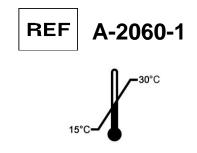
User's Guide



Exicycler[™] 96 Real-Time Quantitative Thermal Block





Real-time Quantitative Thermal Block



User's Guide

Version No.: 1.2 (2016-12)

Please read all the information in booklet before using the unit



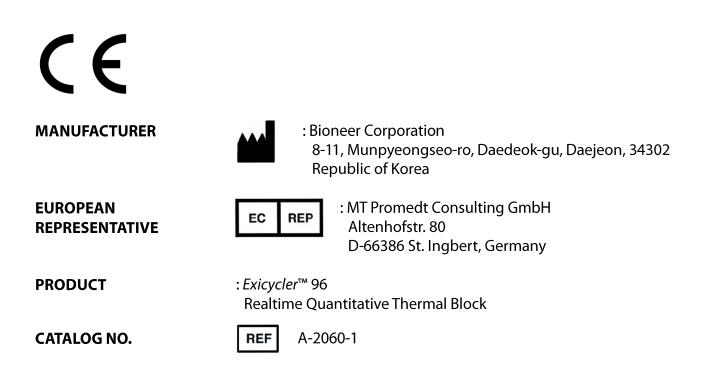
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U.S. and Canadian Safety Standards

Standard for Electrical equipment for measurement, control and laboratory use; Part1: General Requirements, UL 61010-1, 2nd Ed, Rev., October 28, 2008&CAN/CSA-C22. 2 No. 61010-1-04(R2009) Part 2: Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes, CAN/CSA-C22. 2 No. 61010-2-081:04 Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials CAN/CSA-C22.2 NO. 61010-2-010-04 Part 2: Particular requirements for in vitro diagnostic (IVD) medical equipment, CAN/CSA-C22. 2 No. 61010-2-101:04



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Before Starting

How to Use This Guide

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

General Requirements for Installation

This guide assumes that you:

- Are familiar with Microsoft[®] Windows[®] 7 operating system.
- Have basic techniques for handling DNA and RNA samples for PCR.
- Have basic skills for data storage, copying and pasting data into hard drives.
- Have an experience in setting up a network. (Using any data generated by *Exicycler*[™] 96 requires setting up 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 selected from a main menu or shortcut menu. For example:

File> Config> Scan

How to Obtain More Information

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

How to Reach Customer Support

To obtain prompt customer support, please call us at +82-42-930-8777. You can also obtain technical support through www.bioneer.com (Email: exicycler-support@bioneer.com).

Product Use Limitations

- *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. *Exicycler*[™] system can also be used for life science research and other laboratory purposes.
- Gradient function of *Exicycler*[™] 96 is not available in US, EU, Japan and Germany.
- Use of *Exicycler*[™] 96 is covered by U.S. patents Nos. 8,139,210, 8,427,643, and their foreign counterparts or pending patents. The purchase of these products includes a limited, non-transferable immunity from suit under the forgoing patents for using only this amount of product solely for the purchaser's own internal research. No other patent rights to use this product for any other purpose or for commercial purpose, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, are conveyed expressly, by implication, or by estoppels. Further information on purchasing licenses may be obtained by contacting Bioneer at licensing@bioneer.com.

Safety Labels Description on *Exicycler*[™] 96

English	Hot surface. Note that you can hot surface temperature.
Francais	Surface chaude Faites attention à la surface chaude.
English	Do not work long hours at low temperature to Bath. We had a condensate can cause equipment malfunctions.
Francais	Ne pas utiliser le bain à basses températures pendant plusieurs heures. Risque de condensation pouvant entrainer des fonctionnements défectueux de l'équipement.
English	Door Attention Behavior. Open or close attention to when you crash and insertion.
Francais	Manipulation de la porte. Risque de blessures corporelles (pincement/écrasement) lors de. l'ouverture/fermeture de la porte.
English	CRUSH HAZARD Stay clear of this area during Door operation. Injury can result if you shut your fingers in the door.
Francais	Risque d'écrasement Ne pas rester proche de la porte durant son fonctionnement. Risque d'écrasement de doigts en cas de contact avec la porte.

Symbols on *Exicycler*[™] 96

Safety Symbols on Exicycler[™] 96

The following table describes the safety symbols that may be displayed on *Exicycler*[™] 96. Each symbol may appear by itself or with text that explains the relevant hazard.



Hot surface.
Note that you can hot surface temperature.
Do not work long hours at low temperature to Bath.
We had a condensate can cause equipment malfunctions.
Door Attention Behavior.
Open or close attention to when you crash and insertion.
CRUSH HAZARD
Stay clear of this area during Door operation.

Injury can result if you shut your fingers in the door.

Electrical Symbols on Exicycler[™] 96

The following table describes the electrical symbols that may be displayed on *Exicycler*[™] 96.



Indicates **On** position of the main power switch.

Indicates **Off** position of the main power switch.

Indicates a standby switch by which the instrument is switched on to the **Standby** condition.

Indicates a protective grounding terminal in the instruments that must be connected to earth ground.

Indicates a terminal that can receive or supply alternating current or voltage. Indicates a terminal that can receive or supply alternating or direct current or voltage.

Environmental Symbols on the Exicycler[™] 96

The following symbol (WEEE) applies to *Exicycler*[™] 96 placed on the European market.

Do not dispose of *Exicycler*[™] 96 as unsorted municipal waste.

Follow local municipal waste ordinances for proper disposal provisions to reduce environmental impact of waste from electrical and electronic instrument.



European Union customers:

Call your local European office for Bioneer instruments pick-up and recycling.

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*TM 96. The following safety alerts will be used in this section:

	Hazards or dangerous actions that may result in severe injury.
	Hazards or dangerous actions that may result in minor injury or damage.
	Hazards or dangerous actions that may cause a burn.
DANGER	Hazards or dangerous actions that may result in electric shock.

Location Consideration and Installation

General Instrument Safety

- Check power voltage rating before connecting to the instrument and an electrical outlet. *Exicycler*[™] 96 is configured for 100-240VAC (±10%). Using AVR (Automatic Voltage Regulator) or UPS (Uninterrupted Power Supply) is recommended.
- 2) *Exicycler*[™] 96 must be grounded for protection against electric shock. If not, it may cause serious injury and system damage.
- 3) Do not place any objects in front of the main door of *Exicycler*[™] 96 that can interfere with door operation.
- 4) Leave 50 cm space between *Exicycler*[™] 96 and the wall for proper ventilation.
- 5) Do not install *Exicycler*[™] 96 in a dusty environment to prevent false operation or technical damage.
- 6) Keep *Exicycler*[™] 96 away from heat sources.
- 7) *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.
- 8) *Exicycler*[™] 96 must not be installed in an area where it is exposed to combustible or flammable vapor. In case of a gas leak, open windows and let fresh air in. Do not operate any electrical switch during a gas leak. It may cause an explosion or fire.
- 9) Do not disassemble or repair *Exicycler*[™] 96 yourself. It may cause a fire, electric shock, and system damage. A limited warranty does not cover unauthorized alterations or damage due to abuse.

Precautions regarding the electrical environment

- 1) If the power cord is loose, do not use the instrument. Overheated power cord may result in shock or fire.
- 2) Do not operate multiple instruments out of a single wall outlet. The load may cause the instrument to overheat and lead to fire.
- 3) When plugging or unplugging the power cord from a wall outlet, make sure your hands are completely dry. Wet or moist hands may cause electric shock.
- 4) A convenient and safe power cord should be available. The power cord provided by our company should be used.
- 5) Socket outlets should be at least 1.5 m from a sink or wash basin.
- 6) Power cord should not be repaired with insulating tape. Water can still penetrate insulating tape and power cord.

- If you use other power cord, it should be adequate for the electrical capacity for *Exicycler*[™] 96 (250V, 16A, 0.75mm², VDE).
- 8) Plugs should match the socket outlets.
- 9) Unplug the power cord from *Exicycler*[™] 96 when not in operation for a long period of time to prevent the possibility of fire by overheating.
- 10) *Exicycler*[™] 96 is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.
- 11) Improper fuse or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.
- 12) For continued protection against risk of fire, replace fuse types (250V, F10AL, 2ea) according to rating specified for the instrument.

Installation Safety

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

Cautions

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

Operation and Maintenance

Warnings

- 1) The system can be hazardous when misused.
- 2) 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 light source lamp to cool down for at least 10 minutes. Continuous operation without a break will reduce the lamp life span and cause errors.
- 4) Do not place a piece of paper or a plastic cover under *Exicycler*[™] 96. It could cause a fire.
- 5) Do not turn off *Exicycler*[™] 96 right after a Real-Time PCR run is done. Wait until the cooling fan has stopped running completely. Cooling fan remains running for about 3 minutes to cool down the lamp after each Real-Time PCR run.
- 6) Do not cover *Exicycler*[™] 96 with a piece of paper or a plastic cover. This may cause a fire or failure of the system.
- Set 'Power Options Properties' in the Control Panel to 'Turn off monitor', 'Turn off hard disks', 'System standby', and 'System hibernates' to 'Never'. Otherwise, data transfer between *Exicycler*[™] 96 and the computer will be interrupted.

IMPORTANT:

*Exicycler*TM 96 should be operated in a clean condition. Contaminants, such as dust, can cause problems and reduce the life span of *Exicycler*TM 96. Please prevent dust from entering into *Exicycler*TM 96. In order to extend life span of *Exicycler*TM 96, thoroughly remove dust periodically.

