medical electrical equipment. Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility -Requirements and tests.

Compliance is demonstrated by the following mark:

CE

Authorized Representative

MT Promedt Consulting GmbH Altenhofstrasse 80 D-66386 St. Ingbert Germany

Product Use Limitations

The Exicycler[™] system is designed and sold for in vitro diagnostic (IVD) applications in combination with the diagnostic kits manufactured by Bioneer and labeled for diagnostic purposes. The Exicycler[™] system can also be used for life science research and other laboratory purposes.

Introduction

Introduction

The Exicycler[™] 96 Real-Time Quantitative Thermal Block combines a thermal block in a standard 96-well format and an innovative fluorescence detector. The detector monitors the fluorescence emitted as an indicator of amplified nucleic acid product during each PCR cycle in real time.

The thermal block is built in the lower part of the Exicycler[™] 96, which carries out a thermal cycling. The detector is located in the upper part of the Exicycler[™] 96, which measures the fluorescence emitted from samples in the thermal block in real time and transfers data to the computer for analysis. Data transferred from the detector is analyzed with the Exicycler [™] Analysis Software.

The Exicycler[™] 96 Real-Time Quantitative Thermal Block provides a maximum ramping rate of 2.5°C/sec, and features various functions such as time increment, temperature increment, and ramp rate control for myriad applications.

The Exicycler[™] 96 Real-Time Quantitative Thermal Block detector consists of an light source part and a detection part. The light source part is an energy source to excite fluorescent dyes. A short arc lamp that has a long lifetime is bright, and is implemented. A white-light source is divided into particular wavelength groups by band path filters. The band path filters that are set in the Exicycler[™] 96 covers within 480 to 690 ^{nm} and works as a set consisting of an excitation and an emission filter. Five of the filter sets are provided for various applications. Therefore, adding additional band path filters is not required.

The detector, a hight sensitive 2D CCD camera, provides high sensitivity and detects fluorescence signals from a 96-well plate in the thermal block all at one time. This simultaneous detection has a great advantage over sequential detection. The innovative detector invented by Bioneer has also reduced well-to-well variation and has minimized dye-to-dye interference, therefore providing more accurate results. Bioneer's state-of-the-art technologies applied to the Exicycler[™] 96 are to generate a uniform light surface over the thermal block, to detect multiple fluorescent signals emitted from various dyes through the light surface, and to separate the signals within a selective wavelength range of each dye.

The Exicycler[™] 96 Real-Time Quantitative Thermal Block features an auto loading function for automation reducing errors and Self-diagnosis for diagnosing systematical conditions of the Exicycer[™] 96 for users convenience. The Exicycler[™] 96 Software is composed of three main programs.

- 1) A set up program for calibration, diagnosis and confirmation of the instrument's information.
- An operation program for creating a protocol, assigning a probe and plate, saving & displaying data while operating the Exicycer[™] 96.
- 3) An analysis program for data analysis. The analysis program includes Absolute Quantification, Relative Quantification, Multi-Relative Quantification, SNP genotyping, and Existentence / Nonexistentence. Analysis and is applicable to Gene expression, Quantification of cell and virus, and SNP genotyping.

System Components and Specifications

System Components and Specifications

Exicycler[™] 96 Quantitative Thermal Block is warranted by Bioneer against manufacturing defects in materials and workmanship for a limited warranty period of one year. Bioneer will charge for repairing products for the following conditions:

- Failures due to work by the customer himself/herself
- The product is repaired after expiry of the warranty period.

Before you install the Exicycler[™] 96, check the shipped materials with the system component list below:

System Components

Cat. No. A-2060		Check
Main instrument	1	
USB 2.0 high speed cable	1	
Power cable	1	
Software	1 CD	
Operations Guide	1	
96 well reaction plate	1 pack	
Optical sealing tape	1 pack	
Optical tape sealing applicator	1	
Warranties	1	
Customer Service Request Form	1	

Specifications

Physical specifications	
Dimension (mm)	355(W) X 540(D) X 470(H)
Weight (kg)	30 kg
Sample capacity size	96-well plate / 0.2 ml micro tubes
Sample volume	20~100 $\mu\ell$ (50 $\mu\ell$ recommended)
Input voltage	100 ~ 240 VAC
Frequency	50 / 60 Hz
Power	850 Watts (Fuse : 250 V, F10AL)
Operating specifications	
Method of heating / cooling	Peltier
Temperature range	4.0℃ ~ 99.9℃
Temperature accuracy	± 0.3 °C
Temperature uniformity	\pm 0.5 $^{\circ}$ C
Ramping rate	Max. 2.5℃/sec
Lid temperature	Within 90 ~ 120 ℃
Temperature increment range	0.1℃~9.9℃
Time increment range	1 sec ~ 60 sec
Ramp rate control	0.1 ℃/sec
Operating temperature	15 ~ 30 ℃
Operating humidity	20 ~ 80%, no condensation
Communication	USB 2.0 high speed
Operating OS	Window XP & Windows 7 (32 bit OS only,
Operating 03	either in English or in Korean)
Optical Part	
Light source	Short arc lamp
Wattage	120 W
* Lamp life time	3,000 hours
Sensor	16 bit 2D CCD
** Excitation Filter / Emission Filter	5 Set

* Continuously turning on and off the lamp reduces the lamp life time.
* The lamp generates heat when it starts. Make sure that you turn off the Exicycler™ after a cooling fan stops running. It usually takes about 2 minutes to stop the cooling fan. Please refer to a chart below for more information about filter sets.

Position	Excitation (nm)	Emission (nm)	Set	Fluorescent dye
1	Blank	Blank	1	-
2	490	520	1	FAM, SYBR Green I
3	520	550	1	JOE, TET
4	550	580	1	TAMRA, CY3
5	580	610	1	Texas Red, ROX, Red610
6	630	680	1	CY5, Red670

† Filter sets



Warning:

Chemical hazard: Such the florescent dyes may cause eye and skin irritation, and respiratory tract irritation. To treat them, read the MSDS before use and follow the instructions if swallowed or inhaled. Wear appropriate protective eyewear, clothing, and gloves.

System Views

Front view







Inside view



96-well thermal block

Installation

Installation

To run the Exicycler[™] 96 Real-Time Quantitative Thermal Block, camera driver and operation software should be installed in a computer. When first installing the camera driver and operation software, or re-installing these components, please see below for instructions on how to install them yourself.

Setting Up the Computer

Exicycler[™] 96 Real-Time Quantitative Thermal Block operates through a computer. Therefore, to install Exicycler[™] 96, a computer or a laptop is necessary. The computer must meet the following computer requirements listed below. Please contact Bioneer Customer Service Center if you have any questions regarding installation.

Computer Requirements

- Intel Dual Core E2160 (1.8GHz) or higher
- Window XP Operating System for Korean or English Version (Service pack 2 or later)
- 1.0GB RAM or higher
- 1280 * 1024 screen resolution
- USB 2.0 high speed port
- 20GB Hard disk drive minimum
- Microsoft Excel (Option)

For questions regarding problems with the computer or operating system, please contact the computer manufacturer.

- 1) The computer must have at least one communication port for USB data transfer.
- 2) It is recommended that antivirus software be installed in order for the Exicycler[™] 96 to operate safely. A firewall should also be setup to prevent unwanted information coming in from external nextworks. Please contact your IT department to setup the antivirus software and firewall.
- It is recommended that a memory stick not be installed when Exicycler[™] 96 is running. It may cause a technical problem between Exicycler[™] 96 and the computer.

Connecting the Computer and Exicycler[™] 96

There are a main power connector and a communication port connector (USB) in the rear of Exicycler[™] 96. The computer should be turned off when connecting to the Exicycler[™] 96 to prevent any damages to the communication port. Please see below for instructions on how to connect Exicycler[™] 96 to the computer.

- Unpack the Exicycler[™] 96 and make sure all components are included. Refer to the list of components below.
- 2) Exicycler[™] 96 must be installed in an area where it is not exposed to sunlight and must be set on a stable and level surface.
- 3) Set up the computer in the installation site.
- 4) Place the Exicycler[™] 96 in the installation site carefully.
- 5) Connect the USB 2.0 cable to the USB connector on the back of Exicycler[™] 96, then to the computer.
- 6) Connect the power cable to the Exicycler[™] 96, then to the receptable wall circuit.

List of Components

Cat. No. A-2060		Check
Main instrument	1	
USB 2.0 high speed cable	1	
Power cable*	1	
Software	1 CD	
Operations Guide	1	
96 well reaction plate	1 pack	
Optical sealing tape	1 pack	
Optical tape sealing applicator	1	
Warranties	1	
Customer Service Request Form	1	

* Provided according to country standard.

Installing the Operation and Analysis Software

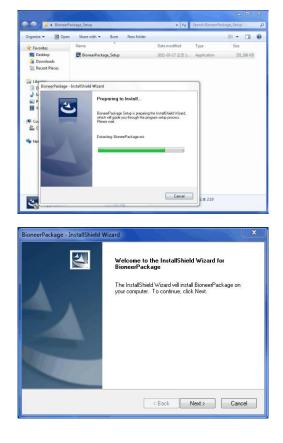
To run the Exicycler[™] 96 Real-Time Quantitative Thermal Block, the operation software and the camera driver must be installed in the computer. When first installing the operation software and the camera driver, or re-installing these components, please see instructions below on how to install them yourself. In case of running the software for only data analysis purpose, please refer to this section for software installation.

IMPORTANT:

Do not power on the Exicycler[™]96 unless you have installed the Operation and Analysis Software and a camera driver. First install the Operation Software and then the camera driver.

To install Bioneer Package and Exicycler3 softwares for an Exicycler[™] 96 device under Window 7, follow the instructions below:

- If a previous version of software has been installed on your computer, please remove the programs and folders from the computer (Refer to 'Troubleshooting 4'). Remaning program folders may cause errors during installation. This section is for the first time installation or re-installation of the software.
- The Bioneer Package has to be installed prior to installing Exicycler3 software. The procedures of Bioneer Package installation is described below.
- Power on the computer, but not the Exicycler[™] 96 Real-Time PCR machine. Place the Exicycler[™] 96 installation CD on the CD-ROM. Go to 'Bioneer Package' folder in the CD drive and run Setup.exe



 To install Bioneer Package, click Next . The installation will proceed in C:\Exicycler3. Enter the desired name in the User Name box and Company Name box.

 Select a setup type to install. Complete is recommended for installing all program features.
 Custom install is recommended only for advanced users. Click Next.

 The InstallWizard is ready for the installation. If you want to review or modify your settings, click **Back**. Click **Install** to start installation.

 This process takes several minutes. Please do not cancel until the installation is complete.

BioneerPackage - InstallShield Wizard
Customer Information
Please enter your information.
Please enter your name and the name of the company for which you work.
User Name:
bioneer
Company Name:
Microsoft
InstallShield
< Back Next > Cancel
BioneerPackage - InstallShield Wizard
Setup Type
Select the setup type to install.
Please select a setup type.
Complete
All program features will be installed. (Requires the most disk space.)
Custom
Select which program features you want installed. Recommended for advanced users.
InstallShield
< Back Next > Cancel
lioneerPackage - InstallShield Wizard
Ready to Install the Program
The wizard is ready to begin installation.
Click Install to begin the installation.
If you want to review or change any of your installation settings, click Back. Click Cancel to exit
the wizard.
InstallShield
< Back Install Cancel
RinnearDackana - InstallShield Wisard X
SioneerPackage - InstallShield Wizard
Secup status
The InstallShield Wizard is installing BioneerPackage
Removing applications

Cancel

After the installation is completed. Please click
 Finish to finish the installation Shiled Wizard.

