Installation and Calibration Guide

Exicycler™ 96 Real-Time Quantitative Thermal Block

BIONEER CORPORATION

Warranty and Liability

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

QC Testing

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

Notice

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

Appearance and specification are subjected to change without notice.

Contents

Before Starting

How to Use This Guide

Who This Guide is Written for

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

General Requirements for Installation

This guide assumes that you:

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

Word Conventions

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

Safety and Warning

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

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

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

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

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

How to Use an Instrument Safely

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

How to Obtain More Information

For more information about Exicycler™ 96 , please visit us online at www.bioneer.co.kr or www.bioneer.com.

How to Reach Customer Support

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

Safety Instructions

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Safety Warnings and Cautions

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

 **Warning: Hazards or unsafe actions that may result in severe injury.**

 **Caution: Hazards or unsafe actions that may result in minor injury or damage.**

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

 **Warnings: Hazards or unsafe actions that may result in Electronic risk.**

Location Consideration and Installation

 **General Instrument Safety**

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

and system damage. A limited warranty does not cover unauthorized alterations or damage by abuse.

Installation Safety

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

Cautions

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

Operation and Maintenance

Warnings

1) The system can be hazardous if misused.
2) Keep the 96-well thermal block area clean to prevent damage and to generate accurate experimental data.
3) After any Real-Time PCR runs including ‘Scan’ or ‘Melting’, allow the Exicycler™ 96 at least 10 minutes to cool down the light source lamp. Continuous operation without a break will reduce the lamp life time and cause errors.

4) Do not place a piece of paper or a plastic cover under the Exicycler™ 96. It could cause fire.
5) Do not turn off the Exicycler™ 96 right after a Real-Time PCR run is done. Wait until the cooling fan stops running completely. The cooling fan still runs for about 2 minutes to cool down the lamp after every Real-Time PCR run.
6) Do not cover the Exicycler™ 96 with a piece of paper or a plastic cover. It may cause a fire or failure.
7) Set 'Turn off monitor', 'Turn off hard disks', 'System standby', and 'System hibernates' to 'Never' using 'Power Options Properties' in the Control Panel. Otherwise, data transfer between the Exicycler™ 96 and the computer will be interrupted.

IMPORTANT:

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

European Safety and EMC Standards (CE)

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

1) Council Directive 98/79/EC of 27 October 1998 concerning in-vitro diagnostic medical devices
2) EN 61010-1 [2001] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1: General requirements for safety.
3) EN 61010-2-010 [2003] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials.
4) EN 61010-2-081 [2001] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes.
5) EN 61010-2-101 [2002] Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
6) EN 61326 [1997+A1] [1998+A2] [2001+A3] [2003 Class A] Electrical equipment for measurement, control and laboratory use - EMC requirements.
7) EN 60601-1-2 [2001] Medical electrical equipment. Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility - Requirements and tests.

Compliance is demonstrated by the following mark:

CE

Authorized Representative

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

Product Use Limitations

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

Introduction

Introduction

The Exicycler™ 96 Real-Time Quantitative Thermal Block combines a thermal block in a standard 96-well format and an innovative fluorescence detector. The detector monitors the fluorescence emitted as an indicator of amplified nucleic acid product during each PCR cycle in real time.
The thermal block is built in the lower part of the Exicycler™ 96, which carries out a thermal cycling. The detector is located in the upper part of the Exicycler™ 96, which measures the fluorescence emitted from samples in the thermal block in real time and transfers data to the computer for analysis. Data transferred from the detector is analyzed with the Exicycler ™ Analysis Software.

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

The Exicycler™ 96 Real-Time Quantitative Thermal Block detector consists of an light source part and a detection part. The light source part is an energy source to excite fluorescent dyes. A short arc lamp that has a long lifetime is bright, and is implemented. A white-light source is divided into particular wavelength groups by band path filters. The band path filters that are set in the Exicycler™ 96 covers within 480 to 680 ㎚ and works as a set consisting of an excitation and an emission filter. Five of the filter sets are provided for various applications. Therefore, adding additional band path filters is not required.

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

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

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

System Components and Specifications

BIONEER CORPORATION

System Components and Specifications

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

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

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

System Components

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

Specifications

Physical specifications	
Dimension (㎜)	355(W) X 540(D) X 470(H)
Weight (㎏)	30 ㎏
Sample capacity size	96-well plate / 0.2 ㎖ micro tubes
Sample volume	20~50 ㎕ (50 ㎕ recommended)
Input voltage	100 ~ 240 VAC
Frequency	50 / 60 ㎐
Power	850 Watts (Fuse : 250 V, F10AL)

Operating specifications	
Method of heating / cooling	Peltier
Temperature range	4.0℃ ~ 99.9℃
Temperature accuracy	± 0.3℃
Temperature uniformity	± 0.5℃
Ramping rate	Max. 2.5℃/sec
Lid temperature	Within 90 ~ 120℃
Gradient range	1℃~20℃
Temperature increment range	0.1℃~9.9℃
Time increment range	1 sec ~ 60 sec
Ramp rate control	0.1℃/sec
Operating temperature	15 ~ 30℃
Operating humidity	20 ~ 80%, no condensation
Communication	USB 2.0 high speed
Operating OS	Windows XP (either in English or in Korean)

Optical Part	
Light source	Short arc lamp
Wattage	120 W
* Lamp life time	3,000 hours
Sensor	16 bit 2D CCD
** Excitation Filter / Emission Filter	5 Types / 5 Types