Introduction

Overview

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

Thermal block is built into the lower part of $Exicycler^{TM}$ 96, which carries out a thermal cycling. The detector is located in the upper part of $Exicycler^{TM}$ 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 $Exicycler^{TM}$ analysis Program.

Exicycler[™] 96 Real-Time Quantitative Thermal Block provides a maximum ramping rate of 4.5°C/sec, and features various functions such as gradient, time increment, temperature increment, and ramp rate control for myriad applications.

Exicycler[™] 96 Real-Time Quantitative Thermal Block detector consists of a light source and a detector. The light source is an energy source to excite fluorescent dyes. A short arc lamp is bright and has a long life span. A white-light source is divided into particular wavelength groups by band path filters. The band path filters in *Exicycler*[™] 96 covers within 475 to 690 nm and works as a set consisting of an excitation and an emission filter. Five filter sets are provided for various applications. Therefore, no additional band path filters is required. Also, Bioneer's imaging technique based on polarization of light enhances detection sensitivity for robust and reliable results. Patented (KR10-1089045, US8427643) polarizing optical apparatus mitigates the common problem of a reflection light, allowing precise quantification and target discrimination.

The detector, a highly sensitive 2D CCD camera, is highly sensitive and detects multiple fluorescence signals from a 96-well plate at once. This simultaneous detection has a great advantage over a sequential detection. This innovative detector, invented by Bioneer, reduces 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 $Exicycler^{TM}$ 96 generate a uniform light surface over the thermal block. This allows detection of multiple fluorescent signals emitted from various dyes through the light surface and separate signals within a selective wavelength range of each dye.

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

- 1) A set up program for calibration test, diagnosis and confirmation of the instrument's information.
- 2) An operation program for creating a protocol, assigning a probe and plate, saving & displaying data while operating *Exicycer*[™] 96.
- 3) Analysis software that comes with *Exicycler*[™] 96 has 4 different tools. The analysis program includes Absolute Quantification, Relative Quantification, SNP genotyping, and Existentence / Non-existentence. It is applicable to Gene expression, Quantification of cell and virus, and SNP genotyping.

Intended purpose

This device is used for the qualitative or quantitative analysis of specific DNA/RNA in samples.

System Components and Specifications

Overview

Exicycler[™] 96 Real-time 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:

- Fault caused by the customer.
- The product is repaired after expiry of the warranty period.

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

System Components

Component Lists		Check
<i>Exicycler</i> [™] 96	1	
USB 2.0 high speed cable	1	
Power cord ¹	1	
Software	1 CD	
User Guide	1	
96 well reaction plate	1 pack	
Optical sealing tape	1 pack	
Optical tape sealing applicator	1	

¹ Provided according to country standard

Specifications

Physical specifications	
Dimension	355mm(W) x 540mm(D) x 470mm(H) 13.98in(W) x 21.26in(D) x 18.50in(H)
Weight	39 kg (85.99lbs)
Sample capacity/ size	Opaque White 96-well PCR Plate / 0.2ml Opaque White 8-Strip PCR Tube
Sample volume	20~100 μl (50 μl recommended)
Power consumption	100-240VAC, 50/60Hz, Max 800VA
Operating temperature	15~30℃ (59~86°F)
Operating humidity	20~80%, no condensation
Thermo module specifications	
Method of heating / cooling	Peltier
Temperature range	4.0℃~99.9℃(39.2~211.82°F)
Max ramp rate	4.5°C/sec (8.1°F/sec)
Temperature accuracy	± 0.3°C (± 0.54°F)
Temperature uniformity	± 0.3°C (± 0.54°F)
Lid temperature	90 ~ 120℃ (194~248°F)
Gradient range	20 ~ 95°C (68 ~ 203°F)
Temperature differential range	1 ~ 20°C (1.8 ~ 36°F)
Temperature increment range	0.1~2.0°C (0.18~3.6°F)
Time increment range	1 sec ~ 60 sec
Ramp rate control range	1 ~ 100%
Computer specifications	
Operating system	Window7 (32-bit OS only)
Processor speed	Intel Dual Core E2160 (1.8GHz) or higher
Memory	1GB or higher
Communication port	USB 2.0 high speed
Screen resolution	1280 x 1024 or higher
Optical Part	
Light source	Short arc lamp(120W)*
Sensor	16 bit 2D CCD
Excitation Filter / Emission Filter**	5 Set

* Continuously turning on and off the lamp reduces the lamp life span.

- * The lamp generates heat when it starts. Make sure that you turn off *Exicycler*[™] 96 when cooling fan stops running. It usually takes about 3 minutes for cooling fan to stop.
- **Please refer to a chart below for more information about filter sets.

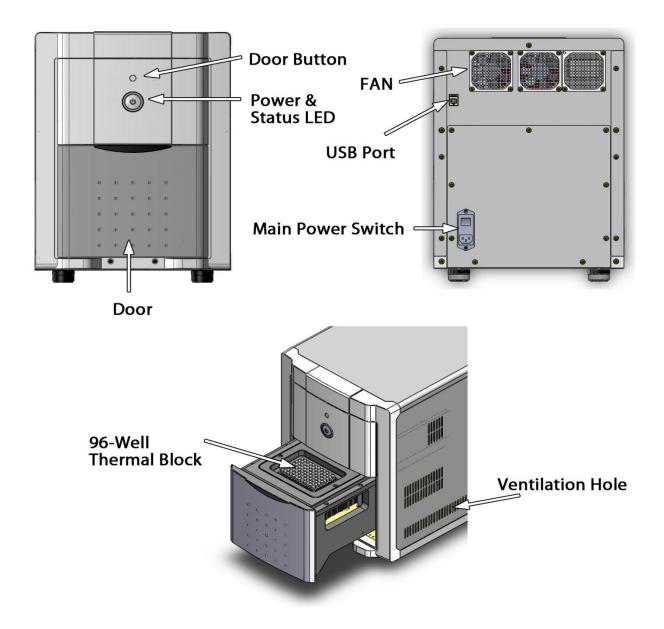
*The specifications of this product may change without notification for performance enhancement.

† Filter sets

Position	Excitation (nm)	Emission (nm)	Set	Fluorescent dye
1	Blank	Blank	1	_
2	475	530	1	FAM, SYBR Green I
3	520	560	1	JOE, TET
4	550	590	1	TAMRA, CY3
5	570	630	1	Texas Red, ROX, Red610
6	630	690	1	CY5, Red670

Chemical hazard: Exposure to following florescent dyes may cause eye and skin and respiratory tract irritation. Read the MSDS before use and follow the instructions if swallowed or inhaled. Wear appropriate protective eyewear, clothing, and gloves.

System Views



Installation and System Operation

Overview

To run *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.

Site Requirements

Exicycler[™] 96 Real-Time Quantitative Thermal Block is for an indoor use. Ensure that the installation site:

- Meets the spatial and weight requirements.
- Meets environmental requirements.
- (The instrument) Is within 1.5 m (4.92 ft) of a 800VA power receptacle.
- (The computer) Is within 1.5 m (4.92 ft) of a 500VA power receptacle.
- Is away from water.

Required Materials

Scissors, pocket knife, or box cutter

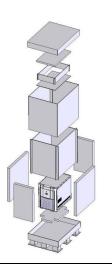
Unpacking *Exicycler*[™] 96

Save the packing materials and box in case you need to ship the instrument to Bioneer for service.

- 1) To unpack *Exicycler*[™] 96:
 - a) Cut the straps securing the instrument box.
 - b) Cut the tape securing the top flaps of the instrument crate, then open the flaps.
 - c) Remove *Exicycler*[™] 96 accessories from the instrument and set them aside.
 - d) Lift and remove the cover from the instrument crate.
 - e) Remove the packing material from *Exicycler*[™] 96, and then inspect the instrument for shipping damage.

If *Exicycler*[™] 96 is damaged, note location and appearance of the damage, and then contact Bioneer Technical Support or your service representative.

- 2) Move *Exicycler*[™] 96 to the desired installation site. Follow these guidelines for lifting and moving:
 - Make sure that you have a secure, comfortable grip.
 - Keep your spine in a neutral position.
 - Bend at the knees and lift with your legs.
 - Do not lift and twist your torso at the same time.



- 3) Open the box containing *Exicycler*[™] 96 accessories, then verify that it contains all the necessary components:
 - Refer to Component Lists.

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. Computer must meet the following 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
- Windows 7 Operating System for Korean or English Version (Service pack 1 or later, 32-bit OS only)
- 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 to install an anti-virus software in order for *Exicycler*[™] 96 to operate safely. A firewall should also be setup to prevent unwanted information coming in from external networks. Please contact your IT department to setup anti-virus software and firewall.
- 3) It is not recommended to insert a flash drive into USB port 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 main power connector and a communication port connector (USB) in the rear of $Exicycler^{TM}$ 96. The computer should be turned off when connecting to $Exicycler^{TM}$ 96 to prevent any damages to the communication port. Please see below for instructions on how to connect $Exicycler^{TM}$ 96 to the computer.

- 1) Unpack *Exicycler*[™] 96 and make sure all components are included.
- 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 at the installation site.
- 4) Place *Exicycler*[™] 96 in the installation site carefully.
- 5) Connect USB 2.0 cable to USB connector on the back of *Exicycler*[™] 96, then to the computer.
- 6) Connect power cable to *Exicycler*[™] 96, then to the receptable wall circuit.