InstallShield Wizard Complete The InstallShield Wizard has successfully installed BioneerPackage. Click Finish to exit the wizard.
< Back Finish Cancel

The R program is also needed for Exicycler software.
 If you click OK, the installation will begin.

BioneerPackage - InstallShield Wizard	×
Exicycler Software requires R.	
ОК	

 R program will be installed automatically. Please wait until the installation is completed.

Setup - R for Windows 2.11.1		×
Installing Please wait while Setup installs R for Windows 2.11.1 on your computer.		R
Extracting files C:₩₩R₩R-2.11.1₩ibrary₩class₩po₩de₩LC_MESSAGES₩R-class	.mo	
	_	
	Ca	ncel

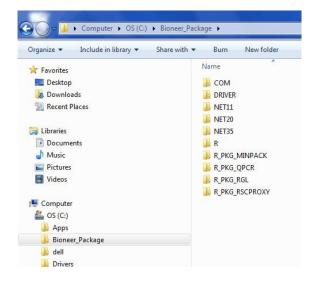
10) Click **OK** for installing R(D)COM interface.



11) The R icon (right) will be shown on the Desktop when the installation is successfully complete.



12) The folders shown here are created after the installation has finished. Please confirm the 'Bioneer_Package' and sub folders.



13) In order to install '.NET Framework 1.1 Package', run dotnetfx_eng.exe file of the C:\Bioneer_Package\Net11 folder.

Organize 🔻 Include in library 🔻	Share with ▼ Burn New folder
🔆 Favorites	Name
Nesktop	🖾 dotnetfx_eng
😹 Downloads	
💯 Recent Places	
浔 Libraries	
Documents	
🕨 🎝 Music	
Pictures	
Videos	
👰 Computer	
4 🏭 OS (C:)	
🔒 Apps	
a 📙 Bioneer_Package	
退 сом	
J DRIVER	
3 NET11	
NET20	
퉳 NET35	
R	

Microsoft .NET Framework 1.1 Setup

 $(\mathbf{?})$

14) The message to confirm the installation of '.NET Framework 1.1 Package' is displayed, and then click Reinstall using recommended settings.

🕶 Program Compatibility Assistant	×
This program might not have installed correctly	
If this program didn't install correctly, try reinstalling using settings the are compatible with this version of Windows.	at
Program: IExpress Setup Publisher: Microsoft Location: C:\Bioneer_Package\NET11\dotnetfx_eng.exe	
Reinstall using recommended settings	
 This program installed correctly 	
Canc	:el
What settings are applied?	

- 15) Click **Yes** for installing '.NET Framework 1.1 Package'.
- 16) The setup window asking about the license agreement is displayed.

1.1	License Agreement
rosoft Let	(A copy of this license is available for printing at http://go.microsoft.com/fwlink?Linkld=12283) SUPPLEMENTAL END USER LICENSE AGREEMENT FOR MICROSOFT SOFTWARE
1	I have read, understood and agree to the terms of the End User License Agreement and so signify by clicking "I agree" and proceeding to use this product.
	⊂ I <u>a</u> gree ● I <u>d</u> onotagree

Would you like to install Microsoft .NET Framework 1.1 Package?

Yes

No

17) If select **I agree** to activate the Install button, Click **Install** to begin the installation.

	License Agreement
rosoft	 (A copy of this license is available for printing at http://go.microsoft.com/fwlink/?LinkId=12283)
	SUPPLEMENTAL END USER LICENSE AGREEMENT FOR MICROSOFT SOFTWARE
r î	I have read, understood and agree to the terms of the End User License Agreement and so signify by clicking "I agree" and proceeding to use this product.
	C I do not agree

All components related to .Net Framework are being installed.

Microsoft .NET Frame Microsoft	work 1.1 Setup
11 1	
K .	Gathering required information
	Cancel

- The installation of .NET Framework 1.1 is complete.
 Click **OK** to finish the installation.
- 20) In the Exicycler3 folder of the provided CD, you can see **setup.exe** file and double click to have InstallSheild Wizard install the required items automatically.

21) Click **Next** and go to the next step.

Installation of Microsoft .NET Framework 1.1 is complete.

icycler3 - InstallShield Wizan	
2	Preparing to Install
0	Exicycler3 Setup is preparing the InstallShield Wizard, which will guide you through the program setup process. Please wait.
	Decompressing: Exicyder3.msi
	1
	Cancel

Exicycler3 - InstallShield W	/izard X
R.	Welcome to the InstallShield Wizard for Exicycler3
	The InstallShield(R) Wizard will install Exicycler3 on your computer. To continue, dick Next.
Z	WARNING: This program is protected by copyright law and international treaties.
	< Back Next > Cancel

- 22) Click Install to begin the installation. If you want to change any settings or cancel installing, click Back or Cancel.
- 23) Please wait while the InstallShield Wizard installs Exicycler3.

and the second s	J Exicycler3 gram features you selected are being installed.
17	Please wait while the InstallShield Wizard installs Exicycler3. This may take several minutes. Status:
tallShield -	

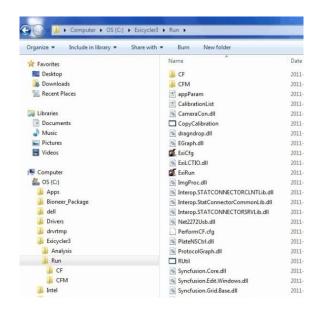
24) The message indicating "InstallShield has successfully installed Exicycler3." is shown. Click Finish to complete the installation

25) On the Desktop, these icons (right) are created if the installation has been successfully completed.



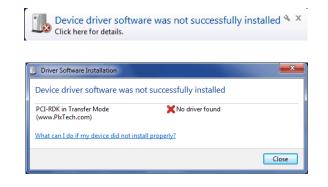


26) In the C:\Exicycler3\Run folder, these folders and files are created. Copy 'calibration' folder from the Exicycler[™] 96 CD into C:\Exicycler3\Run folder. Now you should connect Exicycler[™] 96 system with a computer using provided USB cable.



- 27) Check the USB cable connection between the Exicycler[™] 96 and the computer.
- 28) Power on the Exicycler[™] 96. A power button LED will turn on red when the power is supplied properly.
- 29) Press the power button again for a second to start Self-diagnosis. The POWER button LED starts blinking in green after two short beeps when the selfdiagnosis is complete.
- 30) When Exicycler[™] 96 is powered on for the first time, the computer searches for a new device.
 - A) When an unsigned device is connected, the following message will be displayed. Click the message to view details.
 - B) Because the device driver software is not yet installed, driver installation has to be continued.
 Please click Close.





 To install the device driver and to confirm the device status, go to Control Panel\System and Security\System- Device manager.

32) All devices connected to computer are displayed.

and select Update driver software.

The unsigned device is indicated with an exclamation mark. Point at this device and right-click

 Control Revel 1 - System and Soundy - System
 • (2)
 Tennoh Control Revel 2 - System
 • (2)

 Control Down Homes
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 • (2)
 Tennoh Control Revel 2 - System
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 Windows Added.
 Windows Added.
 • (2)
 Tennoh Control Revel
 • (2)

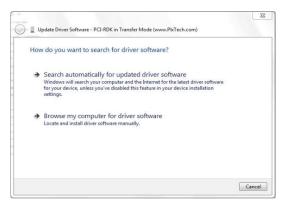
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 Windows Added.
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 • (2)
 Tennoh Control Revel
 • (2)

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File Action View Help	
Þ•• m 🖬 m 🕅	
a 🚔 bioneer-PC	
b 📲 Computer	
Disk drives	
Display adapters	
DVD/CD-ROM drives	
D Dig Human Interface Devices	
DE ATA/ATAPI controllers	
b — Keyboards	
b - P Mice and other pointing devices	
b See Monitors	
b 🔮 Network adapters	
a 🔄 Other devices	
PCI-RDK in Transfer Mode (www.PlxTech.com)	
Portable Devices	
Ports (COM & LPT)	
Processors	
b Sound, video and game controllers	
b 1 System devices	
b - Universal Serial Bus controllers	

33) When a prompt asking how to find device driver is presented, select Browse my computer for driver software.



34) Click **Browse** to search for the appropriate driver software.

	nputer	
Search for driver software in this location:		
C:\Users\bioneer\Documents	•	Browse
	ivers on my comr	outer
Let me pick from a list of device du This list will show installed driver software co software in the same category as the device.		e, and all driver

35) Select C: \Bioneer_Package\driver and click OK.

36) Selcect Install. Installation will be automatically

37) Please wait while installation. This process needs

started.

several minutes.



Windows Security	23
Would you like to install this device software?	
Name: PLX Technology, Inc. (http://www.PlxTech Publisher: PLX Technology, Inc.	
Always trust software from "PLX Technology, Inc.".	n't Install
You should only install driver software from publishers you trust. <u>How can which device software is safe to install?</u>	decide

	23
Update Driver Software - PCI-RDK in Transfer Mode (www.PlxTech.com)	
Installing driver software	

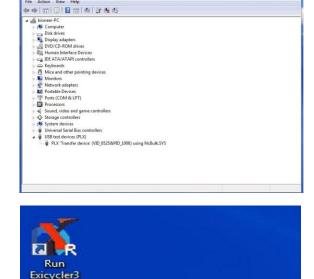
38) A window displays a message indicating that the installation was successful. Click Close to complete the installation.

		Σ
🗓 Up	date Driver Software - PLX 'Transfer device' (VID_0525&PID_1000) using	g NcBulk.SYS
Wind	lows has successfully updated your driver software	
Windo	ws has finished installing the driver software for this device:	
T	PLX 'Transfer device' (VID_0525&PID_1000) using NcBulk.SYS	
		Close

- 39) Open 'Device Manager' and confirm the device driver installed. If USB test devixes (PLX)\PLX'Transfer device' VID_0525&PID_1000) using Ncbulk.SYS has been installed properly, you can see the connected USB indicator.
- Click Run Exicycler3 icon to start Exicycler[™] 96 software.

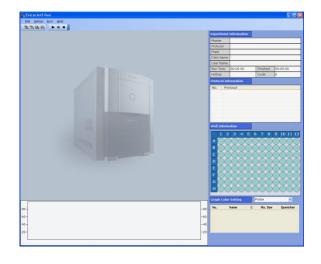
 You can see this screen when the installation is completed successfully. The software version is also indicated.

42) Now you are ready to operate the Exicycler[™] 96 system. To set your protocols, refer the user's manual (provided).





Analysis Exicycler3



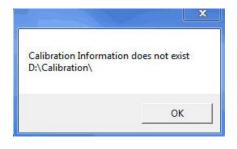
Troubleshooting

1. Appropriate calibration data does not exist

- The CD provided with Exicycler[™] 96 has Calibration data for each device. Calibration data have to be copied into C:\Exicycler3\Run folder in order for normal function. If calibration folder does not exist on this path, following message will be displayed.
- The calbration files do not exist on the appointed path. Click OK to change the path.