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

† Filter sets

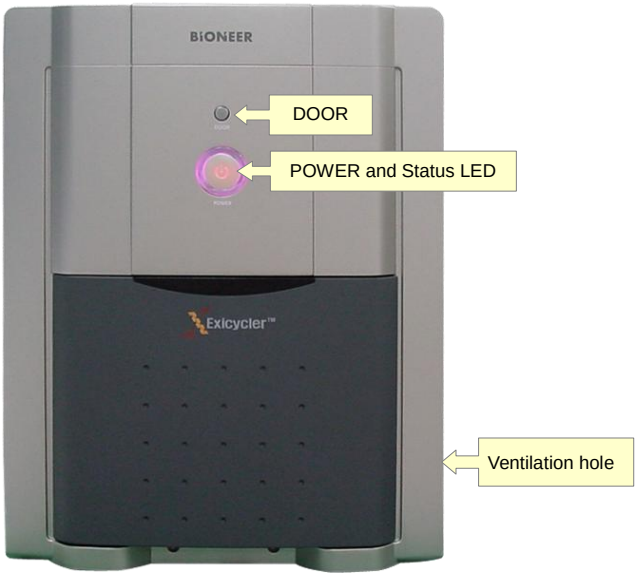
Position	Excitation (㎚)	Emission (㎚)	Set	Fluorescent dye
1	Blank	Blank	1	-
2	490	520	1	FAM, SYBR Green I
3	520	550	1	JOE, TET
4	550	580	1	TAMRA, CY3
5	580	610	1	Texas Red, ROX, Red610
6	640	670	1	CY5, Red670

 Warning:

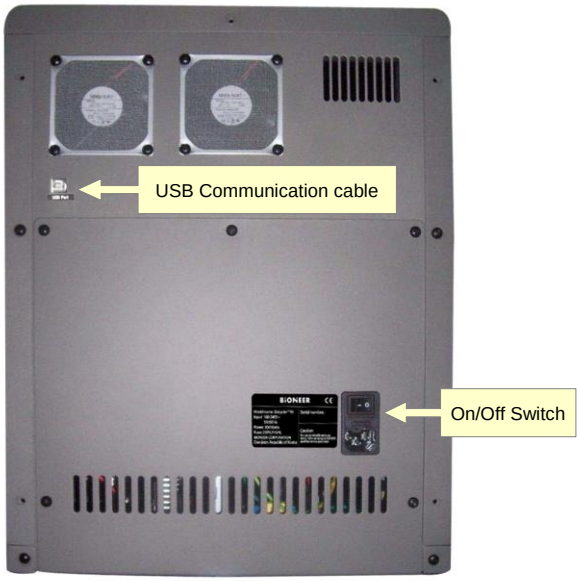
Chemical hazard: Such the florescent dyes may cause eye and skin irritation, and respiratory tract irritation. To treat them, read the MSDS before use and follow the instructions if swallowed or inhaled. Wear appropriate protective eyewear, clothing, and gloves.

System Views

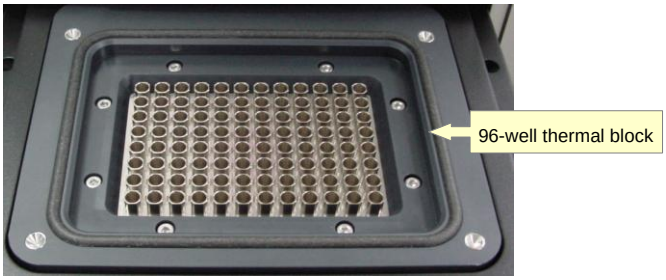
Front view



Rear view



Inside view



Installation

Installation

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

Setting Up the Computer

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

Computer Requirements

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

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

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

Connecting the Computer and Exicycler™ 96

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

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

List of Components

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

* Provided according to country standard.

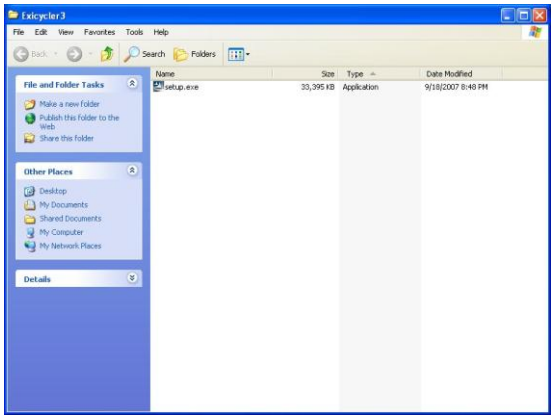
Installing the Operation and Analysis Software

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

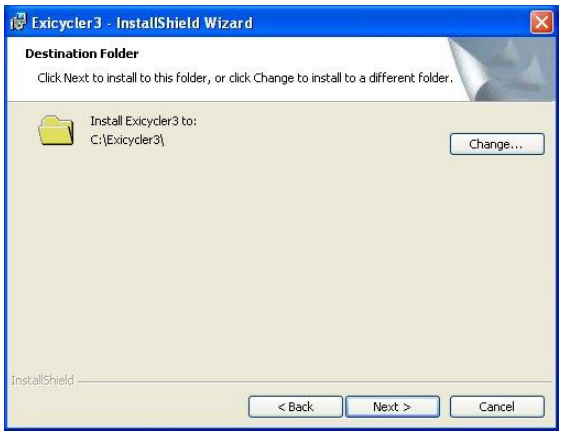
IMPORTANT:

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

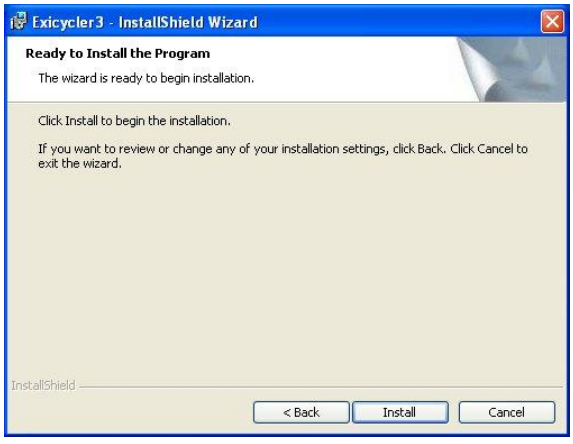
1) Power on the computer only, not the Exicycler™ 96. Insert the installation CD into the CD drive of the computer. Exicycler™ 96 Setup Program starts automatically. If the Setup Program does not start, go to 'My Computer' to locate the CD drive, and then double-click **Setup.exe**.



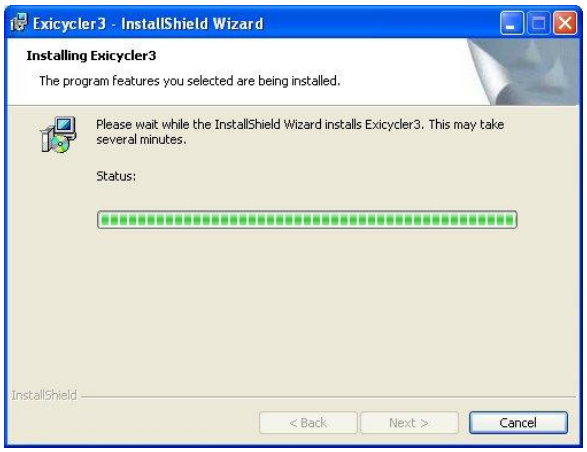
2) The 'Exicycler™ 96 Install Shield Wizard' starts. Click **Next** to start the installation. The installation will proceed in C:\Exicycler3.



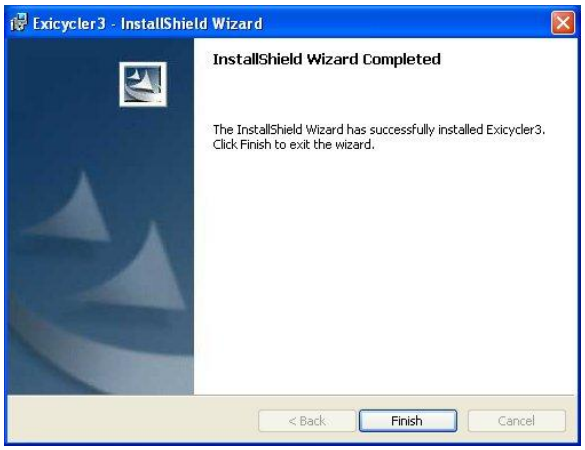
3) Click **Next** to start installing software.



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

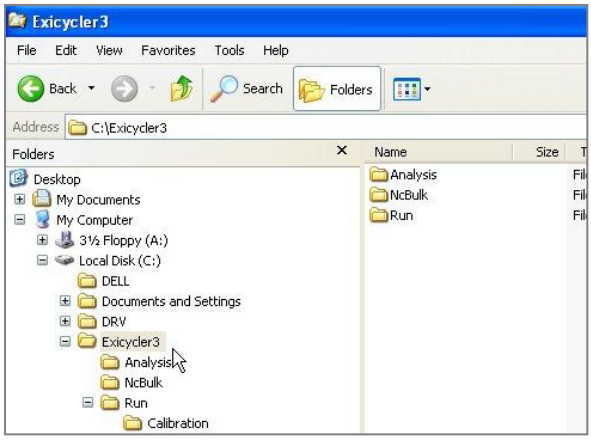


5) When the installation is complete, click **Finish** to finish the Installation Shield Wizard.

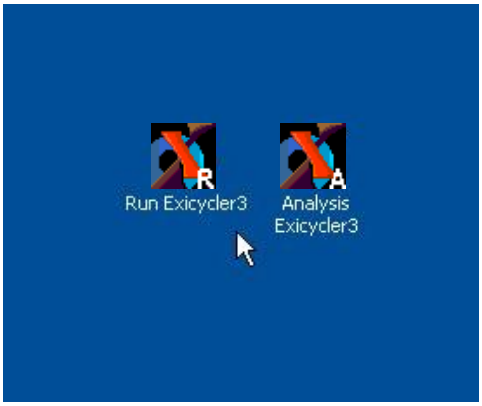


6) Connect the Exicycler™ 96 to the computer when the installation is complete.

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



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



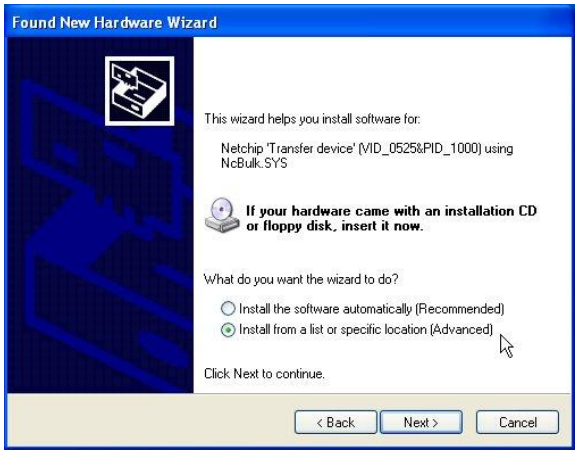
9) Check the USB cable connection between the Exicycler™ 96 and the computer.

