# **Installation and Calibration Guide**

Exicycler<sup>™</sup> 96 Real-Time Quantitative Thermal Block



**BIONEER** CORPORATION

#### 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.

# Contents

Before Starting	4
Safety Warnings and Cautions	9
Location Consideration and Installation	9
Operation and Maintenance	10
European Safety and EMC Standards (CE)	12
Introduction	15
System Components and Specifications	19
System Components	19
Specifications	20
System Views	22
Installation	25
Setting Up the Computer	25
Connecting the Computer and Exicycler™ 96	26
Installing the Operation and Analysis Software	27
System Operation and Calibration Preparation	35
Powering On and Self-diagnosis	35
Reagents and Consumable Products	36
Calibration Using ExiCfg	45
Before Starting Calibration with the Calibration Kit	45
Calibration Plate Preparation	45
Calibration using ExiCfg	
Mask Calibration	
Background Calibration	51
Multi-channel Calibration	55
Starting 'Run Exicycler' software	63
Performing System Diagnosis using ExiCfg	79
Troubleshooting	85
Ordering Information and Warranty	91

# **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<sup>™</sup> 96 Real-Time Quantitative Thermal Block.

#### **General Requirements for Installation**

This guide assumes that you:

- Are familiar with the Microsoft<sup>®</sup> Windows<sup>®</sup> 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<sup>™</sup> 96 Real-Time Quantitative Thermal Block.

#### How to Obtain More Information

For more information about Exicycler<sup>™</sup> 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**

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# **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<sup>™</sup> 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.

Marnings: Hazards or unsafe actions that may result in High temperature and Skin burn.

 $\Delta$  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<sup>™</sup> 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<sup>™</sup> 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.
- 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<sup>™</sup> 96 that can interrupt door operation.
- 7) Leave 30 cm space between the Exicycler<sup>™</sup> 96 and the wall for proper ventilation.
- B) Do not install the Exicycler<sup>™</sup> 96 in a dusty environment to help prevent false operation or technical damage.
- 9) Keep the Exicycler<sup>™</sup> 96 away from heat sources.
- 10)The Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 96. When the computer power is on while connecting, the communication port connector in either the computer or the Exicycler<sup>™</sup> 96 may get damaged.
- Make sure that the USB cable is firmly connected between the Exicycler<sup>™</sup> 96 and the computer. Unstable connection may cause damage of the communication port connector or data transfer errors.
- 4) The built-in camera in the Exicycler<sup>™</sup> 96 is a static-sensitive device. Pay particular attention to any cables connected to the Exicycler<sup>™</sup> 96 to avoid static damage.

#### Cautions

- 1) Ensure the power cable is clean and connect it firmly to the Exicycler<sup>™</sup> 96.
- Operate the Exicycler<sup>™</sup> 96 in a place where the temperature is always between 15<sup>°</sup>C and 30<sup>°</sup>C.
   Poor performance is influenced by extreme temperatures. High temperatures can cause misoperation 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<sup>™</sup> 96 that can interrupt ventilization and cause errors.
- The internal optical components of the Exicycler<sup>™</sup> 96 may get damaged if the instrument falls or is exposes to excessive physical shock.
- Unplug the power cable from the Exicycler<sup>™</sup> 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.

- Keep the 96-well thermal block area clean to prevent damage and to generate accurate experimental data.
- 3) After any Real-Time PCR runs including 'Scan' or 'Melting', allow the Exicycler<sup>™</sup> 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<sup>™</sup> 96. It could cause fire.
- 5) Do not turn off the Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 96 and the computer will be interrupted.

#### **IMPORTANT:**

Exicycler<sup>™</sup> 96 should be operated in clean condition. Contaminants such as dust can cause troubles and reduce the life time of Exicycler<sup>™</sup> 96. Please prevent dust from coming into Exicycler<sup>™</sup> 96. Through removing dust periodically from the Exicycler<sup>™</sup> 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 semi-automatic 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<sup>™</sup> 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<sup>™</sup> system can also be used for life science research and other laboratory purposes.

Exicycler<sup>™</sup> 96 Real-Time Quantitative Thermal Block

# Introduction

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# Introduction

The Exicycler<sup>™</sup> 96 Real-Time Quantitative Thermal Block combines a thermal block in a standard 96well 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<sup>™</sup> 96, which carries out a thermal cycling. The detector is located in the upper part of the Exicycler<sup>™</sup> 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 <sup>™</sup> Analysis Software.

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

The Exicycler<sup>™</sup> 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<sup>™</sup> 96 covers within 480 to 680 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 96 for users convenience. The Exicycler<sup>™</sup> 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<sup>™</sup> 96.
- An analysis program for data analysis. The analysis program includes Absolute Quantification, Relative Quantification, Multi-Relative Quantification, SNP genotyping, Existentence/Nonexistentence. Analysis and is applicable to Gene expression, Quantification of cell and virus, and SNP genotyping.

# **System Components and Specifications**

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# **System Components and Specifications**

Exicycler<sup>™</sup> 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<sup>™</sup> 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 ( <sup>kg</sup> )	<b>30</b> kg
Sample capacity size	96-well plate / 0.2 $^{\text{m}\ell}$ micro tubes
Sample volume	20~50 µl
	(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	± 0.5 °C
Ramping rate	Max. 2.5℃/sec
Lid temperature	Within 90 ~ 120 ୯
Gradient range	1℃~20℃
Temperature increment range	0.1℃~9.9℃
Time increment range	1 sec ~ 60 sec
Ramp rate control	0.1°C/sec
Operating temperature	15 ~ 30 ℃
Operating humidity	20 ~ 80%, no condensation
Communication	USB 2.0 high speed
Operating OS	Windows XP (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 Types / 5 Types

\* 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	640	670	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

BIONEER DOOR POWER and Status LED Extoycler\* Ventilation hole Rear view



#### Inside view



Exicycler™ 96 Real-Time Quantitative Thermal Block

# Installation

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# Installation

To run the Exicycler<sup>™</sup> 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<sup>™</sup> 96 Real-Time Quantitative Thermal Block operates through a computer. Therefore, to install Exicycler<sup>™</sup> 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 <sup>™</sup> 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.
- 3) It is recommended that a memory stick not be installed when Exicycler<sup>™</sup> 96 is running. It may cause a technical problem between Exicycler<sup>™</sup> 96 and the computer.