- You should assign the path of the Calibration folder to be copied automatically. Click **Open** and you will see the following browser.
- 4) After assigning the Calibration folder (ex. D: \Calibration), click OK.

	×
Unable to loa	ad calibration data.
	ОК



Select Calibration Source Folder	Open
No Information	

owse For Folder	X
⊿ j∰ Computer	*
> 🏭 OS (C:)	
4 💽 DVD RW Drive (D:) EXI-05M-1001026	
BioneerPackage_Setup	
🌗 Calibration	=
Exicycler3_Ver3.54.5	
ExiDiagnosis_Ver1.25	
🖻 🍌 Manual	
🖻 👝 SA&D (E:)	
Foxio Burn Disc Viewer	
Network	-
OK Canc	el
Cain	

5) The Copy Calibration window displays the path of calibration folder and serial number of your device. If the serial number matcheds with the connected Exicycler[™] 96 system, click **Copy** button on the right side of the window.

Copy Calibration	
D:\Calibration	Open
Exi - 05M - 1001026	Сору

6) Please wait for a minutes to complete the copy.

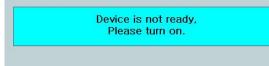
Copy Calibration	
D:\Calibration	Open
Exi - 05M - 1001026	Сору
13/85	Сору

 After copy of Calibration folder is complete, click OK to finish the process. Please confirm the connection between device and computer, and restart the run Exicycler3 software.

	6
Copy Complete.	Run Exicycler

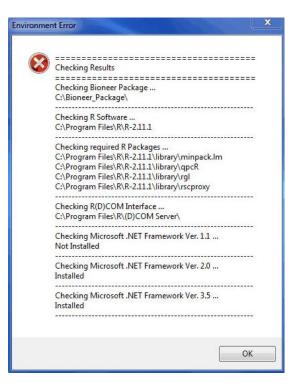
2. The computer does not register Exicycler[™] 96 device when lunching 'Run Exicycler3' software.

This message means that the device power is not on or the appropriate device driver has not yet been installed on the computer. If you see this message despite the instrument power being on, then you should re-install the device driver to solve this problem. The driver installation steps are described in process number 30 of this manual.



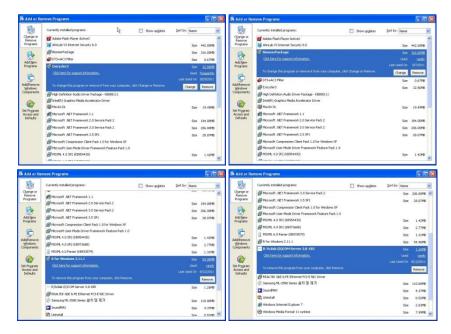
3. An Error message is displayed when lunching 'Run Exicycler3' software.

This message indicates that '.NET Framework 1.1' has not yet been installed. Go to process number 13 and re-install as described.



4. The Bioneer Package is not installed correctly.

If an incorrect installation was performed previously, 'Bioneer Package' may not be installed correctly. Go to **Control Panel-Programs Add/Remove** and remove 4 programs from the computer - '**Exicycler V3**', '**Bioneer package'**, '**R for windows 2.11.1' and 'R/Scilab(D)COM Server3.01-B5'**. After removing them from the computer, reperform the entire installation process.



System Operation and Calibration Preparation

System Operation and Calibration Preparation

Perform the calibration after verifying the installation of the Exicycler[™] 96 and the computer.

Powering On and Self-diagnosis

- Power on the Exicycler[™] 96 by switching on a power supply button in the rear of the instrument. When power is supplied properly, a POWER button LED turns red.
- Press the **POWER** button for a second to start selfdiagnosis.





IMPORTANT:

The POWER button LED starts blinking in green after two short beeps when the self-diagnosis is complete. The Exicycler[™] 96 is now ready to operate. The POWER button LED will turn red when the self-diagnosis fails or excessive physical shock is applied from outside. (e.g. Make sure that there are no objects placed in front of the door) Make sure that the POWER button LED is blinking in green before operating the Exicycler[™] 96 to prevent mis-operation or damage. A result of the self-diagnosis is provided under "ExiConfig".

NOTE:

You can stop the operation here and turn off the Exicycler[™] 96 by press the POWER button for a second when it is blinking in green like Step 2.

3) When the power button is blinking in green, you can either open or close a main door by pressing a **DOOR** button for a second. The power button is blinking in purple when the door is open and is back to green when the door is closed.

Cautions:

Do not place any obstacle in front of the system and do not load a plate or take out it when the door opens or close. It causes serious damages and will give severe injury.

Reagents and Consumable Products

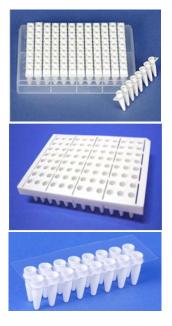
Various reagents and kits are required to perform a Real-Time PCR in the Exicycler[™] 96 Real-Time Quantitative Thermal Block. Please refer to this section to obtain detailed information about the appropriate reagents and kits for your experiments. For ordering, please go to the 8, Ordering Information.

PCR Premix Kit

AccuPower[®] Greenstar[™] PCR PreMix Kit allows an easy and fast amplification in Exicycler[™] 96. The *AccuPower*[®] Greenstar[™] PCR PreMix Kit consists of Greenstar[®] fluorescent dye, HotStart Taq DNA polymerase, and all of the PCR components. Therefore, add just template, primers, and MgCl₂ to start your amplification.

Optical 0.2 mℓ 8-strip tube and 96-well plate

You must use an optical tube or a plate when performing Real-Time Quantitative PCR in the Exicycler[™] 96. A standard tube or a plate can be also used for a conventional PCR without any fluorescent dyes.



Optical sealing tape

Seal up the optical tubes or plates tightly with the optical sealing tape for fluorescence detection. The sealing tape is available in the size of 96-well plate. Therefore, cut up the sealing tape as you need in case of using 0.2 ml 8-strip tubes.

Caution:

You must cut up the sealing tape with a box cutter, not scissors, when needed. The tape adhesive will be carried over to the surface of the sealing tape while cutting up the tape with scissors and make the tubes sealed with the tape stick to the heating lid of the Exicycler[™] 96. The tubes stuck to the lid will drop inside the Exicylcer[™] 96 when the lid is cooled down. Using at least 2 strips of 8-strip tubes at a time is also recommended to prevent this error.

Warning: Chemical hazard

AccuPower[™] Greenstar[™] PCR PreMix and *AccuPower*[™] Dualstar[™] PCR PreMix may cause eye and skin irritation, and respiratory tract irritation. To treat them, read the MSDS before use and follow the instructions if swallowed or inhaled. Wear appropriate protective eyewear, clothing, and gloves.

Calibration Using ExiCfg

Calibration Using ExiCfg

Prepare to perform the calibration with a Calibration Kit (A-2060-A1). The calibration kit is designed for the Exicycler[™] 96 Real-Time Quantitative Thermal Block (Version 3.0) and is not applicable to other Real-Time instruments. The calibration kit is comprised of 11 plates, a mask calibration plate, a background calibration plate, multi-channel calibration plates (9 different optical calibration plates of filter sets) present in the Exicycler[™] 96. The calibration kit is available for reuse up to 3 times. For more information about the calibration kit, please see the following:

Before Starting Calibration with the Calibration Kit

Centrifuge Disposable plastic gloves Calibration Kit (A-2060-A1)

Calibration Plate Preparation

The calibration kit is shipped refrigerated or frozen and must be stored in the freezer upon receipt. Retrieve one Calibration Kit right before starting the calibration.

1) Take out the Calibration Kit from the freezer.



2) Take one of the sealed packs in the Calibration kit.



3) Allow the pack to warm to room temperature for at least

10 minutes.



4) Take out a calibration plate from its packaging.



IMPORTANT:

Pay particular attention to the calibration kit when handling. Wear disposable plastic gloves to help prevent contamination on the sealing tape covered the 96-well plate. Wipe out the surface of the plate with 70% Ethanol when it needs to be cleaned.

NOTE:

Do not vortex the calibration plate. A small amount of calibration solution is pre-aliquoted in each well, and it may be lost while vortexing.

5) Place the calibration plate in a rack and centrifuge for 5 minutes.



NOTE:

During this step, it is important to spin down the calibration solution that remains on the side of the well and to remove air bubbles at the bottom of the well.

 Protect the calibration plate from direct sunlight after the centrifugation. Place the plate directly into the Exicycler[™] 96.



7) Put the calibration kit back into its packaging and return

it to the freezer when the calibration is done.

IMPORTANT:

The Calibration Kit contains photosensitive components. Keep the calibration kit and the plate away from light during calibration. Put the calibration plate back into its packaging right away for reuse.

Calibration using ExiCfg

The calibration must be performed prior to operating the Exicycler[™] 96 Real-Time Quantitative Thermal Block. You can either transfer proper calibration data associated with a serial number from the installation CD to the computer or perform the calibration yourself. Install the Operation and Analysis Software for the calibration data transfer to the computer. For a reliable operation of the Exicycler[™] 96, calibrating with the Exicycler[™] 96 Calibration Kit is recommended. The calibration must be done when moving the physical location of the Exicycler[™] 96 or when changing a light source lamp. A periodical calibration every 6 months helps maintain the optimum condtion of the Exicycler[™] 96.

Use the ExiCfg program to calibrate the Exicycler[™] 96. The ExiCfg calibrates as well as diagnoses the systemical condition of the Exicycler[™] 96. Make sure that you are fully trained for handling the ExiCfg before starting calibration. Otherwise, authorized staff is only allowed to manage the ExiCfg. Mis-use of the ExiCfg by unauthorized staff may cause serious damage in the Exicycler[™] 96.

The calibration is carried out with 4 steps, Warming up the lamp > Mask calibration > Background calibration > Multi-Channel calibration.

Mask Calibration

 Power on the Exicycler[™] 96 by pressing the **POWER** button.

NOTE:

Make sure the POWER button is blinking in green after the self-diagnosis prior to starting the calibration.

2) Go to 'C:\Exicycler3\Run' and double click ExiCfg.exe.

	Calibrated Not calibra	
Lamp warming	205	Extcycler3,0 Config
Mask Calbration		
Background Calibration		
futf-Channel Calibration		



3) Click **Lamp warming** to turn on the lamp. A progress bar of the **Lamp warming** will start right after 'The lamp is turned on' message appears on the 'History' box. Do not click any buttons until the progress bar finishes.

	Calibrated	Not calibrated History
Lamp warming	DX.	Exisciled 30 Config Trying to timo mote laree. Detaching the laree transmission and the large large state in 1.55 C Large transmission considered Dummy cain considered Dummy cain considered Dummy cain considered Dummy cain considered Dummy cain considered The large to turned on.
Background Calibration		
Multi-Channel Calibration		

IMPORTANT:

It usually takes about 30 seconds for the message 'The lamp is turned on' to appear on the 'History' box. If the message does not appear in 5 minutes, turn off the Exicycler[™] 96 and turn it on again after 5 seconds. In case of an abnormal shutdown like this, wait about 5 minutes, then click **Lamp warming** to turn on the lamp again. It takes approximately 10 minutes to reach the maximum light intensity there after. Therefore, wait until the progress bar of the **Lamp warming** finishes before proceeding further.