10) Power on the Exicycler™ 96. A power buttonLED turns red when the power is supplied properly.

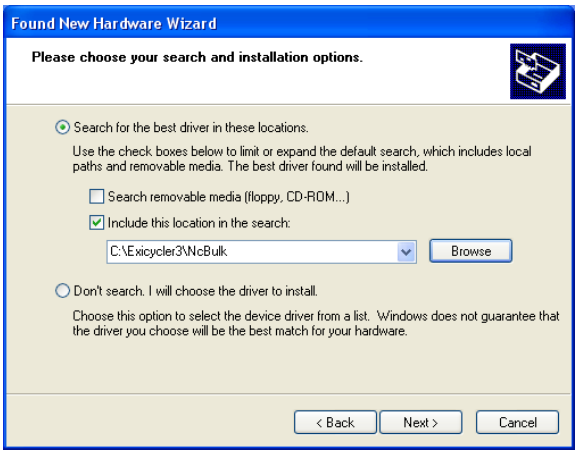
11) Press the power button again for a full second to start Self-diagnosis. The POWER button LED starts blinking in green after two short beeps when the self-diagnosis is complete.

12) When powering on the Exicycler™ 96, 'Found New Hardware Wizard' will appear. Select **Yes, this time only**, then **Next**.

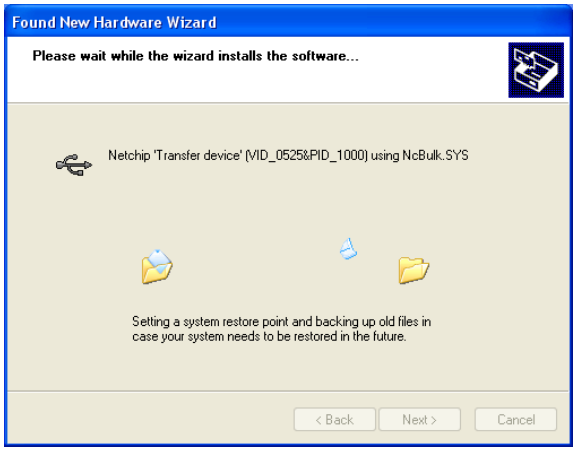
13) Select '**Install from a list or specific location (Advanced)**', then click **Next**.



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

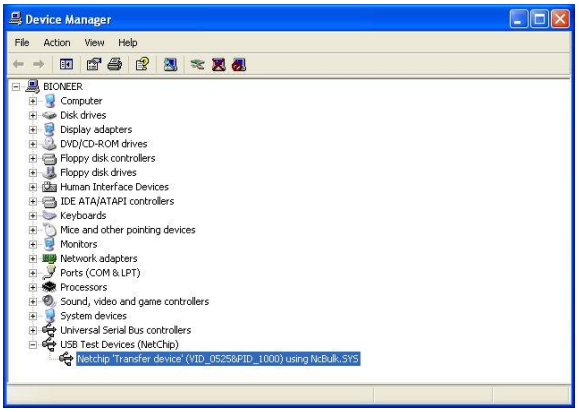


15) The installation starts.

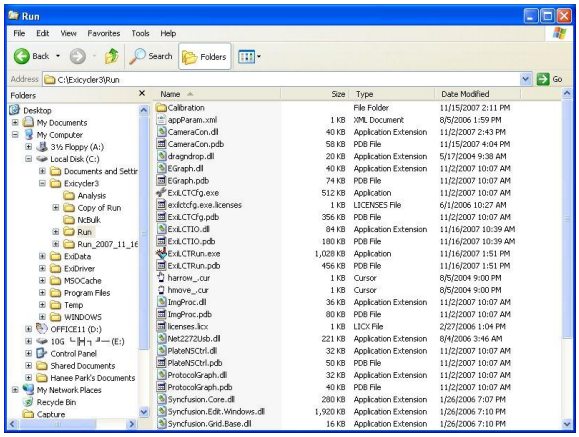


16) When the driver completes the installation, click **Finish**.

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



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



System Operation and Calibration Preparation

BIONEER CORPORATION

System Operation and Calibration Preparation

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

Powering On and Self-diagnosis

1) Power on the Exicycler™ 96 by switching on a power supply button in the rear of the instrument. When power is supplied properly, a POWER button LED turns red.

2) Press the **POWER** button for a second to start self-diagnosis.

IMPORTANT:

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

NOTE:

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

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

Cautions:

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

Reagents and Consumable Products

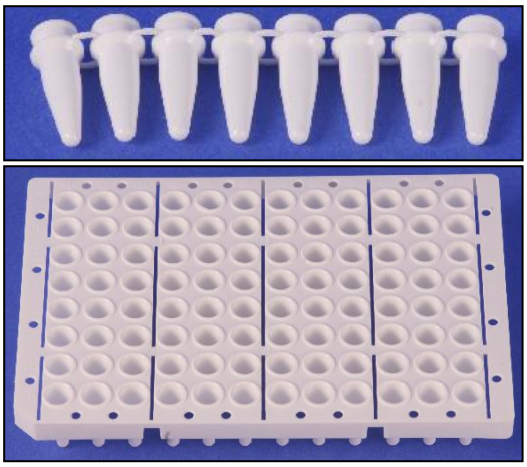
Various reagents and kits are required to perform a Real-Time PCR in the Exicycler™ 96 Real-Time Quantitative Thermal Block. Please refer to this section to obtain detailed information about the appropriate reagents and kits for your experiments. For ordering, please go to the 8. Ordering Information.