# Connecting the Computer and Exicycler<sup>™</sup> 96

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

- Unpack the Exicycler<sup>™</sup> 96 and make sure all components are included. Refer to the list of components below.
- Exicycler<sup>™</sup> 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<sup>™</sup> 96 in the installation site carefully.
- 5) Connect the USB 2.0 cable to the USB connector on the back of Exicycler<sup>™</sup> 96, then to the computer.
- 6) Connect the power cable to the Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup>96 unless you have installed the Operation and Analysis Software and a camera driver. First install the Operation Software and then the camera driver.

 Power on the computer <u>only</u>, not the Exicycler<sup>™</sup> 96. Insert the installation CD into the CD drive of the computer. Exicycler<sup>™</sup> 96 Setup Program starts automatically. If the Setup Program does not start, go to 'My Computer' to locate the CD drive, and then double-click **Setup.exe**.

e Edit View Favorites	Tools	Help				
3 Back + 🕥 + 💋	P	Search 🜔 Folders 🛄 •				
		Name	Size	Type -	Date Modified	
Pile and Folder 1 asks       Image: Start Start       Image: Share this folder	~	Ellisetup.exe	33,395 KB	Application	9/18/2007 8:48 PM	
Other Places	8					
Desktop  My Documents  Shared Documents  My Computer  My Network Places						
Details	*					

 The 'Exicycler<sup>™</sup> 96 Install Shield Wizard' starts. Click Next to start the installation. The installation will proceed in C:\Exicycler3.



3) Click Next to start installing software.



4) Once the installation starts, do not click 'Cancel'.

🙀 Exicycle	er3 - InstallShield Wizard 📃 🗖 🔀
Installing The prog	Exicycler3 ram features you selected are being installed.
IF.	Please wait while the InstallShield Wizard installs Exicycler3. This may take several minutes. Status:
InstallShield	< Back Next > Cancel

5) When the installation is complete, click **Finish** to finish the Installation Shield Wizard.



 Connect the Exicycler<sup>™</sup> 96 to the computer when the installation is complete.



7) Go to a folder 'Exicycler3' on the C drive to verify that the installation is complete. When the installation is successful, new folders appear under the 'Exicycler3' folder.



 Two new icons are created on the desk top (Run Exicycler3 and Analysis Exicycler3).

- Check the USB cable connection between the Exicycler<sup>™</sup> 96 and the computer.
- Power on the Exicycler<sup>™</sup> 96. A power buttonLED turns red when the power is supplied properly.

- 11) Press the power button again for a full second to start Self-diagnosis. The POWER button LED starts blinking in green after two short beeps when the self-diagnosis is complete.
- 12) When powering on the Exicycler<sup>™</sup> 96, 'Found New Hardware Wizard' will appear. Select Yes, this time only, then Next.

Next > Cancel









rd Welcome to the Found New Hardware Wizard Windows will search for current and updated softw

Windows will search for current and updated software by looking on your computer, on the hardware installation CD, or on the Windows Update Web site (with your permission). <u>Read our privacy policy</u>

Can Windows connect to Windows Update to search for software?

Yes, this time only
 Yes, now and every the I connect a device
 No, not this time

< Back

Click Next to continue.

13) Select 'Install from a list or specific location (Advanced)', Found New Hardware Wizard then click Next.



14) Select 'Search for the best driver in these locations', then select 'Include this location in the search' check box.
And then browse 'C:\Exicycler3\NcBulk'. Click Next to start installing the driver.

Found New Hardware Wizard
Please choose your search and installation options.
Search for the best driver in these locations.
Use the check boxes below to limit or expand the default search, which includes local paths and removable media. The best driver found will be installed.
Search removable media (floppy, CD-ROM)
Include this location in the search:
C:\Exicycler3\NcBulk Srowse
O Don't search. I will choose the driver to install.
Choose this option to select the device driver from a list. Windows does not guarantee that the driver you choose will be the best match for your hardware.
< Back Next > Cancel

15) The installation starts.



16) When the driver completes the installation, click **Finish**.

Completing the Found New Hardware Wizard The wizard has finished installing the software for: Netchip Transfer device' (VID_0525&PID_1000) using NcBulk.SYS
Click Finish to close the wizard.

17) Go to 'Device Manager' and check that USB Test Devices (Netchip) is installed. 'Netchip 'Transfer Device' (XXX\_XXX\_XXX) using xxxxx.SYS is shown when the driver installation is successfully completed.

🗒 Device Manager	
File Action View Help	
← → 🔟 🗳 😫 🕿 🕿 🗶	
- 🔍 BIONEER	
🖭 🚽 Computer	
🗄 🥪 Disk drives	
🗉 🕎 Display adapters	
Q DVD/CD-ROM drives	
🗄 🚍 Floppy disk controllers	
🗉 🧸 Floppy disk drives	
Gas Human Interface Devices	
E      IDE ATA/ATAPI controllers	
🗉 🦢 Keyboards	
Mice and other pointing devices	
🗉 🛒 Monitors	
Metwork adapters	
Ports (COM & LPT)	
🗄 🜸 Processors	
😟 🥥 System devices	
🗉 🚭 Universal Serial Bus controllers	
🖃 🕰 USB Test Devices (NetChip)	
🕺 🖨 Netchip 'Transfer device' (VID: 05258PID: 1000) using NcBulk.SYS	

18) Copy the calibration data to the computer by overwriting the Calibration folder in the install CD to C:
\Exicycler3\Run. The calibration data generated from the Exicycler™ 96 is only associated with the Exicycler™ 96. Make sure the serial number in the install CD and in the rear side of the Exicycler™ 96 match when copying the calibration data to the computer.