Installing the Operation and Analysis Program

To run *Exicycler*[™] 96 Real-Time Quantitative Thermal Block, the operation software and the camera driver must be installed in the computer. When installing the operation software and the camera driver for the first time, 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 turn on *Exicycler*[™]96 unless you have installed the Operation and Analysis Program and a camera driver. First install the Operation Software and then the camera driver.

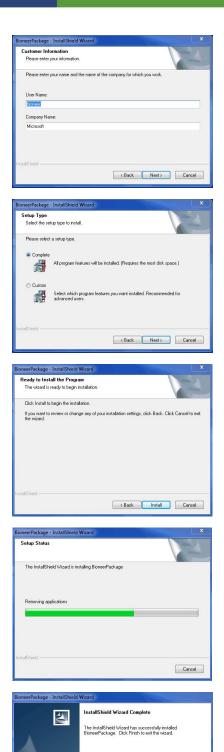
To install Bioneer Package and Exicycler4 softwares for an *Exicycler*TM 96 device under Windows 7, follow the instructions below:

- 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. The Bioneer Package is not installed correctly'). Remaining 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 Exicycler4 software. The procedures of Bioneer Package installation is described below.

Installing Bioneer Package



- 1) Turn on the computer, but not *Exicycler*[™] 96 Real-Time PCR machine. Place *Exicycler*[™] 96 installation CD into 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:₩Bioneer_Package.



Kack Finish Cancel

3) Enter desired name in the User Name box and Company Name box.

- 4) Select a setup type to install. **Complete** is recommended for installing all program features. **Custom** installation is recommended only for advanced users. Click **Next**.
- 5) InstallWizard is ready for installation. If you want to review or modify your settings, click **Back**. Click **Install** to start installation.
- 6) This process takes several minutes. Please do not cancel until installation is complete.

7) When installation is complete, please click **Finish** to finish installation Shield Wizard.

BioneerPackage - InstallShield Wizard Exicycler Software requires R.. OK Setup - R for Wind Installing Please wait while Setup installs R for Windows 2.11.1 on yo R Extracting files... C.W...WRWR-2.11.1WibraryWclassWpoWdeWLC_MESSAGESWR-class.mo Cancel BioneerPackage - InstallShield Wizard × Exicycler Software requires R(D)COM Interface. OK R 2.11.1 - 🗼 🕨 Con r DS (C) Bi Organize 🕶 Burn ☆ Favorites E Desktop 🔋 сом B DRIVER 😹 Download NET11 NET20 NET35 Secent Places 늵 Libraries Documents Music Pictures Videos R R_PKG_MINPACK R_PKG_QPCR R_PKG_RGL R_PKG_RSCPROXY Computer Apps Bioneer_Package Drivers

Exicycler[™] 96 / Real-time Quantitative Thermal Block

- 8) R program is also needed for *Exicycler*[™] software. If you click **OK**, installation will begin.
- 9) R program will be installed automatically. Please wait until installation is complete.

- 10) Click **OK** for installing R(D)COM interface.
- 11) R icon will be shown on the Desktop when installation is successful.
- 12) The folders shown here are created after installation has finished. Please confirm 'Bioneer_Package' and sub folders.

Installing .NET Framework 1.1 Package

🚱 🕞 – 🕌 « Bion	neer_Package > NET11 - 49 Sea
Organize 🔻 Inclu	ude in library 👻 Share with 👻 Burn New
Computer Co	age
Microsoft .NET Framework	1.1 Setup
😢 Would you like	e to install Microsoft .NET Framework 1.1 Package?
	Yes No
Microsoft .NET Framewo	ork 1.1 Setup
and the	License Agreement
	(A copy of this license is available for printing at http://po.microsoft.com.fr/withs/TLinkId=12283) SUPPLEMENTAL END USER LICENSE AGREEMENT FOR MICROSOFT SOFTWARE have read, understood and agree to the terms of the End User License Agreement and so signify by clicking 1 agree" and proceeding to use this product.
	Install
Hicrosoft .NET Framewo	ork 1.1 Setup 🗕 🗖 🗙
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- In order to install '.NET Framework 1.1 Package', run dotnetfx_eng.exe file of the C:₩Bioneer_Package₩Net11 folder.
- 2) Click Yes for installing '.NET Framework 1.1 Package'.
- 3) A setup window asking about the license agreement is displayed.

4) Select I agree to activate install button, Click Install to begin installation.

5) All components related to .Net Framework are being installed.

6) Installation of .NET Framework 1.1 is complete. Click **OK** to finish installation.

Installing Exicycler4 Software



1) In Exicycler4 folder of the provided CD, you can see **setup.exe** file and double click InstallSheild Wizard to install required items automatically.

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

3) Click **Install** to begin installation. If you want to change any settings or cancel installing, click **Back** or **Cancel**.

4) Please wait while InstallShield Wizard installs Exicycler4.

5) Message indicating "InstallShield has successfully installed Exicycler4." is shown. Click **Finish** to complete installation.



- 6) On the Desktop, these icons are created when installation is completed successfully.
- 7) In the C:₩Exicycler4₩Run folder, folders and files that are shown on the image (below) are created. Copy 'Calibration' folder from *Exicycler*[™] 96 CD into C:₩Exicycler4₩Run folder.

BioneerPackage_Setup	Computer + OS (C:)	► Exicycler4 → Run →	• • • • Se	earch Run
	Organize 👻 Include in library 👻	Share with 🔻 🛛 Bu	rn New folder	≡ • □
🎉 Calibration	Videos	Name	Date modified	Туре
Exicycler4_Ver4.00.1		🔒 CF	2/29/2016 3:37 PM	File folder
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	Exi4Data	DataPath.xml	5/28/2013 3:10 PM	XML File
	Exicycler4	S DragNDrop.dll	8/27/2013 1:55 PM	Application extension
	> 🍌 Analysis	Type: XML File Size: 34 bytes	AM	Application extension

8) Now you should connect *Exicycler*TM 96 system to a computer using provided USB cable.





Device driver software was not successfully installed \times × Click here for details.

Driver Software Installation		X
Device driver software was no	t successfully installed	
PCI-RDK in Transfer Mode (www.PbrTech.com)	🗙 No driver found	
What can I do if my device did not insta	all properly?	
		Close



- 9) Check the USB cable connection between the *Exicycler*[™]
 96 and the computer.
- 10) Turn on *Exicycler*[™] 96. The status LED will be blue when the power is supplied properly.
- 11) Press the power button for a second to start selfdiagnosis. The status LED will start blinking in green after two short beeps when the self-diagnosis is complete.
- 12) When *Exicycler*[™] 96 is turned on for the first time, the computer searches for a new device.
- 13) When an unsigned device is connected, the following message will be displayed. Click the message to view details.
- 14) This message appears because device driver software is not installed yet. Driver must be installed in order to continue. Please click **Close**.
- 15) To install device driver and to confirm device status, go to Control Panel₩System and Security₩System - Device manager.

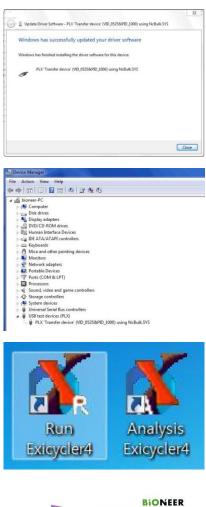
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b Disk drives b - 🔩 Display adapters	
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DE ATA/ATAPI controllers Explored Sector S	
 Mice and other pointing devices Monitors 	
b 🔮 Network adapters	
Other devices	
Portable Devices Ports (COM & LPT)	
Processors	
Update Driver Software - PCI-RDK in Transfer Mode (www.PlxTech.com)	
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Search automatically for updated driver software Windows will search your computer and the Internet for the latest driver software for your device, unless you've disabled this feature in your device installation settings.	
settings.	
 Browse my computer for driver software Locate and install driver software manually. 	
	Cancel
A 1	
G I Update Driver Software - PCI-RDK in Transfer Mode (www.PkrTech.com)	
Browse for driver software on your computer	
Search for driver software in this location:	
Browse Include subfolders	
Let me pick from a list of device drivers on my computer The let will does not all does compare the will be device and all does	-
This list will show installed driver software compatible with the device, and all drive software in the same category as the device.	
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- 16) All devices connected to computer are displayed. Unsigned device is indicated with an exclamation mark. Point at this device and right-click and select Update driver software.
- 17) When a prompt searching for device driver is presented, select **Browse my computer for driver software**.

18) Click Browse to search for the appropriate driver software.

19) Select C: ₩Bioneer_Package₩driver and click OK.

- 20) Select Install. Installation will start automatically.
- 21) Please wait while installing. This process takes several minutes.



- 22) A message indicating that the installation is successful will show when installation is complete. Click Close to complete installation.
- 23) Open 'Device Manager' and confirm that device driver has been installed. If USB test devices (PLX)₩PLX'Transfer device' VID_0525&PID_1000) using Ncbulk.SYS has been installed properly, connected USB will be indicated.
- Exicycler Exicycler 96 V4 4.0 Exicycler 96 V4 / Exicycler 96 V4 Fast Run 4.00.1



24) Click **Run Exicycler4** icon to start *Exicycler*TM 96 software.

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

26) Now you are ready to operate *Exicycler*[™] 96 system.

Troubleshooting

1. Appropriate calibration data does not exist



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- CD provided with *Exicycler*[™] 96 has Calibration data for each device. Calibration data have to be copied into C:₩Exicycler4₩Run folder in order for a normal function. If Calibration folder does not exist on this path, following message will be displayed.
- 2) Click **OK** to change the path.
- 3) 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. E: ₩Calibration), click OK.