PCR Premix Kit

AccuPower® Greenstar™ PCR PreMix Kit allows an easy and fast amplification in Exicycler™ 96. The *AccuPower*® Greenstar™ PCR PreMix Kit consists of Greenstar® fluorescent dye, HotStart Taq DNA polymerase, and all of the PCR components. Therefore, add just template, primers, and $MgCl_2$ to start your amplification.

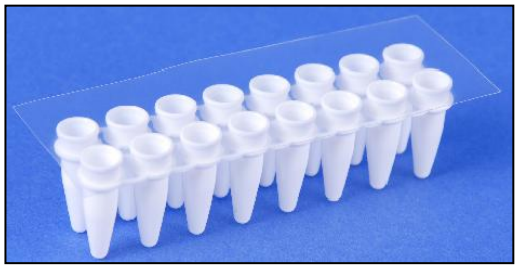
Optical 0.2 ㎖ 8-strip tube and 96-well plate

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



Optical sealing tape

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



Caution:

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

 Warning: Chemical hazard

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

Calibration Using ExiCfg

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Calibration Using ExiCfg

Prepare to perform the calibration with a Calibration Kit (A-2060-1). The calibration kit is designed for the Exicycler™ 96 Real-Time Quantitative Thermal Block (Version 3.0) and is not applicable to other Real-Time instruments. The calibration kit is comprised of 11 plates, a mask calibration plate, a background calibration plate, multi-channel calibration plates (9 different optical calibration plates of filter sets) present in the Exicycler™ 96. The calibration kit is available for reuse up to 3 times. For more information about the calibration kit, please see the following:

Before Starting Calibration with the Calibration Kit

Centrifuge

Disposable plastic gloves

Calibration Kit (A-2060-1)

Calibration Plate Preparation

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

1) Take out the Calibration Kit from the freezer.

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

3) Allow the pack to warm to room temperature for at least 10 minutes.

4) Take out a calibration plate from its packaging.

IMPORTANT:

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

NOTE:

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

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

NOTE:

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

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

7) Put the calibration kit back into its packaging and return it to the freezer when the calibration is done.

IMPORTANT:

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

Calibration using ExiCfg

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

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

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

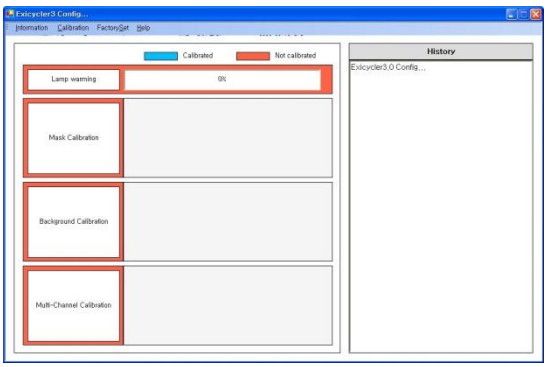
Mask Calibration

1) Power on the Exicycler™ 96 by pressing the **POWER** button.

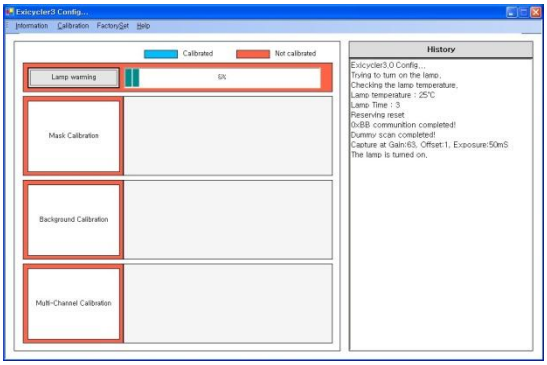
NOTE:

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

2) Go to ‘C:\Exicycler3\Run’ and double click **ExiCfg.exe**.



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.

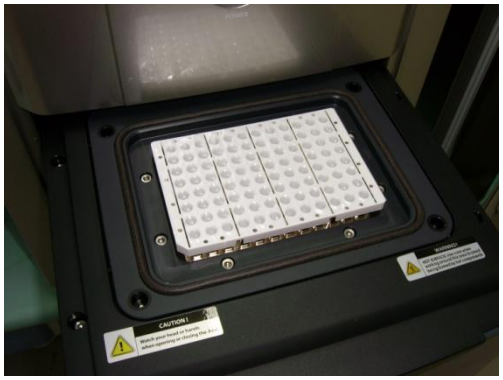


IMPORTANT:

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 5 minutes, turn off the Exicycler™ 96 and turn it on again after 5 seconds. In case of an abnormal shutdown like this, wait about 5 minutes, then click **Lamp warming** to turn on the lamp again. It takes approximately 10 minutes to reach the maximum light intensity there after. Therefore, wait until the progress bar of the **Lamp warming** finishes before proceeding further.