File Edit View Eavoriter Tools	Help					
The Edit Herr Furthers Tools	Thep				100	
🔇 Back 🔹 🔘 - 🏂 🔎 s	🕽 - 🏂 🔎 Search 🍋 Folders 💷 -					
Address 🛅 Ci\Exicyder3\Run				8	🔁 Go	
Folders ×	Name 🔺	Size	Туре	Date Modified		
Desktop	Calibration		File Folder	11/15/2007 2:11 PM		
My Documents	📺 appParam.xml	1 KB	XML Document	8/5/2006 1:59 PM		
🗏 😼 My Computer	SameraCon.dl	40 KB	Application Extension	11/2/2007 2:43 PM		
H 44 31/2 Floopy (A;)	CameraCon.pdb	58 KB	PDB File	11/15/2007 4:04 PM		
🗏 🥪 Local Disk (C:)	🔊 dragndrop.dll	20 KB	Application Extension	5/17/2004 9:38 AM		
Documents and Settin	SEGraph.dl	40 KB	Application Extension	11/2/2007 10:07 AM		
Exicycler3	EGraph.pdb	74 KB	PDB File	11/2/2007 10:07 AM		
C Analysis	* ExiLCTCfg.exe	512 KB	Application	11/2/2007 10:07 AM		
🗄 🦳 Copy of Bup	dexilctcfg.exe.licenses	1 KB	LICENSES File	6/1/2006 10:27 AM		
O N/B /k	ExiLCTCfg.pdb	356 KB	PDB File	11/2/2007 10:07 AM		
E C Bun	SExiLCTIO.dll	84 KB	Application Extension	11/16/2007 10:39 AM		
1 C Rup 2007 11 16	ExiLCTIO.pdb	180 KB	PDB File	11/16/2007 10:39 AM		
🗄 🤭 EviData	*ExiLCTRun.exe	1,028 KB	Application	11/16/2007 1:51 PM		
E C EviDriver	ExiLCTRun.pdb	456 KB	PDB File	11/16/2007 1:51 PM		
E C MSOCarbe	harrowcur	1 KB	Cursor	8/5/2004 9:00 PM		
E Program Eles	2 hmovecur	1 KB	Cursor	8/5/2004 9:00 PM		
I Demo	MagProc.dl	36 KB	Application Extension	11/2/2007 10:07 AM		
IT O WINDOWS	ImgProc.pdb	80 KB	PDB File	11/2/2007 10:07 AM		
H N OFFICE 11 (D)	licenses.licx	1 KB	LICX File	2/27/2006 1:04 PM		
E 52 105 - Ha J-(E)	Net2272Usb.dl	221 KB	Application Extension	8/4/2006 3:46 AM		
H Control Papel	PlateNSCtrl.dl	32 KB	Application Extension	11/2/2007 10:07 AM		
+ Carlot Documents	PlateN5Ctrl.pdb	50 KB	PDB File	11/2/2007 10:07 AM		
Bapee Park's Documents	S ProtocolGraph.dll	32 KB	Application Extension	11/2/2007 10:07 AM		
t S My Network Places	ProtocolGraph.pdb	40 KB	PDB File	11/2/2007 10:07 AM		
Recycle Rip	Syncfusion.Core.dl	280 KB	Application Extension	1/26/2006 7:07 PM		
Capture	Syncfusion.Edit.Windows.dll	1,920 KB	Application Extension	1/26/2006 7:10 PM		
c >	Syncfusion, Grid, Base, dl	16 KB	Application Extension	1/26/2006 7:10 PM		

# System Operation and Calibration Preparation

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# System Operation and Calibration Preparation

Perform the calibration after verifying the installation of the Exicycler<sup>™</sup> 96 and the computer.

# **Powering On and Self-diagnosis**

- Power on the Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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<sup>®</sup> Greenstar<sup>™</sup> PCR PreMix Kit allows an easy and fast amplification in Exicycler<sup>™</sup> 96. The AccuPower<sup>®</sup> Greenstar<sup>™</sup> PCR PreMix Kit consists of Greenstar<sup>®</sup> fluorescent dye, HotStart Taq DNA polymerase, and all of the PCR components. Therefore, add just template, primers, and MgCl<sub>2</sub> 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<sup>™</sup> 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<sup>™</sup> 96. The tubes stuck to the lid will drop inside the Exicylcer<sup>™</sup> 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*<sup>™</sup> Greenstar<sup>™</sup> PCR PreMix and *AccuPower*<sup>™</sup> Dualstar<sup>™</sup> 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.

Exicycler<sup>™</sup> 96 Real-Time Quantitative Thermal Block

# Calibration Using ExiCfg

**BIONEER** CORPORATION

# Calibration Using ExiCfg

Prepare to perform the calibration with a Calibration Kit (A-2060-1). The calibration kit is designed for the Exicycler<sup>™</sup> 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<sup>™</sup> 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-2060-1)

# **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.

6) Protect the calibration plate from direct sunlight after the centrifugation. Place the plate directly into the Exicycler™ 96.
 Image: Control of the control of the

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 while the 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<sup>™</sup> 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<sup>™</sup> 96, calibrating with the Exicycler<sup>™</sup> 96 Calibration Kit is recommended. The calibration must be done when moving the physical location of the Exicycler<sup>™</sup> 96 or when changing a light source lamp. A periodical calibration every 6 months helps maintain the optimum condtion of the Exicycler<sup>™</sup> 96.