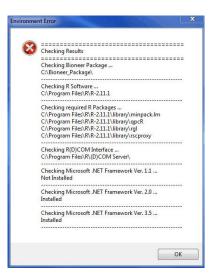
- Copy Calibration window displays the path of Calibration folder and serial number of your device. If the serial number matches with the connected *Exicycler*[™] 96 system, click **Copy** button on the right hand side of window.
- 6) Please wait for a few minutes to complete.
- 7) When copying calibration folder is complete, click **OK** to finish the process. Please confirm the connection between device and computer, and restart Exicycler4 software.

2. The computer does not register *Exicycler*^{IM} 96 when launching 'Run Exicycler4' software.



This message means that the device is not turned on or the appropriate device driver has not yet been installed on the computer. If you see this message despite the instrument is turned on, then you should re-install the device driver to solve this problem. The driver installation steps are described in **'Installing Exicycler4 Software'** section.

3. An Error message is displayed when lunching 'Run Exicycler4' software.



This message indicates that '.NET Framework 1.1' has not yet been installed. Go to 'Installing.NET Framework 1.1 Package' section 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 96 V4 4.0', 'Bioneer package', 'R for windows 2.11.1' and 'R/Scilab(D)COM Server3.01-B5'. After removing them from the computer, repeat the entire installation process.

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Turning On and Self-diagnosis

- 1) Turn on *Exicycler*[™] 96 by switching on the main power switch in the rear of the instrument. When power is supplied properly, a status LED turns blue.
- 2) Press the **POWER** button for a second to start self-diagnosis.
- 3) When the status LED is blinking green, you can either open or close a main door by pressing a **DOOR** button for a second. The status LED is blinking in purple when the door is open and is back to green when the door is closed.









Power Off

Power On

Door Open

Stanby

IMPORTANT:

The status LED starts blinking green after two short beeps when the self-diagnosis is complete. *Exicycler*[™] 96 is now ready to operate. The status 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 status LED is blinking green before operating *Exicycler*[™] 96 to prevent mis-operation or damage. A result of the self-diagnosis is provided under "ExiConfig".

* Status LED color

- a. Red: Error
- b. Blue: Standby
- c. Green: Ready for running
- d. Yellow: Self test
- e. Cyan: Running
- f. Purple: Door open
- g. White: Pause

NOTE:

You can stop the operation here and turn off the *Exicycler*[™] 96 by pressing the POWER button for a second when it is blinking in green like Step 3.

A CAUTION

Do not place any obstacle in front of the system and do not load a plate or take it out when the door is moving. It can cause serious damage and lead to severe injury.

Running Real-Time PCR Using Exicycler[™] 96 Software

Starting 'Run Exicycler4' program



- Ensure that *Exicycler*[™] 96 is in the "Standby" mode. The status LED must be blinking green. Double click **Run** Exicycler4 icon on the desk top to start **Run Exicycler4**.
- 2) System Check window will pop up.

IMPORTANT:

The following error message will appear if $Exicycler^{TM}$ 96 is not turned on. Turn on $Exicycler^{TM}$ 96 by pressing the power switch at the back of $Exicycler^{TM}$ 96 and the power button in front of $Exicycler^{TM}$ 96. Then double click **Run Exicycler4** icon on the desktop again.

IMPORTANT:

The following error message will appear when the calibration has not been performed for *Exicycler*TM 96 or the calibration data is not present. Close Run Exicycler4 program and copy calibration folder into C:**WExicycler4WRun**. (The CD provided with *Exicycler*TM 96 has calibration data for each device.)

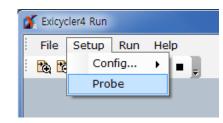
Calibration Info E:\Calibration\	mation does not exist

Device is not ready, Please turn on.

3) The following window will appear when System Check is complete.

Exicycler4 Run						
File Setup Run Help						
tha tha kha kha i ► U ■ 💭		Press and a second				
T	b. Experiment Information ——		Experiment Information Master			
		Protocol				
		Plate	-			
a. Menu		Data Name				
ai mena		User Name	1			
		Run Time	00:00:00	Finished	00:00:00	
		Hottop		Cycle	0	
		Protocol In	formation			
		No. Pro	otocol	7		
	c. Protocol Information					
	d. Well Information —	Well Inform		678	9 10 11 1	
e. Temperature		C D F G H				
		D E F G	v Setting	Probe		
D-		D E F G H -80 Graph Colo	-	Probe C Flu. Dye		
		D E F G H -80 Craph Colo No.	-			
0- 0- 0-		-80 Graph Colo -60 No. -40	-			
e. Temperature		D E F G H -80 Craph Colo No.	-			

- a. **Menu** consists of File, Setup, Run, Window, and Help.
- b. **Experiment Information** displays file name, 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.

IMPORTANT:

9 different probe options are available in *Exicycler*[™] 96. Each probe option includes specifications for 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.

94 54 544 FAM 644 842 94 5788 642 5988 642 6988 642 Modify 94 5788 642 5988 642 644 644 94 5788 642 5988 642 644 644 94 5788 642 5988 642 644 644 94 584 757 773 849 644 94 578 642 644 644 534 94 578 642 644 644 534 94 578 642 645 644 534 94 58 6757 615 849 644			Probe Lis	st			
Add Probe	Vo.	Name	Dye	Quencher	Color	-	Add
Add Probe		Std_FAM	FAM	BHQ			
Add Probe		Std_SYBR_GRE	SYBR_GREEN	None	T. C. C. C. C.		Modify
Add Probe		Std_JOE	JOE	BHQ			
Add Probe		Std_TET	TET	BHQ		E	Delete
Add Probe		Std_TAMRA	TAMRA	BHQ			
Add Probe	6	Std_CY3	CY3	BHQ			
Add Probe		Std_TEXAS_RE	TEXAS_RED	BHQ			Save
о нц.4.827 РАМ ВНО Сlose Add Probe		Std_ROX	ROX	BHQ			
Add Probe		Std_CYS	CYS	BHQ			C1
Setting Probe							
Name b-actin	10	HLA-827	FAM	BHQ		*	0,000
	Ad					-	

Cancel

- 5) Probe Information window will appear. Select one or click **Add** to add additional probe.
- * Additional Probe Setting: ex) Enter 'b-actin' in the field

NOTE:

Quencher

Color

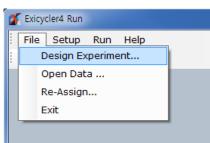
OK

None

2

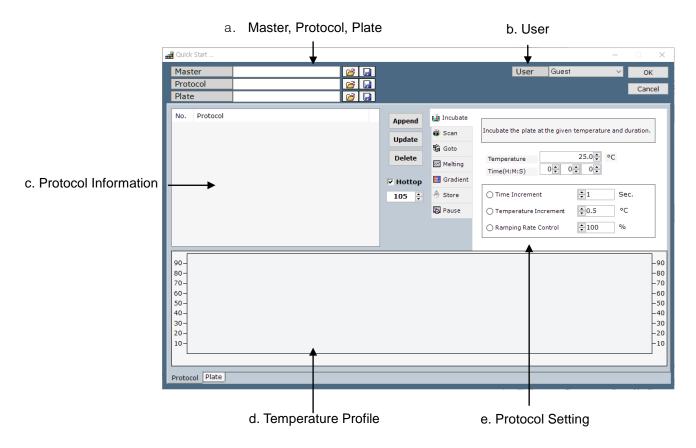
For example: In the **Dye** drop-down list of the **Add Probe** window, select **SYBR_Green**. Select **None** in the Quencher drop-down list. Select your desirable color for display and then click **OK**.





- 6) Check if your new probe is shown under the list, and then click **Save**.
- 7) Go to File > Design Experiment to design a new experiment.

8) Quick Start window will appear as shown below:



- 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.

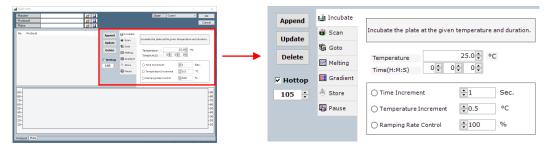
TaqMan probe

NOTE: Example of qPCR protocol

SVBB	Green I
SIDN	Gleen

No.	Protocol	No.	Protocol
1	Incubate at 95.00°C, for 0:5:0	1	Incubate at 95.00°C, for 0:5:0
1 2	Incubate at 95.00°C, for 0:0:10	1 2	Incubate at 95.00°C, for 0:0:10
L 3	Incubate at 60.00°C, for 0:0:20	1 3	Incubate at 60.00°C, for 0:0:20
8 4	Scan	a 4	Scan
\$ 5	Goto Line : 2, Cycle : 40	\$35	Goto Line : 2, Cycle : 40
6	Melting 60°C to 94°C, Every 1.0°C, 1 Sec.	1 6	Incubate at 25.00°C, for 0:1:30
17	Incubate at 25.00°C, for 0:1:30		

9) Click **Incubate** tab and enter a temperature in the **Temperature** field and then a time in the **Time** field.



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

Time Increment sets up time increment per cycle.

Temperature Increment sets up temperature increment per cycle. Ramping Rate reduces 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 *Exicycler*[™] 96. It is not recommended to use Store step when it is excessively humid.
- g. **Pause** is used to pause *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.
- 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 while maintaining the temperature of the previous incubation step. If the **Scan** step is not included in the protocol file, a conventional PCR will be carried out without scanning fluorescence signals.