4) Press the **DOOR** button to open the door.





NOTE:

top-left of the block.

You must perform the Mask calibration first prior to other calibrations.

5) Place the Mask calibration plate with the A1 position at the

IMPORTANT:

It is important to load the calibration plate in the right position. A misplaced plate may cause damage or technical problems.

6) Press the DOOR button again to close the door.



7) Click Mask Calibration button.

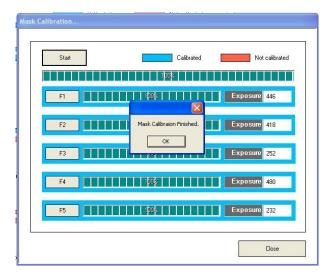
	Calibrated Not calibrate	History
Lamp warming Mask Calibration		Exceeden 0.0 Config Thing to turn on the lane Checking the lane temperature. Lane Time: 3 Lane Time: 3 Lane Time: 3 Mod88 committion concelled: Dummy scien controlled: Dummy scien controlled: Ceature of damin\$0.0 Griget: L. Exposure:\$0m\$ The lame is turned on.
Background Calibration		
fulti-Channel Calibration		

8) Click Start button in the 'Mask Calibration' window.

<u>Start</u>	Calibrat 0%	ed Not calibrated
FT	0%	Exposure 0
F2	Û%	Exposure 0
F3	Û%	Exposure 0
F4	0%	Exposure 0
F5	0%	Exposure 0

NOTE: The calibration time is approximately 10 minutes depending on the condition of the Exicycler[™] 96 and the computer.

9) When the mask calibration is complete, click **OK**.



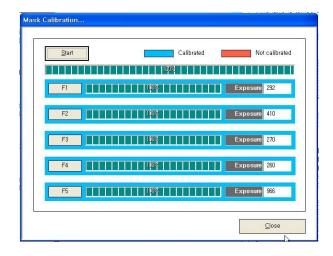
NOTE:

The door will open when the mask calibration is successfully complete. Put the plate back into its packaging and return it to the freezer.

Warnings: High temperature

Wait at least 5 seconds before taking out the calibration plate from the 96-well block. The calibration plate is heated to a temperature of 100°C and may cause a burn.

10) Click Close in the 'Mask Calibration' window.



11) The 'Mask Calibration' frame will turn blue when the calibration is successfully complete.

	Calibrated Not calibrated	History
Lamp warming		The lamp is turned on. Capture Exposure [1]:240 Capture Exposure [1]:291 Calced Level : 46720 at the exposure of 291m Slant : 0.005112
Mask Calibration	Le	Detected Exposure/392 Cachure Exposure/21/345 Cachure Exposure/21/340 Cachure Exposure/21/340 Cachure Exposure/21/340 Cachure Level: 44152 at the exposure of 410m Shant: 0.0006/51067/07317073 Detected Exposure/340.
Background Calibration		Cadute Exposure [3]:270 Calced Level : 50304 at the exposure of 270m Start : 0,005693 Detected Exposure [4]:160 Cadute Exposure [4]:160 Cadute Exposure [4]:259
Mutt-Channel Calibration		Calced Level : 48986 at the exposure of 259m Start : 0.006720804106572080410559 Detected Exposure:380 Cacture Exposure:380 Cacture Exposure:51882 Cacture Exposure:51882 Cacture Exposure:51885 Cacture Levosure:4985 Detected Exposure:986 Ford of Mack Calibration

Background Calibration

1) Press the **DOOR** button to open the door.



NOTE:

This step is omissible when performing the background calibration followed by the mask calibration.

2) Place the Background calibration plate with the A1 position

at the top-left of the block.



IMPORTANT:

It is important to load the calibration plate in the right position. A misplaced plate may cause damage or technical problems.

3) Click Background calibration button.

ation ⊊alibration Factory≦et <u>H</u> elp	
Calibrated	Not calibrated History
Lang warming	The lame is turned on, Ceature Excosure [1]:240 Ceature Excosure [1]:240 Cated Levis 44720 at the excosure of 291m Shart: 0.005112 Detected Excosure [2]:245 Ceature Excosure [2]:245 Ceature Excosure [2]:410 Ceature Excosure [2]:410
Background Calibration	Sinet: 0.0006506707317073 Detected Exosuer410 Catute Exosuer31270 Catute Exosuer31270 Catute Exosuer3270 Catute Exosuer370 Catute Exosuer41180 Catute Exosuer41259 Catute Exosuer41259 Cated Levis 4069 at the exosuer of 259m
Mult-Channel Calibration	Slami 1,0005720390410559 Detected Exposure (5) Capture Exposure (5):720 Capture Exposure (5):782 Capture Exposure (5):982 Capture Exposure (5):985 Calced Level : 49152 at the exposure of 985m Slami 1,0 C2139395073951

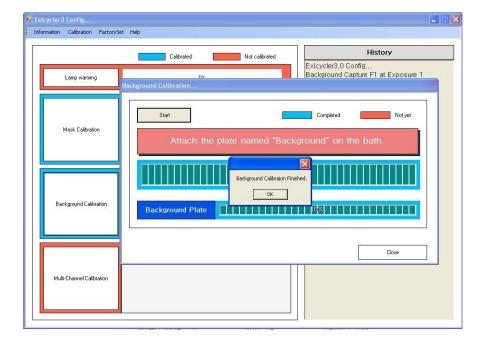
4) Click **Start** in the 'Background Calibration' window.

Attach the plate	e named "Background" on the ba	ath.
	3%	
Background Plate	0%	

NOTE: The background calibration time is approximately 10 minutes depending on the condition of the Exicycler[™] 96.

5) When the background

calibration is complete, click OK.



NOTE: The door will open when the background calibration is successfully complete. Put the plate back into its packaging and return it to the freezer.

Marnings: High temperature

Wait at least 5 seconds before taking out the calibration plate from the 96-well block. The calibration plate is heated to a temperature of 100°C and may cause a burn.

6) Click **Close** in the 'Background Calibration'

window.

Attach the plate na	amed "Background" on the bath.
Background Plate	

 The 'Background Calibration' frame will turn blue when the calibration is successfully complete.

	Calibrated Not calibrated Histor	гу
Lamp warming	Detected Exposure:270 Capture Exposure[4]:180	
Camp warning	Capture Exposure [4]:233	
	Capture Exposure [4]:259	
	Calced Level : 48896 at the	exposure of 259mS
	Slant : 0,0056720890410958	19
	Detected Exposure:260	
Mask Calibration	Capture Exposure [5] : 720	
	Capture Exposure [5]:892	
	Capture Exposure [5]:965	4.005.0
	Calced Level : 49152 at the Slant : 0.0213293650793651	
	Siant : 0.0213230650/95651 Detected Exposure:966	
	End of Mask Calibration	
	Background Capture E1 at E	vnosure 1
	Background Capture F1 at E	
Background Calibration	Background Capture F1 at E	
L.	Background Capture F2 at E	xposure 1
	Background Capture F2 at E	xposure 2
	Background Capture F2 at E	
	Background Capture F3 at E	
	Background Capture F3 at E	
	Background Capture F3 at E	
Multi-Channel Calibration	Background Capture F4 at E	
Multi-Channel Calibration	Background Capture F4 at E: Background Capture F4 at E:	
	Background Capture F4 at E: Background Capture F5 at E:	
	Background Capture F5 at E	

Multi-channel Calibration

1) Press the **DOOR** button to open the door.



NOTE: This step is omissible when performing the background calibration followed by the mask calibration.

2) Click Multi-Channel Calibration button.

	Calibrated Not calibrated	History
Lanp warming Mask Calibration		Detected Exocuse:270 Cataria: Exocuse[4]:280 Cataria: Exocuse[4]:285 Cataria: Exocuse[4]:285 Startic: Exocuse[4]:295 Starti: 0.0067020960106569 Detected Exocuse[5]:070 Cataria: Exocuse[5]:070 Catari
Background Calibration		Detected Exposue:966 End of Mask Calibration Backaround Casture F1 at Exposue 1 Backaround Casture F1 at Exposue 2 Backaround Casture F2 at Exposue 3 Backaround Casture F2 at Exposue 1 Backaround Casture F2 at Exposue 3
Multi-Channel Calibration	R.	Backaround Capture P3 at Exoosure 1 Backaround Capture P3 at Exoosure 2 Backaround Capture P3 at Exoosure 3 Backaround Capture P4 at Exoosure 1 Backaround Capture P4 at Exoosure 3 Backaround Capture P4 at Exoosure 3 Backaround Capture P5 at Exoosure 3 Backaround Capture P5 at Exoosure 3

 Select fluorescence dyes in the 'Multi-Channel Calibration' window for the calibration.

-							
	Start	Add Dye]	<u>C</u> lose			
No.	Name		Status				
01	✓ FAM		0%				
02	SYBR_GREEN		0%				
03	I JOE		0%				
04	TET TET		0%				
05	TAMRA		0%				
06	🗹 СУЗ		0%				
07	TEXAS_RED		0%				
08	ROX ROX		0%				
09	CY5		0%				

NOTE:

9 dyes are available for calibration.

NOTE:

For a selective multi-channel calibration of your desirable dyes, select check boxes of the dyes.

4) Click Start.

	Start	Add Dye	se			
NO.	Name	Status				
01	✓ FAM	0%				
02	SYBR_GREEN	0%				
03	☑ JOE	0%				
04	✓ TET	0%				
05	🗹 TAMRA	0%				
06	I CY3	0%				
07	TEXAS_RED	0%				
08	ROX ROX	0%				
09	CY5	0%				

5) Prepare the calibration plate with a dye **X** (i.e. "FAM") when 'Attach the plate named '**X**' (i.e. "FAM") on the bath' message appears.

	Attach the pla		FAM " on the bat	h.
	Start	Add Dye	CI	ose
No.	Name		Status	
01	FAM		0%	
02	SYBR_GREEN		0%	
03	JOE		0%	
04	TET TET		0%	
05	TAMRA		0%	
06	🗹 СҮЗ		0%	
07	TEXAS_RED		0%	
08	ROX		0%	
09	I CY5		0%	

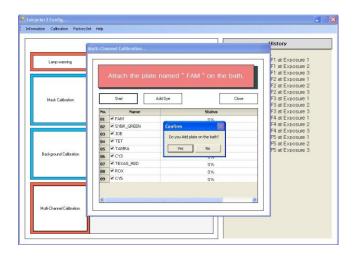
 Place the Multi-Channel calibration plate with the A1 position at the top-left of the block.



IMPORTANT:

It is important to load the calibration plate in the right position. A misplaced plate may cause damage or technical problems.

7) Click Yes in the 'Confirm' window.



NOTE: An approximate calibration time for each dye is 5 minutes.

8) The door will open when the calibration is successfully complete.

NOTE: Put the plate back into its packaging and return it to the freezer.

Marnings: High temperature

Wait at least 5 seconds before taking out the calibration plate from the 96-well block. The calibration plate is heated to a temperature of 100°C and may cause a burn.

9) Prepare the calibration plate with a dye X (i.e. "SYBRGreen") when 'Attach the plate named 'X' (i.e. "SYBR Green") on the bath' message appears.