Use the ExiCfg program to calibrate the Exicycler<sup>™</sup> 96. The ExiCfg calibrates as well as diagnoses the systemical condition of the Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 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.

1	Calibrated	Not calibrated	History
Lamp warning	0%		Exicyclers/O Config
Mask Calibration			
tackground 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 calibra	History
Lamp warming	5N	Exicycles 30 Config Toring to turn on the leno. Checking the lamo temperature. Lamo Time : 3 Reserving reset 0x88 communition completed Dummy scan completed Casture at Gain63, Offset1, Exposure:50mS The lamo is 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<sup>™</sup> 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.



5) Place the Mask calibration plate with the A1 position at the top-left of the block.



# NOTE:

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

## **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 calibrated	History
Lang warming	Exiscolaris 0 config. Toriva to turn on the lane. Checking the lanor temperature, Lanor temperature: 25°C Lanor Time: 3 Reserving mexists: 25°C Reserving mexists: 25°C Dummy science consistent Dummy science consistent Cashue at Gain-53, Offset1, Exoosure:50mS The lano is turned on.
Background Calibration	
Mult-Chaenel Calibration	

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


**NOTE:** The calibration time is approximately 10 minutes depending on the condition of the Exicycler<sup>™</sup> 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.

### 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.

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 Mask Calibration	μ	The lane is turned on. Canhare Excourse [1]:240 Canhare Excourse [1]:240 Canhare Excourse [1]:240 Start : (0.051) Detected Excourse [2]:240 Canhare Excourse [2]:240 Canhare Excourse [2]:240 Canhare Excourse [2]:240 Canhare Excourse [2]:240 Start : (0.005610670731703) Detected Excourse 410
Background Calibration		Cachure Exposure [3]:270 Cached Level : 50004 at the exposure of 270mS Start: 0.005693 Detected Exposure(270 Cachure Exposure [4]:280 Cachure Exposure [4]:283 Cachure Exposure [4]:283 Cachure Exposure [4]:283 Cachure Exposure [4]:284 Cachure Exposure [4]:284 Cachure Exposure [4]:284
Multi-Channel Calibration		Detected Exposure 260 Casture Exosure [5] 700 Casture Exosure [5] 700 Calcute Exosure [5] 705 Calcute Exosure [5] 965 Calcute Exosure 366 Silert - 0.0212634560739651 Detected Exosure 366

## **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.



4) Click Start in the 'Background Calibration'

window.



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



**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' Background Calibration...

window.



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

History
History
etected Exposure:270  apture Exposure[4]:283 apture Exposure[4]:283 apture Exposure[4]:259 apture Exposure[4]:259 apture Exposure[4]:259 apture Exposure[5]:720 apture Exposure[5]:720 apture Exposure[5]:782 apture Exposure[5]:782 apture Exposure[5]:782
alced Level : 44152 at the exposure of secons lettered Exposure:966 and of Mask Calibration ackground Capture F1 at Exposure 1 ackground Capture F1 at Exposure 2 ackground Capture F1 at Exposure 3 ackground Capture F2 at Exposure 1 ackground Capture F2 at Exposure 2 ackground Capture F2 at Exposure 2 ackground Capture F2 at Exposure 3
ackground Capture F3 at Exposure 1 = ackground Capture F3 at Exposure 2 ackground Capture F3 at Exposure 3 ackground Capture F4 at Exposure 1 ackground Capture F4 at Exposure 2 ackground Capture F4 at Exposure 3 ackground Capture F5 at Exposure 1 ackground Capture F5 at Exposure 1

# **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.

😹 Exicycler3 Config	E 🗄 🖻 🖾
Information Calibration FactorySet Help	
Calibrated	History
Lano wemiye	Detected Exposure 270 Cartors Exposure 13180 Cartors Exposure 141:80 Cartors Exposure 141:23 Cartors Exposure 141:23 Cartors Exposure 141:23 Cartors Exposure 141:25 Detected Exposure 141:25 Detected Exposure 151:270 Cartors Exposure 151:270 Cartors Exposure 151:282 Cartors Exposure 151:28 Cartor
Background Calibration	Caled Level : 49152 at the exposure of 965mS Start : 0.0215995079868 Detected Exospan-968 End of Mark Calebration Background Casture F1 at Exospan 2 Background Casture F1 at Exospan 3 Background Casture F1 at Exospan 3 Background Casture F2 at Exospans 1 Background Casture F2 at Exospans 3 Background Casture F2 at Exospans 3
Mult-Channel Calibration	Background Capture F3 at Exoosture 1 Background Capture F3 at Exoosture 2 Background Capture F3 at Exoosture 3 Background Capture F4 at Exoosture 1 Background Capture F4 at Exoosture 3 Background Capture F3 at Exoosture 3 Background Capture F5 at Exoosture 2 Background Capture F5 at Exoosture 3

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

	Attach	your first plate on the bath.
-	Start	Add Dye
No.	Name	Status
01	FAM	0%
02	SYBR_GREEN	0%
03	IOE IOE	0%
04	TET .	0%
05	TAMRA	0%
06	🗹 СҮЗ	0%
07	TEXAS_RED	0%
08	ROX ROX	0%
09	I 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.

	Attach	your first plate on	the bath.
	Start	Add Dye	Close
No.	Name	S	tatus
01	✓ FAM		0%
02	SYBR_GREEN		0%
03	I JOE		0%
04	TET TET		0%
05	Z TAMRA		0%
06	🗹 СҮЗ		0%
07	TEXAS_RED		0%
08	I 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.