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

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



NOTE:

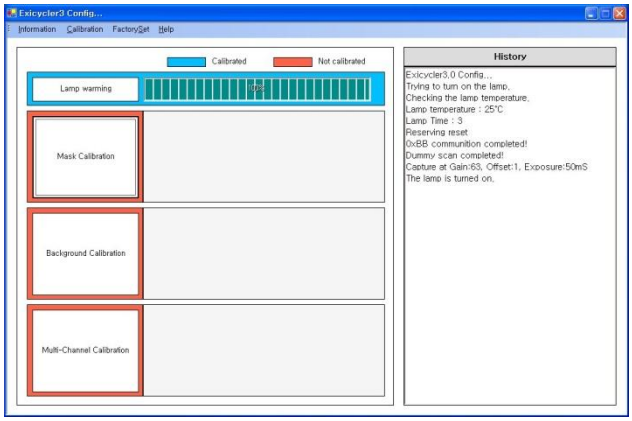
You must perform the Mask calibration first prior to other calibrations.

IMPORTANT:

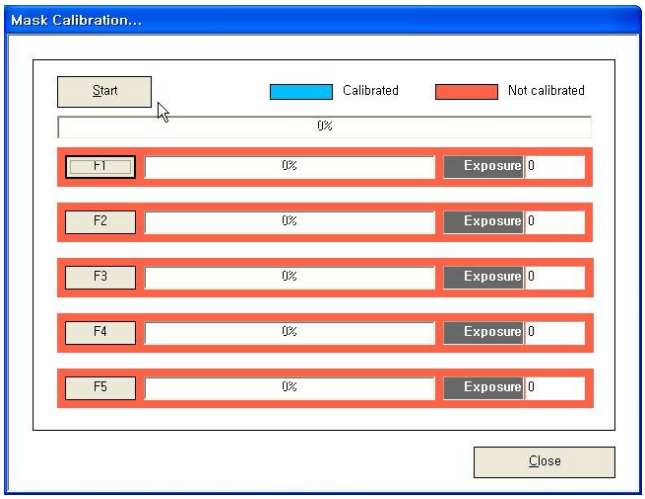
It is important to load the calibration plate in the right position. A misplaced plate may cause damage or technical problems.

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

7) Click **Mask Calibration** button.

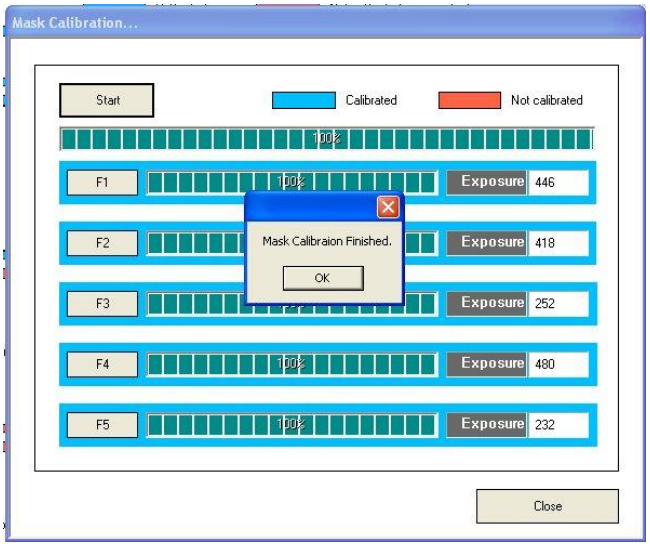


8) Click **Start** button in the 'Mask Calibration' window.



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

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



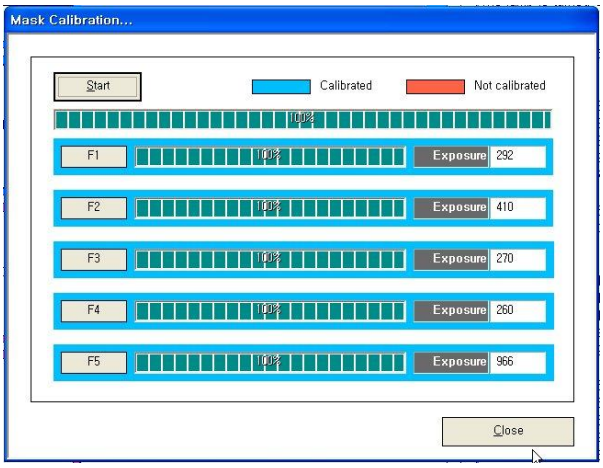
NOTE:

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

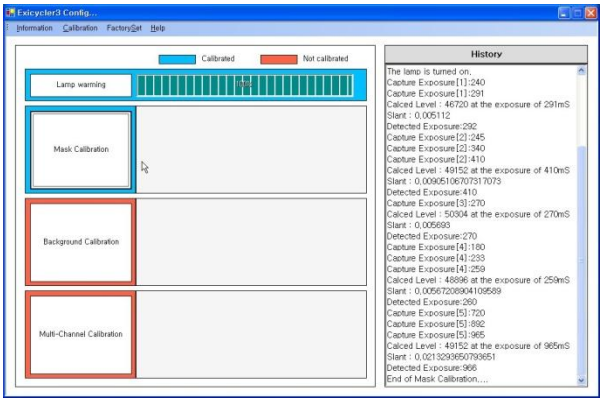
Warnings: High temperature

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

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

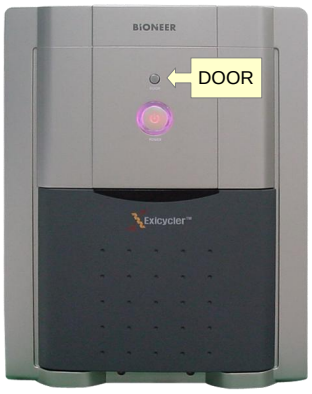


11) The ‘Mask Calibration’ frame will turn blue when the calibration is successfully complete.