Melting sets up starting and ending temperature for melting curve analysis to distinguish specific and non-specific amplification products when SYBR Green is used for Real-Time PCR. Enter starting temperature in the **From** field, ending temperature in the **To** field, a temperature interval within a range of 0.1°C to 1.0°C in the **Between** field, and a hold time within a range of 1 second to 255 seconds in the **Hold Time** field.

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 starting temperature in the **From** field, ending temperature in the **To** field, and time within a range of 1 second to 5 hours 59 minutes and 59 seconds in the **Time** field.

Store keeps the 96-well thermal block at a set temperature when thermal cycling is complete. The heated lid will start cooling down automatically. *Exicycler*TM 96 will keep the main door closed and will maintain thermal block at the set temperature until you stop *Exicycler*TM 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 120°C. The default temperature is 105°C. When the check box is unselected, the heating lid will not be heated.



- 10) Click Append to add a 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 to a selected step.
 - c. **Delete** button is used when you want to delete a selected step from the protocol.
- 11) Click Incubate tab to add another Incubation step and then click Append. Repeat steps 9 through 10 if needed. (i.e. 94°C 30 sec / 55°C 30 sec / 72°C 30 sec).

NOTE:

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



- 12) Click Scan tab and then Append.
- 13) Click **Goto** tab and select a starting step (i.e. "2") in the **Line** drop-down list. Enter a number of cycles (i.e. "40") in the **Cycle** field, and then click **Append**.

NOTE: Melting curve analysis

Melting curve analysis is an assessment of the dissociation-characteristics of double-stranded DNA during heating.

Append	🛄 Incubate		0	1.	3	1.3		
Update	🖨 Scan	Collects the steps.	fluoresc	ence data	aatea	ch gi	/en te	mperatu
opuate	🔓 Goto							
Delete	Melting	Tempera	ature					
Hottop	🚺 Gradient	From	60 🌲	°C ·	Го	94	÷ 0	C
105 💠	A Store		E	Between		1.0	* °	С
	-			Hold Time	1	1		
	Pause		F		-	1	÷ S	ec.
Annend	LOI Pause				-	1		ec.
Append		Incubate the p						
Update	i Incubate		olate at th	ne given te	emper	ature	and du	
	incubate	Incubate the p Temperature Time(H:M:S)	plate at th	ne given ti		ature	and du	

C Time Increment

Ramping Rate

Temperature Increment

-1

÷0.5

21.0

Sec

°C °C/Sec.

- 14) Click **Melting** tab to perform melting analysis. Enter starting temperature in the **From** filed (i.e. "60"), 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").
- 15) Click Append.
- 16) Click **Incubate** to add finish step (i.e. 25°C 1 min 30 sec) and then click **Append.**

NOTE:

A Store

Pause

105 💠

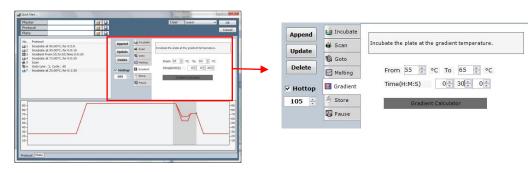
The door of *Exicycler*TM 96 will open automatically when a Real-Time PCR protocol does not include **Store** step. If **Store** step is included in the protocol, you must stop *Exicycler*TM 96 by clicking **Stop** button in order to open the door. Otherwise, *Exicycler*TM 96 will continue to store samples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

IMPORTANT:

To set the temperature on the Hot top Make sure that the Hottop check box is selected. The default value is set to 105 °C. If you turn off the Check box hot top is not heated.

Additional: Setting the temperature gradient mode

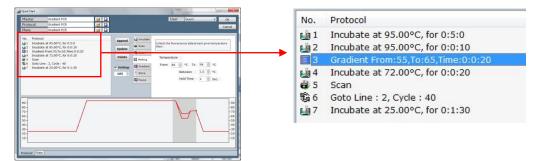
① Click Gradient tap, enter starting temperature in the From field, ending temperature in the To field.



(2) Press 'Gradient Calculator' button below the time setting window, and 'Gradient Calculator' window will appear. It will show predicted temperatures on each well.

Master 20 0 Protocol 20 0 Plate 20 0		User Guest • OK Cancel													
No. Protocol List 11 Inno.adva # 55.00°C, 69 0.5.0 10 12 Inno.adva # 55.00°C, 69 0.5.0 10 13 Inno.adva # 55.00°C, 69 0.5.0 10 14 Inno.adva # 75.00°C, 69 0.0.20 10 14 Inno.adva # 75.00°C, 69 0.0.20 10 14 Inno.adva # 75.00°C, 69 0.0.20 10 15 Gotto Inite 12, Cycle 1:40 10 16 640 Inite 12, Cycle 1:40 10 16 7 Inno.bette # 23.300°C, 69 0.1.30	Append Lil Trouber Update & Scan Delete E Mating O Hottop Oriofest 105 2 A Store	1		🖳 Gradient	Calculator	6					i ante		Inch		
	E Passe	Gradient Calculator	1	1	2	3	4	5	6	7	8	9	10	-11	12
90- 00- 00- 00- 50- 00- 50- 00- 30- 00- 00- 00- 00- 00- 0			-90 +80 -70 -60 -50 -40	54.72	55.0	56.11	57.22	58.33	59.44	60.56	61.67	62.78	63.89	65.0	64.72
Protocol Plate			-90 -20 -10												

3 Click Append.



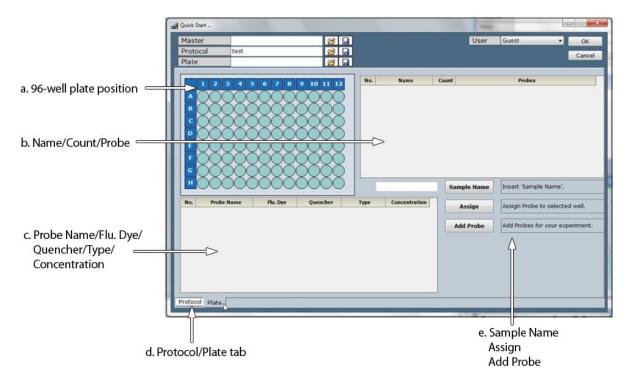
Mast	ter		🞽 🔓
Prote	ocol	test	6
Plate	2		6
No.	Protoco	í	
1	Incubat	e at 94.00°C, for 0:10:0	
2	Incubat	e at 94.00°C, for 0:0:30	
道 3	Incubat	e at 55.00°C, for 0:0:30	
4	Incubat	e at 72.00°C, for 0:0:30	
6 5	Scan		
6 6	Goto Li	ne : 2, Cycle : 35	
7	Melting	60°C to 94°C, Every 1.0°C, 1 Sec.	
8 🛄	Incubat	e at 25.00°C, for 0:1:30	

17) 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.

18) Once the protocol is saved, click **Plate** tab to create a plate file. Plate window will appear as follows:

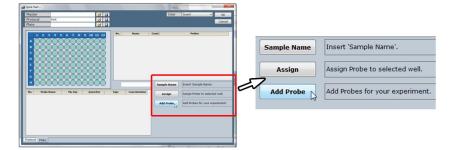


- a. 96-well plate position specifies well locations for samples.
- b. Name/Count/Probe displays a name and probe information for each sample.
- c. **Probe Name/Flu. Dye/Quencher/Type/Concentration** specifies a name, a fluorescence dye, a quencher dye, a type, and a concentration for each sample.
- 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 be used in experiment.

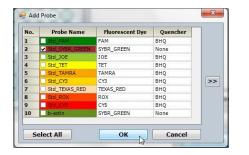
NOTE:

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.

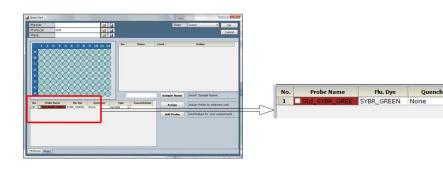
19) Click Add Probe, the Add Probe window will appear.



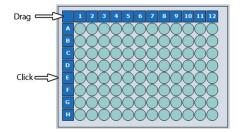
20) Select appropriate probes, and then click **OK**.



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



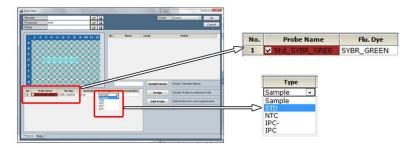
22) Select wells from the 96-well plate diagram.



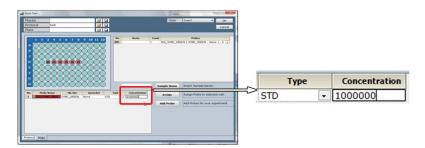
NOTE:

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.

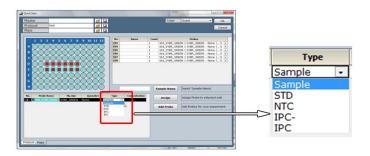
23) Click the probe option cell and then select **STD** in the **Type** drop-down list. Select the check box of **Probe Name** and then click **Assign**.



- a. Sample represents unknown samples.
- b. **STD** stands for a standard sample of a known concentration.
- 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 / Non-existence reaction. It will also diagnose cause of the negative result from 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.
- 24) For example, Click well D3 and enter '1000000' for a concentration of 10⁶-copy and then click **Assign**. Repeat this for wells D4 through D8 with concentrations of serial diluted standards.



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



26) Select cells D3 through E8 and enter a sample name in the **Sample Name** field. Click **Sample Name** to save sample name.

Master 22 a Protocol test 22 a Plate 22 a	User Guest • OK Cancel		
	No. Cast Nation 0 1 000,000,000,000 000,000,000,000,000 000,000,000,000,000,000,000,000,000,00	D TNF-a	Sample Name

27) Verify information on each well in the Name/Count/Probe window.