2 1 North Control 1 North Cont	
Start Add Dye	Close
No. Name Status	
01 FAM 0%	
02 SYBR_GREEN 0%	
03 🗹 JOE 0%	
04 TET 0%	
05 TAMRA 0%	
06 🗹 CY3 0%	
07 TEXAS_RED 0%	
08 ROX 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
Name	Status
FAM	100%
SYBR_GREEN	0%
✓ JOE	0%
✓ TET	0%
🗹 TAMRA	0%
🗹 СҮЗ	0%
TEXAS_RED	0%
ROX	0%
CY5	0%
	Continue Name V FAM V SYBR_GREEN V JOE V TET V TAMRA V CY3 V CY3 V CY5

10) Click Start to repeat steps 4 through 8.

_	Continue	Add Dye	d " TET " on the bath.	
ю.	Name	Status		
11	K HAM	100%	<u>C</u> lose	
12	SYBR_GREEN	0%		
13	IN JOE	0%	Status	i " on the bath.
14	¥ IEI	0%	100%	
15	I TAMRA	0%	100%	1
16	IN CY3	0%	100%	Glose
17	M TEXAS_RED	0%	0%	
8	IN ROX	0%	0%	status
19	ICY5	0%	0%	100%
			0%	100%
			0%	100.20
_			0%	100%
				100%
-				20070

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

			CY5 " on the	e bath.
	Start	Add Dye		<u>C</u> lose
No.	Name			
01	FAM	Multi-Channel Calib	raion Finished	
02	SYBR_GREEN	Multi-Channel Calib	raion rinisheo,	
03	IOE ₪	OK		
04	I TET		80.655	
05	TAMRA		100%	
06	CX3		100%	
07	TEXAS_RED		100%	
08	ROX		100%	
no	✓ CY5		100%	

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

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

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

	Calibrated Not calibrated	History
Lang warming		1/2 Exposure Capture at 205mS Level1 : 5623, Level2 : 4994 Slant : 3,06829268292683, Intercept : 4365, R : 1, Exposure : 14596 Capture at Exposure : 1800mS
Mask Calbration		CY5 F3 Original Exposure Capture at 270mS 1/2 Exposure Capture at 135mS Level1 : 5507, Level2 : 4864 Stant : 8, 367040710707007, Intercept : 4461, R : 1, Exposure : 1355 Capture at Exposure : 1350mS
Background Calibration		CVS F4 CVS F4 Cv5 F4 Level 1: 4863, Level 2: 4843 Level 1: 4863, Level 2: 4843 Start 1: 16820705820706, Intercet 1: 4423, R1 1. Exposure 1: 28430 Creature of Exposure 1: 100mS
Multi-Channel Calibration	Le.	CYS F5 Original Exposure Capture at 998mS 1/2 Exposure Capture at 483mS Level1 : 0, Level2 : 0

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

	Test Function	Select Eilter		
	Vision	Drawer Open	Not calibrated	History
_	Uniformity Setting	Drawer Close		1/2 Exposure Capture at 205mS
	Mask Setting	Bath Up		Level1 : 5623, Level2 : 4994
_	Load Mask Calibration	Bath Down		Slant : 3,06829268292683, Intercept : 4365, R
	Load Background Calibration	Lamp On		: 1, Exposure : 14596
		Lamp Off		Capture at Exposure : Toooms
		14		CY5 F3
M	lask Calibration			Original Exposure Capture at 270mS
				1/2 Exposure Capture at 135mS
				Level1 : 5507, Level2 : 4964
				1 Evpopure : 11535
				Capture at Exposure : 1800mS
				CY5 F4
Back	ground Calibration			Original Exposure Capture at 260mS
Participation				1/2 Exposure Capture at 130mS
				Level1 : 4863, Level2 : 4643
				Slant : 1,69230769230769, Intercept : 4423, H
				Canture at Exposure 1 1800mS
_				
				CY5 F5
2002203				Original Exposure Capture at 966mS
Multi-0	Channel Calibration			1/2 Exposure Capture at 483mS
				Level1 : 0, Level2 : 0
				Siant : U, Intercept : U, HT2 : NaN, Exposure :
_				2147903090

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

16) Power off the Exicycler<sup>TM</sup> 96 by pressing the **POWER** button.

17) Turn off the Exicycler<sup>™</sup> 96 using the main power switch located on the back of the Exicycler<sup>™</sup> 96.



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

Lamp Status Error Log Self Test Exit	ĪU	Machine	Information	ractor	y <u>s</u> et	<u>n</u> eib
Exit		Lamp St Error Los Self Tes	atus 9 t			
	-	E <u>x</u> it				

# Running Real-Time PCR Using Exicycle™ 96 Software

**BIONEER** CORPORATION

# Starting 'Run Exicycler' software

Ensure that the Exicycler<sup>™</sup> 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<sup>™</sup> 96 is not powered on. Power on the Exicycler<sup>™</sup> 96 by pressing the power switch in the back of the Exicycler<sup>™</sup> 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<sup>™</sup> 96 or if the calibration data is lost accidently. Close the Run Exicycler3 and perform the calibration.

Fail to load calibration information	Fail to load calibration information				
Fail to load calibration information		Call be load			
		Fail to load	calibrati	on inform	atior

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.

😽 Exio	cycler	B PCR			
: <u>F</u> ile	<u>S</u> etup	<u>R</u> un	<u>W</u> ine	dow	<u>H</u> elp
	Co Pro	nfig obe	•	6	Ŧ

#### **IMPORTANT:**

9 different probe options are available in the Exicycler<sup>™</sup> 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 List			
No.	Name	Dye	Quencher	Co	Add
1	Std_SYBR_GRE	SYBR_GREEN	None		
2	Std_JOE	JOE	BHQ		Modify
3	Std_TET	TET	BHQ		
4	Std_TAMRA	TAMRA	BHQ	= l	Delete
5	Std_CY3	СҮЗ	BHQ		
6	Std_TEXAS_RE	TEXAS_RED	BHQ		
7	Std_ROX	ROX	BHQ		Save
8	Std_CY5	CY5	BHQ		-
9	Std_FAM	FAM	BHQ	V (	

6) Enter SYBR\_AQ in the Name field.