_		bath.
	Continue	Add Dye
No.	Name	Status
01	✓ FAM	100%
02	SYBR_GREEN	0%
03	IOE IN IOE	0%
04	✓ TET	0%
05	TAMRA	0%
06	🗹 СҮЗ	0%
07	TEXAS_RED	0%
08	✓ ROX	0%
09	CY5	0%

10) Click Start to repeat steps 4 through 8.

	Continue	Add Dye	d " TET " on the bath.	
No.	Name	Status		
	✓ FAM	100%	<u>C</u> lose	
	SYBR_GREEN	0%		
	I JOE	0%	Status	i " on the bath.
	✓ TET	0%	100%	T a state of the second se
	Z TAMRA	0%	100%	
	CX3	0%	100 %	Close
	TEXAS_RED	0%	0%	
	ROX .	0%	0%	Status
09	CV2	0%	0%	100%
			0%	100%
			0%	100%
			0%	100%
			570	100%

11) When the multi-channel calibration is complete, click **OK**.

	Priceon the	plate named '	CTJ OITU	15 Datit.
_	Start	Add Dye		Close
NO.	Name			
)1	FAM	Multi-Channel Calil	usian Finished	
32	SYBR_GREEN	Multi-Channel Cam	oraion Finisheo,	
13	IOE IOE	OK		
)4	TET TET		100 10	
35	TAMRA		100%	
06	CY3		100%	
17	TEXAS_RED		100%	
38	ROX		100%	
19	I CY5		100%	

12) Click Close in the 'Multi-Channel Calibration' window.

	Attach the	plate named '		e bath.	
	Start	Add Dye		<u>C</u> lose	
No.	Name		Status		
01	FAM		100%		
02	SYBR_GREEN		100%		
03	IOE ≥		100%		
04	✓ TET		100%		
05	TAMRA		100%		
06	I CV3		100%		
07	TEXAS_RED		100%		
08	ROX		100%		
09	CY5		100%		

13) The 'Multi-Channel Calibration' frame will turn blue when the calibration is successfully complete.

	Calibrated Not calibrated	History
Lamp warming		1/2 Exposure Capture at 205mS Level1 : 5623, Level2 : 4994 Slant : 3,068236882683, Intercept : 4365, R° : 1, Exposure : 14596 Capture at Exposure : 1800mS
Mask Calibration		CV5 F3 Original Exposure Capture at 270mS 1/2 Exposure Capture at 155mS Level 1: 5507, Level 2: 4564 Sinnt : 3, 87407407407, Intercept : 4461, R ⁺ 1. E. Exposure : 11555
Background Calibration		Casture at Exposure : 1800mS CY5 F4 Ordinal Exposure Casture at 280mS 1/2 Exposure Casture at 130mS Level1 : 4643, Level2 : 4643 Slant : 1, 69230786250786, Intercept : 4423, P ⁺ 1.1 Exposure : 28430
Multi-Channel Calibration	P.	Cedure at Exposure : 1800mS CV5 F5 CV5 F5 L2 Exposure Casture at 996mS L2 Exposure Casture at 485mS Level : 0, Level 2: 0 Slant : 0, Intercept : 0, R*2 : NaN, Exposure : - 214745948

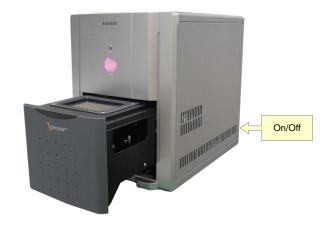
14) Go to **Calibration>Test Function>Lamp Off** from the top menu bar.

Exicycler3	Config		
Information	Calibration FactorySet Help		
	Test Function	Select Eilter	
	⊻ision	Drawer Open Not calibrat	History
	Uniformity Setting Mask Setting	Drawer Close Bath Up	1/2 Exposure Capture at 205mS
	Load Mask Calibration Load Background Calibration	Bath Down Lamp On	Slant : 3,06829268292683, intercept : 4365, R^2 : 1, Exposure : 14596
		Lamp Off	Capture at Exposure : 1800mS
		24	CY5 F3
M	lask Calibration		Original Exposure Capture at 270mS 1/2 Exposure Capture at 135mS
			Level1 : 5507. Level2 : 4984
			Slant : 3,87407407407407, Intercept : 4461, R^2
			: 1, Exposure : 11535
			Capture at Exposure : 1800mS
			CV5 F4
1.000			Original Exposure Capture at 260mS
Back	ground Calibration		1/2 Exposure Capture at 130mS
			Level1 : 4863, Level2 : 4643
			Slant : 1,69230769230769, Intercept : 4423, R^2 : 1, Exposure : 26430
			Capture at Exposure : 1800mS
			CY5 F5
			Original Exposure Capture at 966mS
Multi-	Channel Calibration		1/2 Exposure Capture at 483mS
			Level1 : 0, Level2 : 0
			Slant : 0, Intercept : 0, R^2 : NaN, Exposure : - 2147483648
			Capture at Exposure : 1800mS

15) Wait for at least 5 minutes to switch off main power of the Exicycler[™] 96. The cooling fan will continue to run to cool down the lamp, althought you already turned the lamp off. Shutting down Exicycler[™] 96 before cooling down the lamp will reduce the lamp lifetime.

16) Power off the Exicycler[™] 96 by pressing the **POWER** button.

17) Turn off the Exicycler[™] 96 using the main power switch located on the back of the Exicycler[™] 96.



Go to Information>Exit from the top menu bar to close
 ExiCfg program.

!		<u>C</u> alibration	FactorySet	<u>H</u> elp
	<u>M</u> achine Lamp St Error Log <u>S</u> elf Tes	9		
24	E <u>x</u> it			R

Running Real-Time PCR Using Exicycle™ 96 Softwar

Starting 'Run Exicycler' software

 Ensure that the Exicycler[™] 96 is in the "Standby" position. The **POWER** button must blink in green. Double click **Run Exicycler3** icon on the desk top to start **Run Exicycler3**.



2) System Check window will pop up.

System is ready...

IMPORTANT:

The following error message will appear if the Exicycler[™] 96 is not powered on. Power on the Exicycler[™] 96 by pressing the power switch in the back of the Exicycler[™] 96 and double click **Run Exicycler3** on the desk top again.

Device is not ready, Please turn on.

IMPORTANT:

The following error message will appear whether the calibration has not been performed in the Exicycler[™] 96 or if the calibration data is lost accidently. Close the Run Exicycler3 and perform the calibration.



- 💃 Exicycler3 Run <u>File S</u>etup <u>R</u>un <u>H</u>elp 🗞 🗞 🗞 💫 🕴 🕨 🔳 🥊 Experiment Information Master b. Experiment Information Protoco Plate Data Name User Name a. Menu Run Time 00:00:0 Finished 00:00:00 Hotto Cycle col Infor c. Protocol Information No. Protocol Well Inform d. Well Information 8 9 10 11 12 В e. Temperature Profile e Graph Color Setting Flu. Dye Quencher en -80 60 -60 40 -40 -20 20
- 3) The following window will appear when System Check is complete.

- a. Menu consists of File, Setup, Run, Window, and Help.
- b. Experiment Information displays a file name, a user name, and elapsed and estimated finish time.
- c. Protocol Information displays a cycling protocol in detail.
- d. Well information displays sample and probe specification for each well.
- e. Temperature Profile displays a temperature curve of the cycling protocol.
- 4) Go to **Setup > Probe** from the top menu.

	👗 Exicycler3 Run						
ł.	<u>F</u> ile	<u>S</u> e	tup	<u>R</u> un	<u>H</u> e	lp	
ł.			Con	fig	►		
			Prot	ре			

IMPORTANT:

9 different probe options are available in the Exicycler[™] 96. Each probe option includes specifications of a fluorescence dye and a quencher. You can either select one of existing probe options in **Probe List** or add your own. Ensure you specify an appropriate probe option for accurate data analysis.

Probe Information window will appear.
 Select one or click Add to add additional probe.

Probe Information						
		Probe Lis	t			
No.	Name	Dye	Quencher	Color	^	Add
1	Std_FAM	FAM	BHQ			
2	Std_SYBR_GR	SYBR_GREEN	None			Modify
3	Std_JOE	JOE	BHQ			
4	Std_TET	TET	BHQ			Delete
5	Std_TAMRA	TAMRA	BHQ			
6	Std_CY3	CY3	BHQ			
7	Std_TEXAS_R	TEXAS_RED	BHQ			Save
8	Std_ROX	ROX	BHQ			
9	Std_CY5	CY5	BHQ			
10	GI	FAM	TAMRA		~	Close

6) Enter SYBR_AQ in the Name field.

Add Probe	
	Setting Probe
Name	SYBR_AQ
Dye	SYBR_GREEN
Quencher	None 🔽
Color	
OK	Cancel

NOTE:

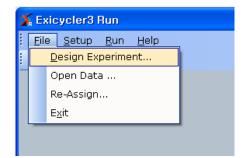
In the **Dye** drop-down list of the **Add Probe** window, select **SYBR_Green**. Select **None** in the Quencher drop-down list. Select your desirable color for display and then click **OK**.

 Check if your new probe is shown under the list, and then click Save.

		Probe Lis	t			
No.	Name	Dye	Quencher	Color	^	Add
1	Std_FAM	FAM	BHQ			
2	Std_SYBR_GR	SYBR_GREEN	None			Modify
3	Std_JOE	JOE	BHQ		≡	
4	Std_TET	TET	BHQ			Delete
5	Std_TAMRA	TAMRA	BHQ			
6	Std_CY3	CY3	BHQ			
7	Std_TEXAS_R	TEXAS_RED	BHQ			Save
8	Std_ROX	ROX	BHQ			
9	Std_CY5	CY5	BHQ			
10	GI	FAM	TAMRA		~	Close

Exicycler[™] 96 Real-Time Quantitative Thermal Block

 B) Go to File > Design Experiment to design the new experiment.



9) Quick Start window will appear as follows:

		b. User
	a. Master, Protocol, Plate	
	🚅 Quick Start	
	Master 22 G	User Guest V OK Cancel
	Update	Incubate Scan Incubate the plate at the given temperature and duration. Goto
c. Protocol Information	→ Delete Hottop	■ Melting Temperature 25.0 \$ °C Time(H:M:S) 0 \$ 0 \$ 0 \$ 0 \$
	105 🗘 🛱	Pause O Time Increment 1 Sec.
		Ramping Rate 21.0 °C/Sec.
	90- 80- 70-	-90 -80 -70
	60- 50- 40-	-70 -60 -50 -40
	30- 20- 10-	-30 -30 -20 -10
	Protocol Plate	
	d. Temperature Profile	

e. Protocol setting

- a. Master, Protocol, and Plate: You can create, save, or open a file.
- b. User: Select or create the user name for personal account management.
- c. Protocol Information: Displays a cycling protocol in detail.
- d. Temperature Profile: Displays a temperate curve of the cycling protocol.
- e. Protocol setting: Specifies protocol specifications such as temperature, time, and a number of cycles.
- Click Incubate tab and enter a temperature in the Temperature field and then a time in the Time field.

Append	🏥 Incubate	
Update	🖨 Scan	Incubate the plate at the given temperature and duration.
Delete	🔓 Goto	Temperature 25.0 °C
Delete	Melting	Time(H:M:S)
🔽 Hottop	🐣 Store	
105 🛟	Pause	○ Time Increment 🗘 1 Sec.
		○ Temperature Increment
		○ Ramping Rate \$1.0 °C/Sec.

a. **Incubate** sets up a temperature and a time for the thermal block.