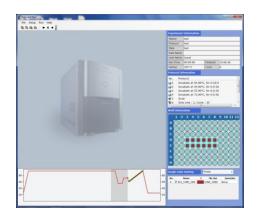


- 28) Enter a plate name in the **Plate** field (i.e. Test) and then click 😡 button to save the plate file.
- 29) The protocol file and plate file are saved. Enter a master file name in the **Master** field (i.e. Test) and then click 😡 button to save the master file.

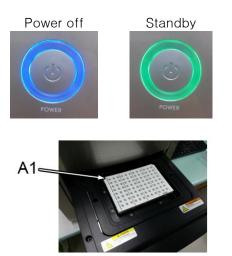
Master test 201 Protocol test 201 Plate test 201		-	User	Guest	•	ок
No. Telefore 10 Includes at 95.00°C, for 0.50 10 10 Includes at 95.00°C, for 0.100 10 10 Includes at 95.00°C, for 0.100 10	Any lands teach					Cancel
90- 60- 70- 60- 50-			Quick Start	test		6
40- 30- 20- 10-		23	Protocol	test		
Protocol Plate			Flate	test		

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 Quick Start menu. The master file includes both protocol and plate file.



30) Click **OK** at the top right corner. Go back to the main window.



- 31) Ensure the status LED in front of the *Exicycler*[™] 96 is blinking green.
- 32) Press the **DOOR** button for a full second to open the door. Load the 96-well plate with A1 positioned at top-left corner of the block.
- 33) Verify the master file and go to **Run > Run** from the top menu bar or click the **Run** button.

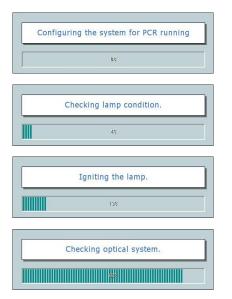


Dataname	2	
Please in	put the dataname	to save the result.
Sample_	AQ	
	ОК	Cancel

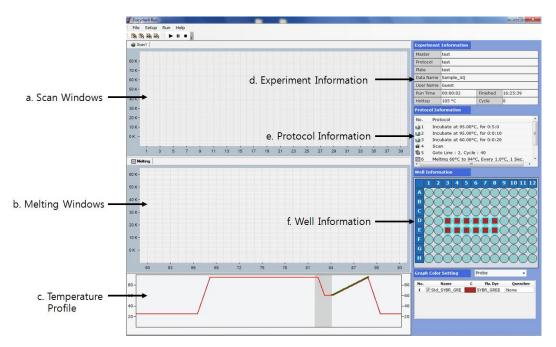
🚺 Exicycler4 Run	
File Setup	Run Help
🕴 🕰 🗗 🛍	🕨 Run
	() Pause
	Stop

34) 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.

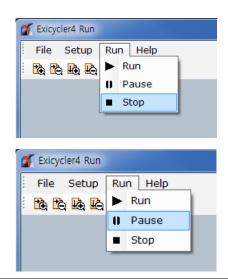
35) The following three message boxes will appear in the following order.



- a. This message will appear when closing the door of the *Exicycler*[™] 96.
- b. This message will appear when the lamp condition is being checked.
- c. This message will appear during lamp ignition.
- d. This message will appear when lamp is turned on and initialization of *Exicycler*[™] 96.
- 36) The main window will appear when the progress bar finishes without any problems.



- 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 of the current experiment.
- e. Protocol Information indicates current progress of the experiment.
- f. Well Information displays details of each well when selecting either Probe or Type in the Graph Color Setting drop-down list. To display the curve of amplification, select a well from 96-well plate diagram and click the check box from the probe option.



- 37) Go to **Run > Stop** to stop *Exicycler*[™] 96 or click **Stop** button.
- 38) Go to **Run > Pause** to pause *Exicycler*[™] 96 or click **Pause** button.

IMPORTANT:

If the pop up window remains more than 5 minutes after running $Exicycler^{TM}$ 96 or if the temperature profile does not display anything in the main window, turn off $Exicycler^{TM}$ 96 and turn it on again. Start **Run Exicycler4** again and run $Exicycler^{TM}$ 96 again. If the same error keeps occurring, please contact us for customer service.

IMPORTANT:

It is recommended to re-start *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 span and cause errors.

NOTE:

Do not turn off *Exicycler*TM 96 while the heating lid is warming up. While the lid is heated to the set temperature, 96-well block maintains at 25°C. The thermal cycling will start when the heating lid reaches 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 *Exicycler*[™] 96 Analysis Program to generate analysis data. Please refer to **Analyzing Data using Analysis Program** for data analysis.

Reagents and Consumable Products

Various reagents and kits are required to perform a Real-Time PCR in *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 71 page, **Ordering Information**.

PCR Premix kit

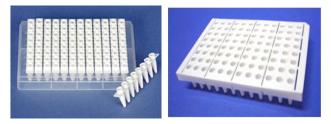
AccuPower[®] Greenstar[™] qPCR PreMix kit allows an easy and fast amplification in *Exicycler*[™] 96. AccuPower[®] Greenstar[™] qPCR PreMix kit consists of Sybr[®] Green I fluorescent dye, HotStart Taq DNA polymerase, and necessary qPCR components. Therefore, only template, primers, and DW need to be added 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 *Exicycler*TM 96. A standard tube or a plate can be also used, but only for a conventional PCR without 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 when using 0.2 ml 8-strip tubes.





ACAUTION

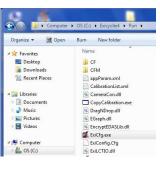
You must cut up the sealing tape with a box cutter, not scissors. The tape adhesive can be carried over to the surface of sealing tape when cutting up the tape with scissors. This will make the tubes stick to the heating lid of *Exicycler*TM 96. Tubes stuck to the lid will drop inside *Exicylcer*TM 96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

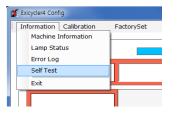
Marning Chemical hazard:

AccuPower[®] Greenstar[™] qPCR PreMix may cause eye and skin irritation, and respiratory tract irritation. Read MSDS before use and follow the instructions if swallowed or inhaled. Wear appropriate protective eyewear, clothing, and gloves. Performance Maintenance

Performing System Diagnosis using ExiCfg







1) Turn on *Exicycler*[™] 96 by pressing the **POWER** button.

2) Go to 'C:₩Exicycler4₩Run' and double click **ExiCfg.exe** to start the program.

3) Go to Information > Self Test.

NOTE:

⁶USB Communication is NOT initialized' message box will pop up if *Exicycler*[™] 96 is turned off or if the cable connection between *Exicycler*[™] 96 and the computer is not firmly connected.

	Start
Door Motor	
Bath Motor	
Excitation Motor	
Emission Motor	
Lamp Fan	
Sensor	
Peltier Heating	
Peltier Cooling	
Hot lid	
Bath Fan	

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) **OK** signs will show up in green, when all of the selfdiagnosis tests pass completely without any problems.

NOTE:

Self-diagnosis is complete with two short beeps.

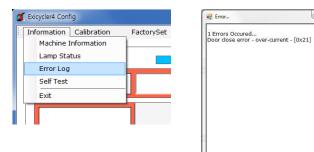


6) When any of the self-diagnosis tests fail, **NG** signs will appear in red.

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.





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Guide to AccuPower[®] Fluorescence test kit for calibration

Prepare to perform the calibration with a Fluorescence test kit. The Fluorescence test kit is designed for $Exicycler^{TM}$ 96 Real-Time Quantitative Thermal Block. Therefore, it is not applicable to other Real-Time instruments. The Fluorescence test kit is comprised of 11 plates, a mask fluorescence test plate, a background fluorescence test plate, multi-channel fluorescence test plates (9 different optical luorescence test plates of filter sets) present in *Exicycler*TM 96. The Fluorescence test kit is available for reuse up to 3 times. For more information about the Fluorescence test kit, please see the following:

Before Starting Calibration with the Fluorescence test kit

Centrifuge, Disposable plastic gloves, *AccuPower*[®] Fluorescence test kit for *Exicycler*[™] 96 (A-2060-A1)

Fluorescence test plate preparation

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



- 1) Take out the Fluorescence test kit from the freezer.
- 2) Take out a sealed packs in the Fluorescence test kit.





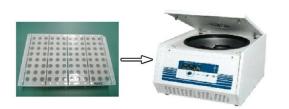
- 3) Allow the pack to thaw in room temperature for at least 10 minutes.
- 4) Take out a Fluorescence test plate from its packaging.

IMPORTANT:

Pay particular attention to the Fluorescence test kit when handling. Wear disposable plastic gloves to prevent contamination on the sealing tape covering 96-well plate. Wipe the surface of the plate with 70% ethanol when it needs to be cleaned.

NOTE:

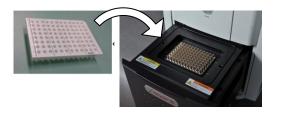
Do not vortex the Fluorescence test plate. A small amount of Fluorescence test solution is pre-aliquoted in each well, and it may be lost while vortexing.



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

NOTE:

It is important to centrifuge the Fluorescence test kit prior to use. This will spin down remains of fluorescence dye on the side of the well and to remove air bubbles at the bottom of the well.



- Protect the Fluorescence test plate from direct sunlight after centrifugation. Place the plate directly into *Exicycler*[™] 96.
- 7) Put the Fluorescence test kit back into its packaging and return it to the freezer when the calibration is done.