	Setting Probe	
Name	SYBR_AQ	
Dye	SYBR_GREEN	
Quencher	None	
Calas		

### 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 List			
No.	Name	Dye	Quencher	Co 🛆 🦷	Add
3	Std_TET	TET	BHQ		
4	Std_TAMRA	TAMRA	BHQ		Modify
5	Std_CY3	CY3	BHQ		
6	Std_TEXAS_RE	TEXAS_RED	BHQ		Delete
7	Std_ROX	ROX	BHQ		
8	🗌 Std_CY5	CY5	BHQ		
9	Std_FAM	FAM	BHQ		Save
10	SYBR_AQ	SYBR_GREEN	None		



9) Quick Start window will appear as follows:



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	ы Incubate	· · · · · · · · · · · · · · · · · · ·
Undate	🎁 Scan	duration.
opulate	🖏 Goto	
Delete	Melting	Temperature 94.0 °C
🔽 Hottop	🧾 Gradient	
100 💲	🐣 Store	C Time Increment Sec.
	🐻 Pause	C Temperature Increment 20.5 °C
	$\triangleright$	C 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. Gradient is used to evaluate an optimum annealing temperature condition for amplification.
- f. Store keeps the 96-well thermal block at a set temperature until you stop the Exicycler<sup>™</sup> 96. It is not recommended to use the Store step when it is excessively humid.
- g. Pause is used to pause the Exicycler<sup>™</sup> 96 when it is necessary to check samples during experimentation. The door of Exicycler<sup>™</sup> 96 will open when the Pause step is inserted in the protocol file. Click Run to resume the experiment.
- h. 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.

**Gradient** is used to select an optimum annealing temperature for amplification and programs a temperature gradient up to 20°C across the rows of a sample block. Enter the starting temperature in the **From** field, the ending temperature in the **To** field, and a time within a range of 1 second to 99 hours 59 minutes and 59 seconds in the **Time** field.

**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<sup>™</sup> 96 will keep the main door closed and will continue to store the thermal block at the set temperature until you stop the Exicycler<sup>™</sup> 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.



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).

Outo: Start      Protocol     Proto	Append Update Delete 100 0 0	Incubate Scan Notting Gradient Store Pause C Tan C Tan C Tan C Tan	Control User     Guest       te the plate at the given term n.     0       porstore     72.0       o(table)     0       o (table)     0       a parature tacrement     0       a pinging Bate     0	Cancel
9 - 70 - 70 - 70 - 70 - 70 - 70 - 70 - 70				-90 -80 -78 -60 -50 -30 -30 -10

#### NOTE:

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

#### Exicycler™ 96 Real-Time Quantitative Thermal Block

13) Click Scan tab and then Append.

4 Ould: Start Master 22 7 Protocol 22 7 Plate 22 7		Cancel
No. Profection 1 Incolute al 94,00°C, for 0:000 12 Incolute al 94,00°C, for 0:000 12 Incolute al 94,00°C, for 0:000 13 Incolute al 72,00°C, for 0:000 14 Incolute al 72,00°C, for 0:000 15 Scan	Append Lil Incubate Uddate & Scan Delete :: retting '> Hottop :: A Store S Pauce	Collects the fluorescence data at this step.
00- 00- 00- 00- 00- 00- 00- 00-		

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.

ы Incubate 🖨 Scan	Repeat the given steps for the number of times.
🖏 Goto	
Melting	Line 2
📕 Gradient	Cycle 35
🐣 Store	4
🖏 Pause	

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

≦ Outick Blant Master Protocol Plate Ne. Protocol all Incohes at 54 00°C. for 8 1610 all Incohes at 54 00°C. for 8 1610 all Incohes at 55 00°C. for 8 1610 all Incohes at 50°C. for 8 16100 all Incohes at 50°C. for 8 16100 all Incohes at 50°C. for 8	Append & Incubete Uddate & Scan Uddate & Goto Delete = retling P Hottop = Gradeat 100 = 4 Store	User Guest  Repeatthe piven steps for the num  Line 2   Cycle 35	ber of times.
Protect Plate			-90 -90 -90 -90 -90 -90 -90 -90 -90 -90

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").

🖨 Scan	Collects the fluorescence data at each given temperature steps.
Goto	Tomporatura
Melting	
📑 Gradient	From 60 • oc 10 94 • oc
A Store	h≷ Between 1.0 ♀ °C
Pause	Hold Time 1 💲 Sec.

#### Exicycler™ 96 Real-Time Quantitative Thermal Block

#### 17) Click Append.

a Quick Start			
Master 😼 🔒			🖳 User Guest 🔽 OK
Protocol 🧭 🔒			Cancel
Plate 🔂 🔂			
No. Protocol	Append	🔒 Incubate	
2 Incubate at 94,00°C, for 0:0:30	lindate	🗳 Scan	temperature steps.
4 Incubate at 72,00°C, for 0:0:30	opene	🛱 Goto	Tennesature
Scan     Scan	Delete	Melting	remperature
7 Melting 60°C to 94°C, Every 1,0°C, 1 Sec.	Hottop	Gradient	From 60 🗘 °C To 94 🗘 °C
Fe .	100	A Store	Between 1.0 🗢 °C
	100	Pause	Hold Time 1 🗘 Sec.
90 -			-90
so -			-80
10 - 00 - 00 - 00 - 00 - 00 - 00 - 00 -			
50 -			- 50
40 - 20 -			- 40
20 -			- 20
10 -			- 10
Protocol Plate			

 Click Store tab to set up a temperature for the 96-well block after cycling, and the click Append.