Time Increment sets up time increment per second.

Temperature Increment sets up temperature increment per second.

Ramping Rate sets up a ramping rate.

- b. Scan measures fluorescence signals emitted from samples.
- c. Goto specifies a starting step of a thermal cycling and a number of cycles.
- d. **Melting** sets up starting temperature and ending temperature for melting curve analysis to distinguish specific and nonspecific amplification products when SYBR Green is used for Real-Time PCR.
- e. **Store** keeps the 96-well thermal block at a set temperature until you stop the Exicycler[™] 96. It is not recommended to use the **Store** step when it is excessively humid.
- f. Pause is used to pause the Exicycler[™] 96 when it is necessary to check samples during experimentation. The door of Exicycler[™] 96 will open when the Pause step is inserted in the protocol file. Click Run to resume the experiment.
- g. Hottop sets up a temperature for the heating lid.

NOTE:

Scan is used to detect fluorescence signals from samples. The fluorescence signals are measured for about 25 seconds at the set temperature of the previous incubation step. If the **Scan** step is not included in the protocol file, a convetional PCR will be carried out without scanning fluorescence signals.

Melting sets up starting temperature and ending temperature for melting curve analysis to distinguish specific and nonspecific amplification products when SYBR Green is used for Real-Time PCR. Enter the starting temperature in the **From** field, the ending temperature in the **To** field, a temperature interval within a range of 0.3°C to 2°C in the **Between** field, and a hold time within a range of 1 second to 99 hours 59 minutes and 59 seconds in the **Hold Time** field.

Store keeps the 96-well thermal block at a set temperature after the thermal cycling is complete. The heated lid will start cooling down automatically. The Exicycler[™] 96 will keep the main door closed and will continue to store the thermal block at the set temperature until you stop the Exicycler[™] 96. If the **Store** step is not inserted into the protocol file, the main door will open automatically when the thermal cycling is done.

Hottop Check Box sets up a temperature for the heating lid within a range of 90°C to 110°C. The default temperature is 100°C. When the check box is unselected, the heating lid will not be heated.

- 11) Click **Append** to add the new step into the protocol.
 - a. **Append** button is used when you want to add new steps into the protocol.
 - b. Update button is used when you want to make changes on steps.
 - c. Delete button is used when you want to delete steps from the protocol.

Append	ы Incubate
Update	🎒 Scan
	😼 Goto
Delete	Melting
🗸 Hottop	🐣 Store
105 💲	🐻 Pause

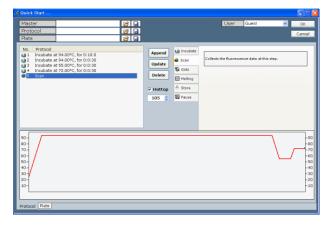
12) Click Incubate tab to add another Incubation step and then click Append. Repeat steps 2 through 4 if needed. (i.e. 94°C 30 sec / 55°C 30 sec / 72°C 30 sec).

a Quick Start		
Master 22 Review		User Guest 💌 OK
Plate 2		Cancel
No. Protocol Troubate at 04.00PC, for 0.10.0 Troubate at 05.00PC, for 0.030 Troubate at 05.00PC, for 0.030 Troubate at 72.00PC, for 0.030	Append Lit Incubite Update & Scan Delete Inteling V Hottop A Stare 105 S Reve	Incubits the plate at the given temperature and duration. Temperature 72.0 ° C C 0 0 0 30 ° C Time (H4HS) C 0 0 0 30 ° C Time forement 0 50 ° C Temperature Encrement 0 0.5 ° C Anoming Rate 0 0 C/Sec.
90- 80- 70- 60- 50- 40- 30- 20- 10- Protocol Flate		

NOTE:

To edit **Incubate** steps, click one of the steps in the **Protocol Information** window and edit. Click **Update** to change the protocol.

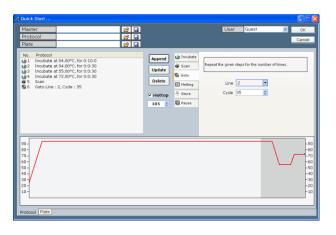
13) Click Scan tab and then Append.



14) Click Goto tab and select a starting step (i.e. "2") in the Line drop-down list. Enter a number of cycles (i.e. "35") in the Cycle field, and then click Append.

🏥 Incubate	
👸 Scan	Repeat the given steps for the number of times.
🖏 Goto	
Melting	Line 2
🐣 Store	Cycle 35
🐻 Pause	

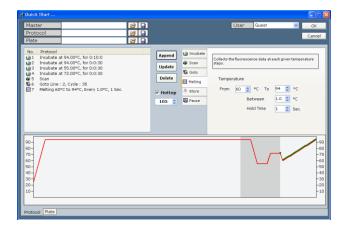
15) The protocol is created and saved. The **TemperatureProfile** window displays an estimated temperature curve as follows:



16) Click Melting tab to perform melting analysis. Enter the starting temperature in the From filed (i.e. "60"), the ending temperature in the To field (i.e. "94"), a temperature interval in the Between field (i.e. "1"), and a hold time in the Hold Time field (i.e. "1").

ы Incubate	
🖨 Scan	Collects the fluorescence data at each given temperature steps.
🚱 Goto	
Melting	Temperature
🐣 Store	From 60 🗢 °C To 94 🗢 °C
🐻 Pause	Between 1.0 🔷 °C
	Hold Time 1 💲 Sec.

17) Click Append.



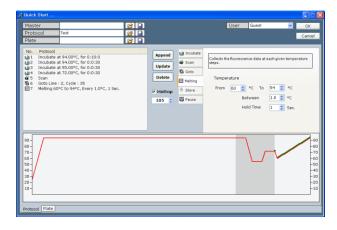
NOTE:

The door of the Exicycler[™] 96 will open automatically when the Real-Time PCR has run by a protocol that does not include the **Store** step. If the **Store** step is included in the protocol, you must stop the Exicycler[™] 96 first by clicking **Stop** button in order to open the door. Otherwise, the Exicycler[™] 96 will continue to store samples in the thermal block at the set up temperature.

IMPORTANT:

In order to edit the heating lid option, select the check box in the **Hottop** and enter a desirable temperature. The default temperature is 100°C. The heating lid will not be heated if the check box is unselected.

18) Enter a protocol file name in the **Protocol** field at the top left (i.e. "Test") and then click button to save the protocol.



NOTE:

Click button from the Quick Start to open the saved Master, Protocol and Plate files when needed.

- 19) Once the protocol is saved, click Plate tab to create a plate file. The Plate window will appear as follows:
 a. 96-well plate position
 b. Name/Count/Probe
 c. Probe Name, Flu. Dye
 d. Protocol/Plate Tab
 a. 96-well plate position specifies well locations for samples.
 - b. **Name/Count/Probe** displays a name, probe information for each sample.
 - c. **Probe Name/Flu. Dye/Quencher/Type/Concentration** specifies a name, a fluorescence dye, a quencher dye, a type, and a concentration for each sample.
 - d. Protocol/Plate tab switches between the Protocol Information and the Plate Information windows.
 - e. Sample Name enters a sample name.
 Assign specifies the information of each well such as probe and type.
 Add Probe adds the probe to use in experiment.

NOTE:

Ensure that you specify appropriate information (i.e. probe set up and sample type) for each well before running experiment in order to generate accurate data.

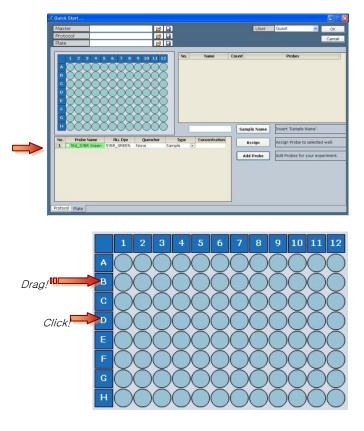
	af Ouick Start	
20) Click Add Probe, the Add Probe window will appear.	Master 27 27 27 27 27 27 27 27 27 27 27 27 27	User Guest V OK
		Probes ample Name Insert "Sample Name",
	No. Probe Name Flu. Dye Quencher Type Concentration	Assign Probe to selected well.
Add probe		Add Probe Add Probes for your experiment.
	Protocol Piete	

21) Select appropriate probes, and then click **OK**.

No.	Probe Name	Fluorescent Dye	Quencher
1	Std_FAM	FAM	BHQ
2	Std_SYBR_GREEN	SYBR_GREEN	None
З	Std_JOE	JOE	BHQ
4	Std_TET	TET	BHQ
5	Std_TAMRA	TAMRA	BHQ
g 6	Std_CY3	CY3	BHQ
7	Std_TEXAS_RED	TEXAS_RED	BHQ
8	Std_ROX	ROX	BHQ
9	Std_CY5	CY5	BHQ

- a. Probe Name: displays the name assigned by the user.
- b. Fluorescent Dye: displays a name of fluorescence dye assigned by the user.
- c. Quencher: displays a name of a quencher assigned by the user.
- 22) The probe is loaded and shown as follows:

23) Select wells from the 96-well plate diagram.

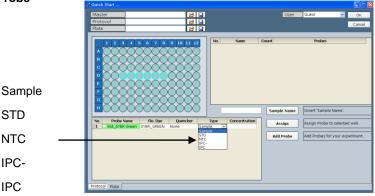


NOTE:

Use the left mouse button when selecting a single well. When selecting a range of cells, click the first cell in the range, and then drag to the last cell. You can also select cells in a row or column by pressing CTRL and clicking the row or column heading. To select all cells in 96-well plate, click the cell at the top left corner of the diagram.

Exicycler[™] 96 Real-Time Quantitative Thermal Block

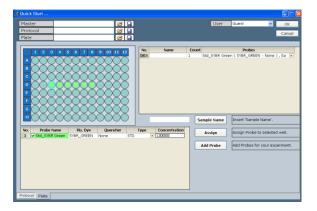
24) Click the probe option cell and then select STD in the Type drop-down list. Select the check box of Probe Name and then click Assign.



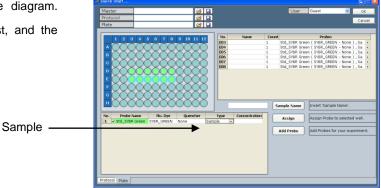
- Sample represents unknown samples. a.
- b. STD stands for a standard sample of a known concentration. Absolute Quantification

STD NTC IPC-IPC

- c. NTC stands for No Template Control and is a sample without a template.
- d. IPC stands for Internal Positive Control and is a sample that monitors the PCR run during Existence / Nonexistence reaction. It will also diagnose the cause of the negative result of PCR.
- e. IPC- is a sample that is used as a template and contains a reagent to prevent the IPC reaction during Existence / Nonexistence reaction. IPC- is not amplified during the Real-Time PCR.
- 25) Click the well D3 and enter concentration. For example, enter '1000000' for a concentration of 106copy and then click Assign. Repeat this for wells D4 through D8 with concentrations of serial diluted standards.



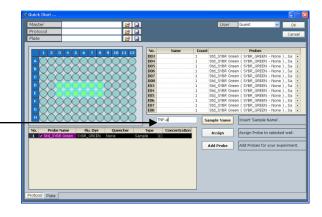
26) Select wells E3 through E8 in the plate diagram. Select Sample in the Type drop-down list, and the click Assign.