Background Calibration

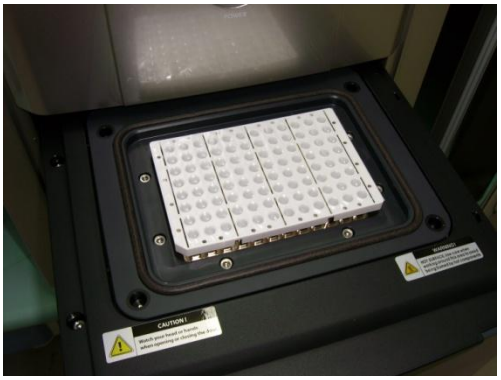
1) Press the **DOOR** button to open the door.



NOTE:

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

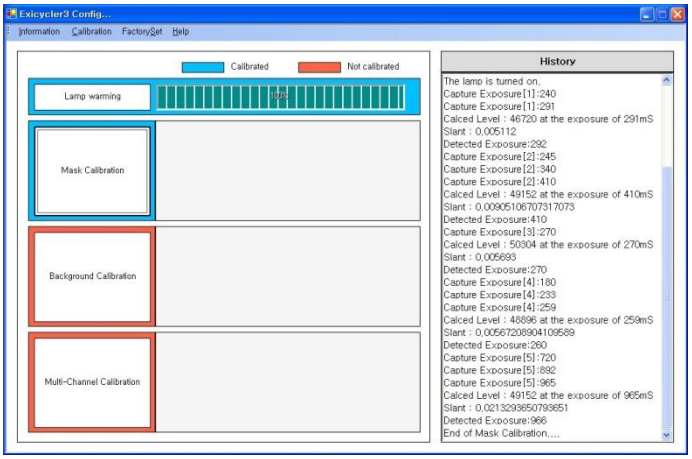
2) Place the Background calibration plate with the A1 position at the top-left of the block.



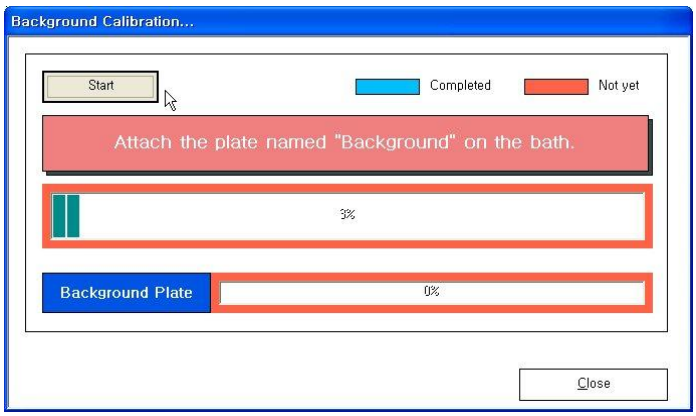
IMPORTANT:

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

3) Click **Background calibration** button.

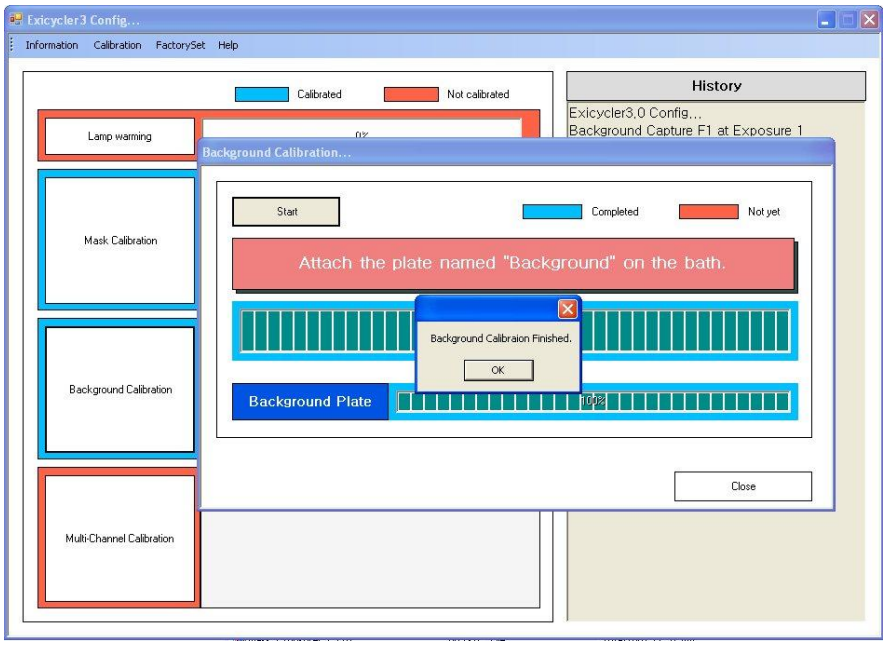


4) Click **Start** in the 'Background Calibration' window.



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

5) When the background calibration is complete, click **OK**.