📓 Incubate			
🗑 Scan	Keep the plate at the given temperature and finish the PCR.		
G Goto			
Melting	Hold Temperatur 8.0		
🔣 Gradient			
🐣 Store			
Pause			

#### NOTE:

The door of the Exicycler<sup>™</sup> 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<sup>™</sup> 96 first by clicking **Stop** button in order to open the door. Otherwise, the Exicycler<sup>™</sup> 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.

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.



- 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.

	a Quick Start	
21) Click Add Probe, the Add Probe window will appear.	Master 22 G Protocol test 22 G Plate 22 G	User Guest V OK Cancel
Add probe	1         2         3         6         7         0         9         0         1         1         Name         C           0	Sample Name Insert Sample Name'. Assign Assign Probe to selected well.
Add probe —	Protocol Plate	Add Probe Add Probes for your experiment.

22) Select appropriate probes, and then click OK.

	Add	Probe		
	lo.	Probe Name	Fluorescent Dye	Quencher
1		Std_FAM	FAM	BHQ
2	2 [	Std_SYBR_GREEN	SYBR_GREEN	None
3	}	Std_JOE	JOE	BHQ
4	ł (	Std_TET	TET	BHQ
5	i	Std_TAMRA	TAMRA	BHQ
)ve 6	j	Std_CY3	СҮЗ	BHQ
7	7	Std_TEXAS_RED	TEXAS_RED	BHQ
8	3	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.
- 23) The probe is loaded and shown as follows:



24) 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.

25) 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.
- IPC stands for Internal Positive Control and is a sample that monitors the PCR run during Existence / d. 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.
- 26) Click the well D3 and enter concentration. For example, enter '1000000' for a concentration of 10<sup>6</sup>-copy and then click Assign. Repeat this for wells D4 through D8 with concentrations of serial diluted standards.



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



- 28) 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
  Sample Name Field
  Sample Name Field
- 30) Enter a plate name in the **Plate** field (i.e. Test) and then clickbutton to save the plate file.



31) 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.

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



33) Ensure the **POWER** button in the front of the Exicycler<sup>™</sup> 96 is blinking in green.

34) 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.







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

😽 Exicycler3 PCR				
: <u>F</u> ile <u>S</u> etup	<u>R</u> un	<u>W</u> indow	<u>H</u> elp	
		<u>l</u> un <u>'</u> ause <u>I</u> top	-3	
😽 Exicycler3 PCR				
: <u>F</u> ile <u>S</u> etup	<u>R</u> un	<u>W</u> indow	<u>H</u> elp	
i 🔥 🏠 🖡	à I	▶ 0 ■	Ę	
		2		

36) 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.

Dataname		
Please input the d	lataname to :	save the result.
Counts AD		
Sample_Auj		
	Ok	Cancel

- The following three message boxes will appear in the following order.
  - a. The message will appear when closing the door of the Exicycler<sup>™</sup> 96 if the door was open.
  - b. The message will appear when checking the lamp condition.

08	
Checking lamp condition.	
4%	_

Configuring the system for PCR running

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



The message will appear when checking if the lamp d. is on and initializaing the Exicycler<sup>™</sup> 96.

Checking optical system.

- 38) The main window will File Setup Run Window Help appear when the 🖨 Scan1 **Experiment Information** 4 progress bar finishes 60 K 50 K without any problems. ample AC 40 K lser Name 30 K 00:15:36 Fi 00:24:14 100 °C Cycle 20 K 10 K 4 > пκ Incubate at 94.00°C, for 0:10:0 Incubate at 94.00°C, for 0:0:10 Incubate at 60.00°C, for 0:0:30 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 Goto Line : 2, Cycle : 40 Incubate at 72.00°C, for 0:2:30 Melting 60 K 50 K 9 10 11 12 40 K 30 K 20 K 10 K 0 K 63.0 66.0 69.0 72.0 75.0 78.0 81.0 84.0 87.0 90.0 93.0 60.0 4 > Ten ture Pro - 90 - 80 - 70 - 60 - 50 - 40 Graph Color Setting Probe 70 60 50 40 Flu. Dye Qu SYBR\_GREEN None
  - Scan window displays a curve of amplification in real time during PCR. a.
  - Melting windows displays a melting curve in real time when the melting step is included in the protocol file. b.
  - Temperature Profile displays a temperature profile and in real time c.
  - Experiment Information displays information for the experiment currently in progress. d.
  - Protocol Information highlights the step currently running. e.
  - Well Information displays set up details for each well when selecting either Probe or Type in the Graph Color f. 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.

39) Go to Run > Stop to stop the Exicycler<sup>™</sup> 96 or click the Stop button.



 Go to Run > Pause to pause the Exicycler<sup>™</sup> 96 or click the Pause button.



#### **IMPORTANT:**

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

#### **IMPORTANT:**

It is recommended to re-start the Exicycler<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> 96 by pressing the **POWER** button.



2)	Go to 'C:\Exicycler3\Run' and double click $\ensuremath{\textbf{ExiCfg.exe}}$ to
star	t the program.

1	Calibrated	Not calibrated	History
Lamp warning	01		Exicycler3.0 Config
Mask Calbration			
Background Calibration			
hill-Chunal Calibration			

#### 3) Go to Information > Self Test.



#### NOTE:

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

#### 4) Click Start in the Self Test window.

Self Test	
	<u>S</u> tart
Door Motor	4
Bath Motor	
Excitation Motor	
Emission Motor	
Lamp Fan	
Sensor	
Peltier Heating	
Peltier Cooling	
Hot lid	
Bath Fan	

#### **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.

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.