Exicycler™ 96 Real-Time Quantitative Thermal Block

27) Select cells D3 through E8 and enter a sample name in the Sample Name filed. Click Sample Name to save the sample name.

Sample Name Field -



28) Verify information on each well in the

Name/Count/Probe window.

Name/Count/Probe

Master 🗃 🖬					User	Guest	×	0
Protocol 🛛 🖉 🖟 Plate 🔗 🕞								Ca
	No.	Na	me	Count	t	Probe	5	-
	D03			1		reen (SYBR_GRI		
• • • • × × × × × × × × × × × × × × × ×	D04		-	1		een (SYBR_GRE		
	D05			1	Std_SYBR G	reen (SYBR_GRI	EN - None),	Sa
	D05			1		reen (SYBR_GRI reen (SYBR_GRI		
	D07			1		reen (SYBR_GR		
	E03			1		reen (SYBR_GRI		
	E04			1		reen (SYBR_GRI		
	EOS			1		reen (SYBR_GRI		
	E06	TNF-a		1		reen (SYBR_GRI		
	E07			1		reen (SYBR_GRI		
	E08	TNF-a		1	Std_SYBR G	reen (SYBR_GRI	EN - None) , 1	Sa
] s	ample Name	Insert 'Sam	ple Name'.	
No. Probe Name Flu. Dye Quencher	Type	Conce	ntration	10	Assign	Assign Prob	e to selected w	rell.
					Add Probe	Add Probes	for your experi	ine
								_

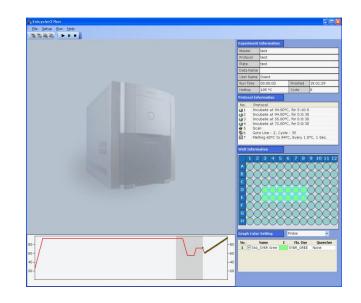
29) Enter a plate name in the **Plate** field (i.e. Test) and then click **button** to save the plate file.

Master Protocol								_		
	test	1					User	Guest	~	OK.
Protocol	test	1 i i								ance
Plate	test	1							- La	nici
12	3 4 5 6 7 1	8 9 10 11 12	NO.		Name	Count		Probes		
				TNF-a		1		ion (SYBR_GREEN - N		
		$X \times X \times X$		TNF-a		1		en (SYBR_GREEN - N		
				TNF-a		1		en (SYBR_GREEN - N		
				TNF-a		1		en (SYBR_GREEN - No		
	- X X X X X	$X \times X \times X$		TNF-a		1		en (SYBR_GREEN - No		
				TNF-a		1		en (SYBR_GREEN - N		
				TNF-a		1		en (SYBR_GREEN - N		
				TNF-a		1		en (SYBR_GREEN - N		
				TNF-a		1		en (SYBR_GREEN - No		
FOX				TNF-a TNF-a		1		en (SYBR_GREEN - No en (SYBR_GREEN - No		
G				TNF-a TNF-a		1		ien (SYBR_GREEN - Ni ien (SYBR GREEN - Ni		
			200	INP-d		1	SCG_STER GIE	IGHT (STER_ORDER - N	me), 50	2.1
)0000				5	ample Name	Insert 'Sample Nam	e'.	
	e Name Flu. Dye BR Green SYBR_GREE	Quencher None	Type Sample	Co	ncentration	10	Assign	Assign Probe to sele	ected wel	Ι.
							Add Probe	Add Probes for your	experim	ent
							neerrobe			_
votocol Plate			_	_		_				

30) The protocol file and plate file are saved. Enter a master file name in the Master field (i.e. Test) and then click button to save the master file.

NOTE: Save the master file, the protocol file, and the plate file by clicking button. You can open the saved master file by clicking button from the Quick Start. The master file is loaded along with the protocol file and the plate file.

 Click **OK** at the top right to display the go back to the main window.

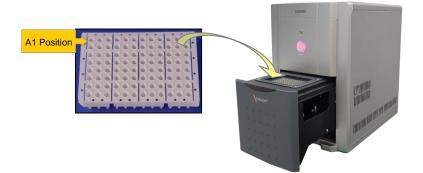


32) Ensure the **POWER** button in the front of the Exicycler[™] 96 is blinking in green.



33) Press the **DOOR** button for a full second to open the door. Load the 96-well plate with samples with the A1 position at the top-left of the block.





Exicycler™ 96 Real-Time Quantitative Thermal Block

34) Verify the master file and go to Run > Run from the top menu bar or click the Run button.

- 35) Enter a data name (i.e.Sample_AQ) in the Dataname window and click Ok. If you do not enter the data name, default name will be used.

Datanam	ie	
Please in	put the dataname to	save the result.
Sample_/	٨Q	
	OK	Cancel

- 36) The following three message boxes will appear in the following order.
 - a. The message will appear when closing the door of the Exicycler[™] 96 if the door was open.
 - b. The message will appear when checking the lamp condition.

Checking Jamp condition	
	Checking lamp condition.

Configuring the system for PCR running

0%

c. The message will appear while the lamp is turning on.

Igniting the lamp.	
13%	

 d. The message will appear when checking if the lamp is on and initializing the Exicycler[™] 96.

Checking optical system.

88%

37) The main window will appear when the progress bar finishes without any problems.

File Setup Run Help Tà Tà Tà Tà Tà Ti Ti 19 21 23 25 27 29 31 33 35 37 39 Mating Ma
Scall Experiment Information 0 K- Master AQ1 0 K- Data Name Sample_AQ 0 K- Data Name Suest 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Data Name 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Data Name 0 K- Data Name Suest
0.K - 0.K - 0.
0 K- 0 K- 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Well Information 0 K- 0 K- 0 K- 0 K- 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 0 K- 0 K- 0 K-
0 K- 0 K- 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Mething 0 K- 1 4 5 Can 1 5 C 1 2 3 4 5 6 7 8 9 10 11 12 Mething 0 K- 0 K- 1 2 3 4 5 6 7 8 9 10 11 12 0 K- 1 2 3 4 5 6 7 8 9 10 11 12 0 K- 1 2 3 4 5 6 7 8 9 10 11 12 0 K- 0 K-
0 K- 0 K- 0 K- 0.0000 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 I Moking Incubate at 94.00°C, for 0.0100 I A Maine Scan I A B F R A A A A A A A A A A A A A A A A A A
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0 K- 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Mething 0 K- 0 K- 0 K- 0 K- 0 K-
Mathing 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Mathing Image: Second secon
IX - IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
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1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
Mething 7 Mething Gove to 94+C, Every 1.0*C, 1 Sec. 13 1 1 1 12 3 4 5 6 7 8 9 10 11 12 0 K - 1 2 3 5 6 7 8 9 10 11 12
0 K- 0 K- 1 2 3 4 5 6 7 8 9 10 11 12 A
60 63 66 69 72 75 78 81 84 87 90 93
Graph Color Setting Probe 💌
-80 No. Name C Flu. Dye Quencher
-60 1 Std_SYBR_GRE None
-40
Pre-Cooling

- a. Scan window displays a curve of amplification in real time during PCR.
- b. Melting windows displays a melting curve in real time when the melting step is included in the protocol file.
- c. Temperature Profile displays a temperature profile and in real time
- d. Experiment Information displays information for the experiment currently in progress.
- e. Protocol Information highlights the step currently running.
- f. Well Information displays set up details for each well when selecting either Probe or Type in the Graph
 Color Setting drop-down list. To display the curve of amplification, select well from the 96-well plate diagram and click the check box from the probe option.

Exicycler[™] 96 Real-Time Quantitative Thermal Block

38) Go to Run > Stop to stop the Exicycler[™] 96 or click the Stop button.



39) Go to Run > Pause to pause the Exicycler[™] 96 or click the Pause button.



IMPORTANT:

If the pop up window remains more than 5 minutes after running the Exicycler[™] 96 or if the temperature profile does not display anything in the main window, turn off the Exicycler[™] 96 and turn it on again. Start **Run Exicycler3** again and run the Exicycler[™] 96 again. If the same error keeps occurring, please contact us for customer service.

IMPORTANT:

It is recommended to re-start the Exicycler[™] 96 at least 10 minutes after the previous Real-Time PCR run has finished. Continuous operation without a break will reduce the lamp life time and cause errors.

NOTE:

Do not turn off the Exicycler[™] 96 while the heating lid is warming up. While the heating lid is heated to the set temperature, the 96-well block maintains at 25°C. The thermal cycling will start when the heating lid reaches to the set temperature and the lamp is stabilized.

NOTE:

*.ex3 file is created under a folder designated by the user after the experiment is complete. The user can analyze the *.ex3 file using the Exicycler[™] 96 Analysis Software to generate analysis data. Please refer to **Analyzing Data using Analysis Software** for data analysis.

Performing System Diagnosis using ExiCfg

 Power on the Exicycler[™] 96 by pressing the **POWER** button.



2) Go to 'C:\Exicycler3\Run' and double click **ExiCfg.exe** to start the program.

	Calibrated	Not calibrate	History
Lamp warming	D%	Ē	cycler3 Config V3.55.0
Mask Calibration			
Background Calibration			
Multi-Channel Calibration			

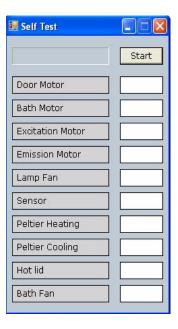
3) Go to Information > Self Test.

Exicycler3 Config.		
Information Calibration Machine Information	FactorySet	
Lamp Status Error Log	Calibrated Not calibrate	History Exicycler3 Config V3.55.0
Self Test	0%	excyclera comig va.ss.o
Exit		
Mask Calibration		
Background Calibration		
~		
Multi-Channel Calibratio	n	
	_	

NOTE:

'USB Communication is NOT initialized' message box will pop up if the Exicycler[™] 96 is powered off or if the cable connection between the Exicycler[™] 96 and the computer is not firmly connected.

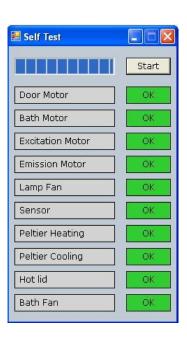
4) Click Start in the Self Test window.



IMPORTANT:

Make sure that there are no objects placed in front of the door while performing Self Test.

- 5) All of the self-diagnosis tests pass completely without
- any problems, OK signs will show up in green.



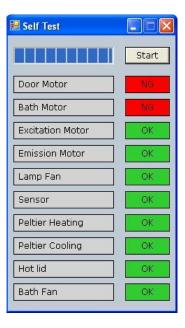
NOTE:

The self-diagnosis is complete with two short beeps.

Exicycler[™] 96 Real-Time Quantitative Thermal Block

6) When any of the self-diagnosis tests fail, **NG** signs will

appear in red.



NOTE:

Any problems caused during self-diagonsis are recorded in 'Error log'.

7) Go to Information>Error Log from the top menu bar to

see the problems.

i, E	xicycler3 Config.	Error	
I	nformation Calibration Machine Information	1 Errors Occured Door close error - over-current - [0x21]	
	Lamp Status	-	
	Error Log		
	Self Test		
	Exit		

Troubleshooting

Troubleshooting

Please take recommend action for each observation. Contact Bioneer Customer Service Center if problem continues after the recommended action.