IMPORTANT:

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

Calibration using ExiCfg

The calibration must be performed prior to operating $Exicycler^{TM}$ 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 $Exicycler^{TM}$ 96, calibrating $Exicycler^{TM}$ 96 with a Fluorescence test kit is recommended. Calibration must be done when moving $Exicycler^{TM}$ 96 to a different location or when changing a light source lamp. A periodical calibration of every 6 months helps to maintain the optimum condition of $Exicycler^{TM}$ 96.

Use the ExiCfg program to calibrate $Exicycler^{TM}$ 96. The ExiCfg calibrates as well as diagnosessystemical condition of $Exicycler^{TM}$ 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 to $Exicycler^{TM}$ 96.

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

Mask Calibration



1) Turn on *Exicycler*[™] 96 by pressing the **POWER** button.

NOTE:

Make sure the status LED is blinking green after the self-diagnosis prior to starting the Calibration.



- Go to 'C:₩Exicycler4₩Run' and double click ExiCfg.exe.
- 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.

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 3 minutes, turn off *Exicycler*TM 96 and turn it on again after 5 seconds. In case of an abnormal shutdown like this, wait about 3 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 Fluorescence test plate on the block.

NOTE:

You must perform the Mask Calibration test prior to any other calibrations.

IMPORTANT:

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



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

nation Calibration	FactorySet		
	Cali	orated Not	calibrate
Lamp warming		1do%	
Mask Calibration			
ration	<u> </u>	Dar Links	
ration		Dark 1.0000, 1	- 10
	Stop Cal	brated Not	calibrate
	Stop Cal		calibrate
		brated Not a	calibrate
Start	9%		calibrate
Start 1	0% 0%	Exposure 0 Exposure 0	calibrate
Start :	0%	Exposure 0	calibrate
Start 1	0% 0%	Exposure 0 Exposure 0	calibrate

Close

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

NOTE:

The Calibration time is approximately 10 minutes depending on the condition of $Exicycler^{TM}$ 96 and the computer.



9) When the Mask Calibration is complete, click **OK**.

NOTE:

The door will open when the Mask Calibration is successfully completed. Put the plate back into its packaging and return it to the freezer.

Marning High temperature

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



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

Calented	Not calibrate History
	Capture Exposure(3):413 Calced Level (40004 at the opposure of 413ed) Marr (0.0048)0094(007)479
Mask Calibration	Detected Exposure:414 Capture Exposure(4) 300
	Capture Exposure(4):374 Capture Exposure(4):412 Calced Level : 46696 at the exposure of 412e6
Background Calibration	Slavit : 0.00869423076823077 Detected Exposure:412 Capture Exposure(12) 150
	Capture Exposure(5):252 Capture Exposure(5):252 Capture Exposure(5):423
Multi-Osennel Galibration	Calced Level : 49664 at the exponence of 421mS Start : 0.00915948275962059
A CONTRACTOR OF	Detected Exposure:422 Mask Calibration complete.

11) The 'Mask Calibration' frame will turn blue when the Mask Calibration is completed.

Background Calibration



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

NOTE:

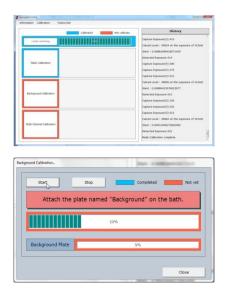
This step is omissible when performing the Background Calibration followed by the Mask Calibration.



2) Place the Background Fluorescence test plate on the block.

IMPORTANT:

It is important to load the Fluorescence test 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 takes approximately 10 minutes depending on the condition of *Exicycler*[™] 96.



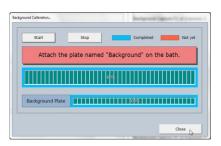
5) When the Background Calibration is complete, click OK.

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.

Marning High temperature

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



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

7) The 'Background Calibration' frame will turn blue when the calibration is completed.

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.

		History
	Calibrated Not calibrate	Mask Calibration complete.
		Background Capture F1 at Exposure
		Background Capture F1 at Exposure
		Background Capture F1 at Exposure
		Rackground Capture F2 at Exposure
		Rackground Capture F3 at Exposure
		Background Capture 72 at Exposure
		Background Capture F3 at Exposure
sten		Background Capture F3 at Expense
		Background Capture F3 at Exposure Background Capture F4 at Exposure
		Background Capture F4 at Exposure :
		Background Capture F4 at Exposure
		Background Capture F5 at Expense
ration		Background Capiture FS at Exposure
		Background Capture FS at Exposure .
		Background Calibration complete.
No	Name	
No.	Name	
No. 01	Name	
01	FAM	
01 02	 ✓ FAM ✓ SYBR_GREEN 	
01 02 03	 ✓ FAM ✓ SYBR_GREEN ✓ JOE 	
01 02 03 04	 ✓ FAM ✓ SYBR_GREEN ✓ JOE ✓ TET ✓ TAMRA 	
01 02 03 04 05	 ✓ FAM ✓ SYBR_GREEN ✓ JOE ✓ TET ✓ TAMRA ✓ CY3 	
01 02 03 04 05 06	 ✓ FAM ✓ SYBR_GREEN ✓ JOE ✓ TET ✓ TAMRA ✓ CY3 ✓ TEXAS_RED 	
01 02 03 04 05 06 07	 ✓ FAM ✓ SYBR_GREEN ✓ JOE ✓ TET ✓ TAMRA ✓ CY3 	

2) Click Multi-Channel Calibration button.

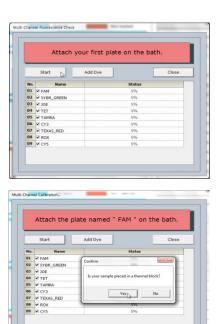
3) Select fluorescence dyes in the 'Multi-Channel Calibration' window for the Calibration.

NOTE:

9 dyes are available for calibration.

NOTE:

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



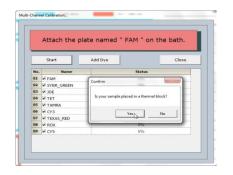
4) Click Start.

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

6) Place the Multi-Channel Fluorescence Test plate on the block.

IMPORTANT:

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



- 7) Click Yes in the 'Confirm' window.
- 8) The door will open when the Calibration is successfully complete.

NOTE:

An approximate calibration time for each dye is 5 minutes.

NOTE:

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

High temperature

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

A	ttach the pla	te named " bath		N " on the
	Continue	Add Dye	j, l	Close
No.	Name		Status	
01	FAM		19595	
02	SYBR_GREEN		0%	
03	90E		096	
04	TET 🕅		096	
05	2 TAMRA		0%6	
06	CV3		096	
07	TEXAS_RED		096	
08	ROX		0%6	
09	CY5		096	

Attach the plate named " CY5 " on the bath.

ОК

Attach the plate named " CY5 " on the bath Add Dye

100%

Status 10045 10045

10000

Add Dve

Start

SYBF JOE TET TAM

Start

 No.
 Name

 01
 ✓ FAM

 02
 ✓ SYBR_GREEN

 03
 ✓ JOE

 04
 ✓ TET

 05
 ✓ TAMRA

 06
 ✓ (Y3

 07
 ✓ TEXAS_RED

 08
 ✓ ROX

 09
 ✓ (Y5

 No.
 FAM

 01
 Ø FAM

 02
 Ø SYBI

 03
 Ø JOE

 04
 TET

 05
 TAM

 06
 CY3

 07
 TEX.

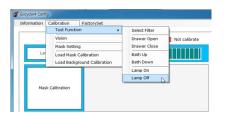
 08
 ROX

 09
 CY5

- 9) Prepare the Fluorescence Test plate with a dye X (i.e. "SYBR Green") when 'Attach the plate named ' \mathbf{X} ' (i.e. "SYBR Green") on the bath' message appears.
- 10) Click Continue to repeat step 9.
- 11) When the Multi-channel Calibration is complete, click OK.

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

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



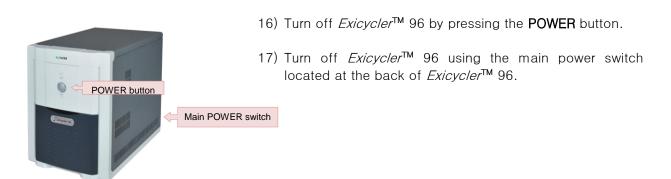
14) Go to Calibration>Test Function>Lamp Off from the top menu bar.

Information Calibration Machine Information	FactorySet
Lamp Status	Calibrated
Error Log Self Test	0%
Exit	<u> </u>
Mask Calibration	

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

IMPORTANT:

Wait at least 3 minutes to switch off main power of *Exicycler*TM 96. The cooling fan will continue to run to cool down the lamp. Shutting down *Exicycler*TM 96 before cooling down the lamp will reduce the lamp life span.



Cleaning the main body

Note:

- Turn off *Exicycler*[™] 96 Real-time Quantitative Thermal Block, then remove the power cord.
- Allow the instrument to cool until the bath block reach room temperature.

Cleaning the Block Wells

If you use any cleaning or decontamination method, except those recommended in the manual, you risk damaging the Instrument. Clean the block wells once a weekly or as needed.

Always wear protective glasses and gloves when servicing the instrument. Also, make sure you disconnect the instrument from power cord before you begin any service procedure.

During instrument operation, the temperature of the bath block can be as high as 100°C. Before performing the procedure, keep hands away until the bath block reach room temperature.