🖶 Exicycler3 Config		Error	
Information       Calibration         Machine Information       Lamp Status         Error Log       Self Test         Exit       Exit	Factory <u>S</u>	1 Errors Occured Door close error - over-current - [0x21]	

Exicycler™ 96 Real-Time Quantitative Thermal Block

# Troubleshooting

**BIONEER** CORPORATION

# 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.	<ul> <li>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<sup>™</sup> 96.</li> <li>Check a fuse box located down by the power switch in the rear of the Exicycler<sup>™</sup>96. Change the fuse if it has blown.</li> </ul>
Errors occur either after or during Self-diagnosis (POWER button in red).	<ul> <li>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.</li> <li>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.</li> </ul>
No communication detects between the computer and the Exicycler™ 96 .	<ul> <li>Make sure that the USB cable is firmly connected to the computer.</li> <li>Ensure you use the USB cable provided with the Exicycler<sup>™</sup> 96 in order to connect the computer and the Exicycler<sup>™</sup> 96.</li> <li>Check if the Exicycler<sup>™</sup> 96 is in the "Standby" position. The POWER button must blink in green. Power off the Exicycler<sup>™</sup> 96 and back on again if the POWER button still blinks in red.</li> </ul>
The POWER button or the DOOR button does not work.	<ul> <li>Make sure that the power cable is firmly connected to the wall circuit, and then switch on the power supply button in the rear of the Exicycler<sup>™</sup> 96.</li> <li>The Self-diagnosis may be still running. Wait until the Self-diagnosis is complete and the POWER button blinks in green.</li> </ul>
The Exicycler™ 96 has stopped running.	<ul> <li>Check if the electricity is supplied properly. Operate an AVR or UPS if needed.</li> <li>Disable Screen Saver and Monitor Power in the Control Panel.</li> <li>Ensure you use the USB cable provided with the Exicycler<sup>™</sup> 96 to connect the computer and the Exicycler<sup>™</sup> 96.</li> <li>Check if the USB driver is installed properly. If not, reinstall it again.</li> <li>Do not plug in any other USB cables in the computer while the Exicycler<sup>™</sup> 96 is running to prevent friction between the Exicycler<sup>™</sup> 96 and the computer.</li> </ul>

Observation	Recommended Action
The Exicycler™ 96 does not start a thermal cycling.	• The Exicycler <sup>™</sup> 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 ${ m Mg2}^+$
	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.
	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
PCR sample is evaporated.	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 <sup>+</sup> concentration if
	needed.
	• Either examine fluorescence signal of the probe used for Real-Time PCR
No fluorescent signal is detected	or use a new intercalating dye.
although PCR product is amplified.	Perform the calibrations again to adjust the light intensity of the lamp.
	Background Calibration finished will appear if the Background
	Calibration is successful.
Pop-up message during calibration process:	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.
	Can't close program during PCR will appear if you attempt to close the
	Exicycler™ 96 software while PCR is still running.

Observation	Recommended Action
Pop-up message during calibration process (continued):	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 does not match the ID from the Exicycler™ 96 software. Set up the
	instrument ID 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 top menu while PCR is still running.
Error message when running the	Can't Pause during Melting Protocol will appear if you click Pause button
Exicycler™ 96 software:	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.
	Can't read Plate Information will appear if you attempt to open an
	outdated plate file.

Observation	Recommended Action
	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 <b>Run</b> 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 <b>Run</b> button when the Exicycler™ 96 ID and the calibration data ID
	do not match.
Error message when running the Exicycler™ 96 software (continued):	No matching calibration data with machine will appear if the Exicycler™
	96 ID 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 <b>Cancel</b> 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.
Exicycler<sup>™</sup> 96 Real-Time Quantitative Thermal Block

# **Ordering Information and Warranty**

**BIONEER** CORPORATION

### **Ordering Information and Warranty**

Product	
A-2060	Exicycler™ 96 Real Time Quantitative Thermal Block
Kit	
K-6200	AccuPower® Greenstar™ qPCR PreMix, Exicycler 8-well Strip,
	12 strips / 96 tests, $50\mu\ell$ reaction
K-6203	AccuPower® Greenstar™ qPCR PreMix, Exicycler 96-well Plate,
	1 plate / 96 tests, $50\mu\ell$ reaction
K-6110	AccuPower® DualStar™ qPCR PreMix, Exicycler 8-well Strip,
	12 strips / 96 tests, $50\mu\ell$ reaction
K-6113	AccuPower® DualStar™ qPCR PreMix, Exicycler 96-well Plate,
	1 plate / 96 tests, $50\mu\ell$ reaction
A-2060-A1	Exicycler™ 96 Calibration Kit
Tube & Sealing T	ape
3111-50	0.2ml 8-strip PCR Tube for Real-Time PCR (opaque white)
3111-52	96-well Semi-skirted PCR Plate for Real-Time PCR
	(opaque white)

3111-41AB Sealing Film for Real-Time PCR Plate

## 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 disasters.

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						
Service Issue	Issue 1						
	Issue 2						
	Issue 3						
	Issue 4						
Customer Information	Name						
	Company						
	Name						
	Contact	Phone:		Fax:			
	E - mail						

Headquarters	Bioneer, Inc.	Bioneer Co., Ltd. China	Order
49-3, Munpyeong-dong, Daedeok- gu, Daejeon 306-220, Korea Phone:+82-42-930-8599 Fax:+82-42-930-8600 Website: www.bioneer.com	1000 Atlantic Avenue, Alameda, CA 94501 USA Toll free : 1-877-264-4300 Fax : 1-510-865-0350 E-mail: infousa@bioneer.com	403 Room, Building 88, number 887, Zuchongzhi Road, Zhangjiang High Technology Park, PuDong new District, Shanghai 201203, China Tel: +86-21-5080-0969/1191/1651 Fax: +86-21-5080-1620 E-mail: ascn@bioneer.com	Korea: order@bioneer.co.kr 1588-9788 International: sales@bioneer.com +82-42-930-8599