Observation	Recommended Action		
The Exicycler™ 96 does not start.	 Make sure the power cable is firmly connected to the wall circuit, and then switch on the power supply button in the rear of the Exicycler[™] 96. Check a fuse box located down by the power switch in the rear of the Exicycler[™]96. Change the fuse if it has blown. 		
Errors occur either after or during Self-diagnosis (POWER button in red).	 The lamp may still be cooling down or the Self-diagnosis is still running. If the POWER button does not turn back to green in 5 minutes, power off the Exicycler™ 96 and back on again. If the same error occurs, please contact Bioneer Customer Service Center for help. Power off the Exicycler™ 96 if unexpected errors occur. Power back on the Exicycler™ 96 again to cool down the lamp and wait for 3 minutes. Press the POWER button to start Self-diagnosis. Please contact Bioneer Customer Service Center if the same error keeps occurring. 		
No communication detects between the computer and the Exicycler™ 96 .	 Make sure that the USB cable is firmly connected to the computer. Ensure you use the USB cable provided with the Exicycler[™] 96 in order connect the computer and the Exicycler[™] 96. Check if the Exicycler[™] 96 is in the "Standby" position. The POWER butt must blink in green. Power off the Exicycler[™] 96 and back on again if t POWER button still blinks in red. 		
The POWER button or the DOOR button does not work.	 Make sure that the power cable is firmly connected to the wall circuit, and the switch on the power supply button in the rear of the Exicycler[™] 96. The Self-diagnosis may be still running. Wait until the Self-diagnosis is complete and the POWER button blinks in green. 		
The Exicycler™ 96 has stopped running.	 Check if the electricity is supplied properly. Operate an AVR or UPS if needed. Disable Screen Saver and Monitor Power in the Control Panel. Ensure you use the USB cable provided with the Exicycler[™] 96 to connect the computer and the Exicycler[™] 96. Check if the USB driver is installed properly. If not, reinstall it again. Do not plug in any other USB cables in the computer while the Exicycler[™] 96 is running to prevent friction between the Exicycler[™] 96 and the computer. 		

Observation	Recommended Action		
The Exicycler™ 96 does not start a thermal cycling.	• The Exicycler [™] 96 may still be in the "Standby" position in order to warm up the lamp. Warming up the lamp takes about 10 minute. The thermal cycling will start automatically after the lamp warm up is finished.		
Low intensity fluorescence signal is detected.	 Either examine fluorescence signal of the probe used for Real-Time PCR or use a new kit. The PCR product amplified may be too long or there may be non specific products. Perform gel electrophoresis to determine the presence of amplification product or adjust an annealing temperature or Mg2⁺ concentration if needed. Perform the calibrations again to adjust the light intensity of the lamp. 		
Excessively high intensity fluorescence signal is detected.	 Adjust concentration of the probe used for Real-Time PCR. Make sure you use an appropriate probe system. Adjust and optimize the PCR sample conditions. 		
The software shuts down abnormally.	The USB driver has not been installed properly. Reinstall the USB driver.		
PCR sample is evaporated.	 Check if the heating lid works or if the sample tubes or plates are completely sealed with the sealing tape. Ensure the heating lid option is set up correctly. The Hottop Check Box must be selected and correct temperature must be set up. A small amount of evaporation is not critical. It is recommended that you use Bioneer kits and reagents to prevent PCR sample evaporation. 		
No PCR products are amplified.	 Perform gel electrophoresis to determine the presence of amplification products. Adjust an annealing temperature or Mg2⁺ concentration if needed. 		
No fluorescent signal is detected although PCR product is amplified.	 Either examine fluorescence signal of the probe used for Real-Time PCR or use a new intercalating dye. Perform the calibrations again to adjust the light intensity of the lamp. 		
Pop-up message during calibration process:	 Background Calibration finished will appear if the Background Calibration is successful. Can't Read Data from EEPROM will appear if the computer can not read data from the Exicycler[™] 96. Check if the USB cable is firmly connected between the computer and the Exicycler[™] 96. 		

Observation	Recommended Action		
	Can't close program during PCR will appear if you attempt to close the		
	Exicycler™ 96 software while PCR is still running.		
	Do you want to stop PCR? will appear if you click Stop button during the Multi-		
	channel Calibration.		
	Do you add plate on the bath? will appear to make sure that the calibration		
	plate is loaded in the thermal block prior to PCR.		
	Mask Calibration finished will appear if the Mask Calibration is complete.		
	Multi-Channel Calibration finished will appear if the Multi-channel Calibration is		
	successful.		
	Need to set up Machine ID will appear if an instrument ID has not been set up		
	for the Exicycler™ 96. Assign the ID using the Exicycler™ 96 software.		
	No matching calibration data with machine will appear if the instrument ID		
Pop-up message during	does not match the ID from the Exicycler™ 96 software. Set up the instrument ID		
calibration process (continued):	using the Exicycler™ 96 software.		
	Turn off the lamp will appear if you attempt to end ExiCfg while the lamp is still		
	on. To close ExiCfg, turne off the lamp first and then close the ExiCfg.		
	Please load Mask Calibration Information first will appear if you attempt to		
	perform the Uniform Calibration before the Mask Calibration.		
	USB Communication is NOT Initialized will appear if the computer and the		
	Exicycler™ 96 do not communicate with each other. Check if the USB cable		
	between the computer and the Exicycler [™] 96 is firmly connected.		
	You must insert New Dye Name will appear if a new name is not assigned for a		
	custom dye.		
	You must select at least one dye will appear if you start the Multi-channel		
	Calibration without selecting dyes. You must select at least one fluorescent dye		
	for the calibration.		
	Can't exit program during PCR will appear if you click Stop button in the		
	Exicycler™ 96 software while PCR is still running.		
	Can't open data during PCR will appear if you select File > Open Data from the		
Error message when running the	top menu while PCR is still running.		
Exicycler™ 96 software:	Can't Pause during Melting Protocol will appear if you click Pause button while		
	the melting step is still running.		
	Can't read data from EEPROM will appear if errors occur during PCR and the		
	computer cannot read data from the Exicycler™ 96.		

Observation	Recommended Action	
	Can't read Plate Information will appear if you attempt to open an outdated	
	plate file.	
	Can't Stop during Melting Protocol will display if you attempt to stop the	
	Exicycler™ 96 software while the melting step is running.	
	Communication between machine and S/W was not initialized will display if	
	you click Run button when the Exicycler™ 96 is not ready.	
	Do you want to stop PCR? will display if you click Stop button while PCR is still	
	running.	
	Fail to load calibration information will appear if the computer fails to read the	
	calibration data from the Exicycler™ 96. Go to C: \Exicycler3 to check if the	
	calibration folder and file exist.	
	Melting Protocol can't be in Cycle Region will appear if the melting step is	
	inserted within a thermal cycling.	
	Need to replace the Lamp will appear if the lamp intensity drops below 60% of	
	the normal lamp intensity. Replace the lamp.	
	No matching calibration data with Machine. Can't Run! will appear if you click	
Error message when running the	Run button when the Exicycler™ 96 ID and the calibration data ID do not match.	
Exicycler™ 96 software	No matching calibration data with machine will appear if the Exicycler™ 96 ID	
(continued):	and the calibration data ID do not match.	
	Please insert any protocol before inserting Goto Protocol will appear if the	
	Goto step is inserted in the first line of a protocol file.	
	Please insert Data name will appear if you have not entered a name for data	
	prior to PCR running.	
	Probe Information was changed, do you want to cancel this? will appear if	
	you click the Cancel button without saving the edited probe Information.	
	Probe using same filter is assigned will appear if you assign more than 2	
	probe options within the same wavelength range.	
	Ramping rate option Must be inserted between Incubation steps will appear	
	if you insert a ramping rate option in a wrong position of the protocol file.	
	Set Probe Name will appear if you have not entered a new name for the custom	
	probe in the Add Probe window.	
	UserName was already in User List will appear when entering an existing	
	name for User set-up.	
	You must select Dye and Quencher will display if the dye and quencher set up	
	has not been selected in the Add Probe window.	

Ordering Information and Warranty

Ordering Information and Warranty

Product			
A-2060	Exicycler™ 96 Real Time Quantitative Thermal Block		
Accessories			
A-2060-A1	AccuPower [®] Calibration Kit for Exicycler [™] 96		
Plastic consu	mables		
3111-41	Adhesive Optical Sealing Film, 100 sheets		
3111-50	0.2 ml Opaque White 8-strip PCR Tube, 250 Strips		
3111-52	Opaque White 96-well Semi-skirted PCR Plate, 25 Plates		
Premix & Reagent			
K-6200	AccuPower® Greenstar™ qPCR PreMix, Exicycler 8-well strip, 50 μl, 12 strips		
K-6203	AccuPower® Greenstar™ qPCR PreMix, Exicycler 96-well plate, 50 µl, 1 plate		
K-6253	AccuPower $\ensuremath{\mathbb{R}}$ 2X Greenstar qPCR Master Mix / 100Rxn, 50 μL reaction		
K-6254	AccuPower $\ensuremath{^{ extsf{e}}}$ 2X Greenstar qPCR Master Mix / 200Rxn, 50 μL reaction		
K-6110	AccuPower® DualStar™ qPCR PreMix, Exicycler 8-well strip, 50 µl, 12 strips		
K-6113	AccuPower® DualStar™ qPCR PreMix, Exicycler 96-well plate, 50 µl, 1 plate		
K-6600	AccuPower® Plus DualStar™ qPCR PreMix, Exicycler™ 96, 12 strips, 50 µl		
K-6603	AccuPower® Plus DualStar™ qPCR Master Mix(2X), 2.5ml, 100 rxn		

Warranty

This Bioneer brand product, as supplied and distributed by Bioneer Corporation, is warranted by Bioneer against manufacturing defects in materials and workmanship for a limited warranty period of one year.

Product	Exicycler™ 96 Real-Time Quantitative Thermal Block		
Catalog No.	A-2060		
Serial No.			
Date of Purchase	/ (dd/mm/yy)		
Warranty Period	For 12 months from purchasing date		

1. How to request warranty service

Please fill out the service request form attached to this manual and submit it to us by fax or mail. For prompt service, please have the problem log and the experimental file ready before contacting us. For more details, please contact Bioneer Customer Service Center or your local distributor. You may call us at 1588-9788 for minor problems. The service request result will be notified to you within 7 business days and the system will be repaired or replaced within 14 business days.

2. Repairs under warranty

During the one-year warranty period, Bioneer will repair all defective products free of charge.

3. Exclusion from warranty

The product is excluded from warranty if:

- The product has been found to be defective after expiry of the warranty period.
- The product has been subjected to misuse, abuse, or unauthorized repair, whether by accident or other cause.
- Product is damaged beyond repair due to nature disaste

Warranty Service Request Form					
Product	Exicycler ™ 96 Real-Time Quantitative Thermal Block				
Catalog No.	A-2060	Serial No.			
Date of request					
Date of purchase					
	* Please list one service issue or concern per line				
	Issue 1				
Service	Issue 2				
Issue	Issue 3				
	Issue 4				
	Name				
Customer	Company				
	Name				
Information	Contact	Phone:		Fax:	
	E - mail			·	

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