To clean the block wells:

- 1) Open the Front door.
- 2) Remove the sample from the bath block and set it aside.
- Use a cotton swab soaked in pure isopropyl alcohol or DNA Zap to clean the wells thoroughly. Make certain that the isopropyl alcohol or DNA Zap has evaporated completely before reloading a sample tray.

MARNING CHEMICAL HAZARD

Isopropyl alcohol is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation.

Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions.

Remove any remaining isopropyl alcohol from the cover.

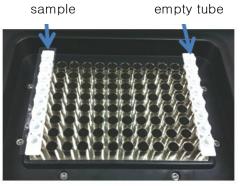
Note:

If the platen becomes contaminated with amplified DNA, raise the hot-top cover to a cleaning position, wipe the platen with a cloth or cotton swab soaked in bleach, then rinse with water.

- 1) Clean the heated platen once a month or as needed.
- 2) If the hot-top cover become contaminated with amplified DNA, raise the hot-top cover to a cleaning position. Wipe the cover with a cloth swab soaked in Isopropyl alcohol or DNA zap, then wipe the cover with a damp cloth.

Precautions for PCR tube loading

Do not put a tube column bias one if you experiment with PCR Strip tube. This can be caused by a phenomenon in skewing the Hot Lid cause evaporation of such samples, equipment failure. So please put the empty tube to balance the opposite direction. Please also well positioned for insertion into the correct PCR tube. If Tube is inserted incorrectly can not get the normal data, in this case, you must do the experiment again.



PCR tube balancing



Correctly located strip tube



Incorrectly located strip tubetube

Replacing Fuses

The instrument has two 250V, F10AL and 5×20mm fuses.

Required Materials

- Two 250V, F10AL and 5×20mm fuses
- Small screw-driver(-)

Procedure

1) Turn off the instrument and disconnect the power cord from the back of *Exicycler*[™] 96.

A DANGER ELECTRICAL SHOCK HAZARD

Severe electrical shock, which could cause physical injury or death, can result from working on an instrument with high voltage power supply. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.



2) Insert a small screw-driver(-) into the slot in the upper portion of the power entry module, and open the fuse door.



3) Pull out the fuse compartment to inspect the two fuses in the fuse compartment.





- 4) Pull out the blown fuse(s) from the fuse compartment and replace with new 250V, F10AL, 5×20mm fuses.
- 5) Place the fuse compartment back into the power entry module and close the fuse door.
- 6) Press the fuse door until it locks in place.
- 7) Connect the instrument power cord.

Returning *Exicycler*[™] 96 for Service

1) Decontaminate the instrument.

If the bath block became contaminated with radioactivity, use a commercially available decontaminant to remove the contamination. If the block is not decontaminated, the instrument cannot be returned to Bioneer for service.

- 2) Complete certification for instrument decontamination.
- 3) Fax or e-mail (<u>exicycler-support@bioneer.com</u>) the service request form to the customer center.
- 4) Pack the instrument in the provided packaging, without any accessories or power cords. Include service request form in the box.

Note:

Request for instruments repair without the service request form can lead to delayed in service.

5) Affix the provided postage to the box, then ship the instrument to the designated facility. The repair process requires 1 to 3 weeks.

Troubleshooting

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

Problems	Recommended Action
<i>Exicycler</i> ™ 96 does not start.	 Make sure the power cable is firmly connected to the wall circuit, and then switch on the power supply button at the rear of <i>Exicycler</i>[™] 96. Check a fuse box located at the rear of <i>Exicycler</i>[™] 96. Change the fuse if it has blown.
Errors occur either after or during self-diagnosis (Status LED in red).	 The lamp may still be cooling down or the self-diagnosis is still running. If the status LED does not turn back to green in 3 minutes, turn off <i>Exicycler</i>[™] 96 and turn it on again. If the same error occurs, please contact Bioneer Customer Service Center for help. Turn off <i>Exicycler</i>[™] 96 if unexpected errors occur. Turn on <i>Exicycler</i>[™] 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 detected between the computer and <i>Exicycler</i> ™ 96.	 Make sure that the USB cable is firmly connected to the computer. Ensure you use the USB cable provided with <i>Exicycler</i>[™] 96 to connect the computer and <i>Exicycler</i>[™] 96. Check if <i>Exicycler</i>[™] 96 is in the "Standby" mode. The status LED must blink in green. Turn off <i>Exicycler</i>[™] 96 and back on again if the status LED 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 then switch on the power supply button at the rear of <i>Exicycler</i>[™] 96. The self-diagnosis may be still running. Wait until the self-diagnosis is complete and the POWER button blinks in green.
<i>Exicycler</i> ™ 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 <i>Exicycler</i>[™] 96 to connect the computer and the <i>Exicycler</i>[™] 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 <i>Exicycler</i>[™] 96 is running to prevent friction between <i>Exicycler</i>[™] 96 and the computer.
<i>Exicycler</i> [™] 96 does not start a thermal cycling.	 Exicycler[™] 96 may still be in the "Standby" mode in order to warm up the lamp. Warming up the lamp takes about 10 minutes. The thermal cycling will start automatically after the lamp warm up has finished.

Intensity of fluorescence signal is low.	 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 of fluorescence signal.	 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.	1. The USB driver is not been installed properly. Re-install the USB driver.
PCR sample has 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 cannot read data from Exicycler[™] 96. Check if the USB cable is firmly connected between the computer and Exicycler[™] 96. Can't close program during PCR will appear if you attempt to close 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. Did 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 Multi-channel Calibration is successful. Need to set up Machine ID will appear if an instrument ID has not been set up for Exicycler[™] 96. Assign the ID using Exicycler[™] 96 software.

Pop-up message during calibration process.(continue)	 9. Multi-Channel Calibration finished will appear if the Multi-channel Calibration is successful. 10. Need to set up Machine ID will appear if an instrument ID has not been set up for <i>Exicycler</i>[™] 96. Assign the ID using <i>Exicycler</i>[™] 96 software. 11. No matching calibration data with machine will appear if the instrument ID does not match the ID from <i>Exicycler</i>[™] 96 software. Set up the instrument ID using <i>Exicycler</i>[™] 96 software. 12. Turn off the lamp will appear if you attempt to end ExiCfg while the lamp is still on. To close ExiCfg, turn off the lamp first and then close the ExiCfg. 13. Please load Mask Calibration Information first will appear if you attempt to perform the Uniform Calibration before the Mask Calibration. 14. USB Communication is NOT Initialized will appear if the computer and <i>Exicycler</i>[™] 96 are not communicating with each other. Check if the USB cable between the computer and the <i>Exicycler</i>[™] 96 is firmly connected. 15. You must insert New Dye Name will appear if a new name is not assigned to a custom dye. 16. 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.
Error message when running <i>Exicycler</i> ™ 96 software.	 Can't exit program during PCR will appear if you click Stop button in <i>Exicycler</i>[™] 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. 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 <i>Exicycler</i>[™] 96. 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 <i>Exicycler</i>[™] 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 <i>Exicycler</i>[™] 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 <i>Exicycler</i>[™] 96. Go to C: WExicycler4 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. No matching calibration data with Machine. Can't Run! will appear if you click Run button when <i>Exicycler</i>[™] 96 ID and the calibration data ID do not match.

Error message when running <i>Exicycler</i> ™ 96 software. (continue)	 13. No matching calibration data with machine will appear if the <i>Exicycler</i>[™] 96 ID and the calibration data ID do not match. 14. Please insert any protocol before inserting Goto Protocol will appear if the Goto step is inserted in the first line of a protocol file.
	15. Please insert Data name will appear if you have not entered a name for data prior to PCR running.
	16. Probe Information was changed, do you want to cancel this? will appear if you click the Cancel button without saving the edited probe Information.
	17. Probe using same filter is assigned will appear if you assign more than 2 probe options within the same wavelength range.
	18. 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.
	19. 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.
	21. You must select Dye and Quencher will display if the dye and que ncher set up has not been selected in the Add Probe window.

Ordering Information and Warranty

Ordering Information

Product				
A-2060-1	Exicycler [™] 96 Real Time Quantitative Thermal Block			
Accessories				
A-2060-A1	AccuPower [®] Fluorescence test kit for <i>Exicycler</i> ™ 96			
Plastic consuma	ables			
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 & Reage	ent			
K-6200	<i>AccuPower[®] Greenstar</i> ™ qPCR PreMix, <i>Exicycler</i> ™ 8-well strip, 50 µl, 12 strips			
K-6203	<i>AccuPower[®] Greenstar</i> ™ qPCR PreMix, <i>Exicycler</i> ™ 96 -well plate, 50 µl, 1 plate			
K-6253	AccuPower [®] 2X Greenstar [™] qPCR Master Mix / 100Rxn, 50 µL reaction			
K-6254	AccuPower [®] 2X Greenstar [™] qPCR Master Mix / 200Rxn, 50 µL reaction			
K-6600	<i>AccuPower[®] Plus DualStar</i> ™ qPCR PreMix, <i>Exicycler</i> ™ 96, 12 strips, 50 µl			
K-6603	<i>AccuPower[®] Plus DualStar</i> ™ 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-2060-1		
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 +81-42-930-8777 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 $1\sim3$ weeks.

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 natural disaster.

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

Revision History

Rev. No.	Rev. Date	Reason	Revision contents	etc
1.0	21/July/2014	First issue	First description	
1.1	11/July/2016	Document Form changes	BQ-042-101-01 Rev.1 (2015-11-06)	
1.2	8/Dec/2016	Document Form changes	BQ-042-101-01 Rev.2 (2016-11-04)	

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