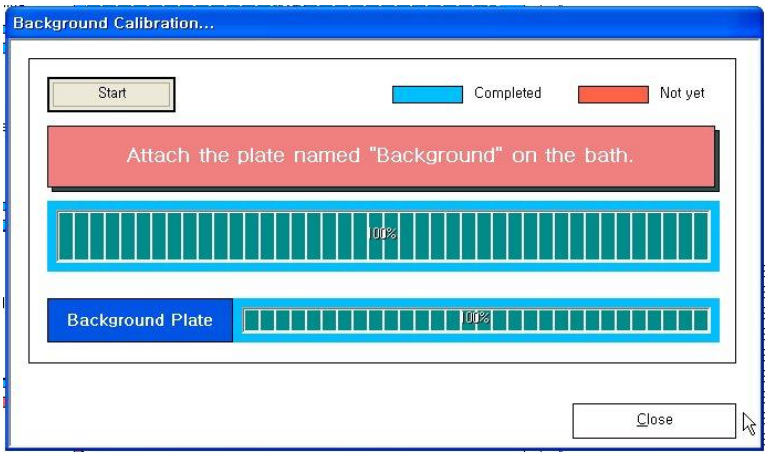


NOTE: The door will open when the background calibration is successfully complete. Put the plate back into its packaging and return it to the freezer.

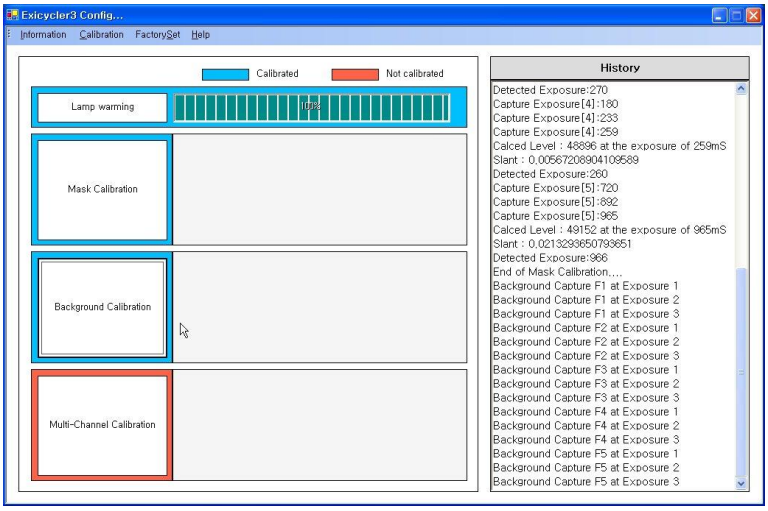
Warnings: High temperature

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

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

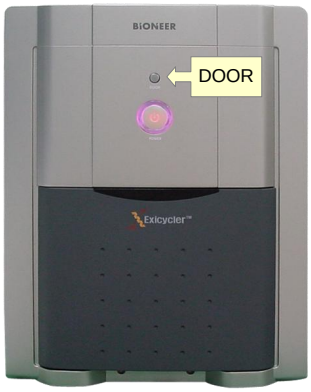


7) The 'Background Calibration' frame will turn blue when the calibration is successfully complete.



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.

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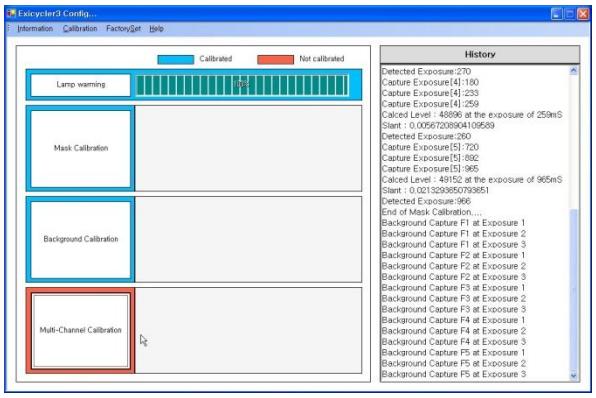
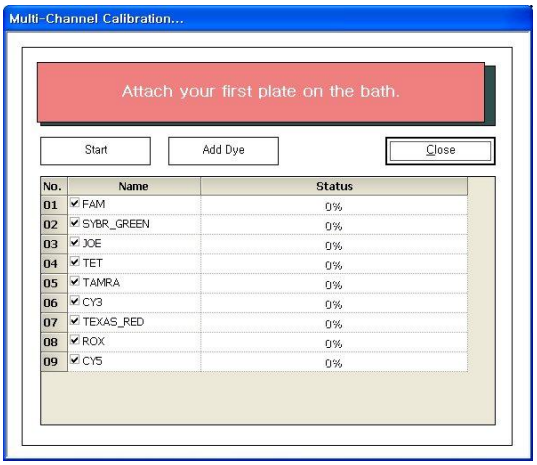
2) Click **Multi-Channel Calibration** button.



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



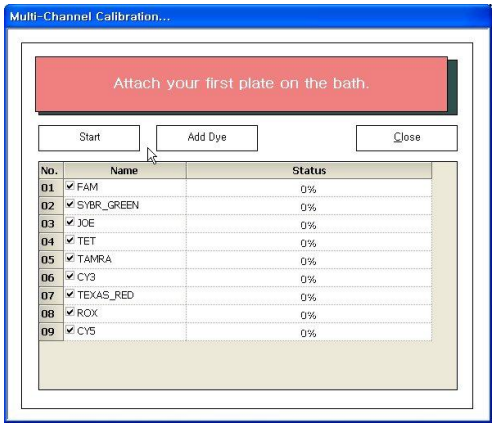
NOTE:

9 dyes are available for calibration.

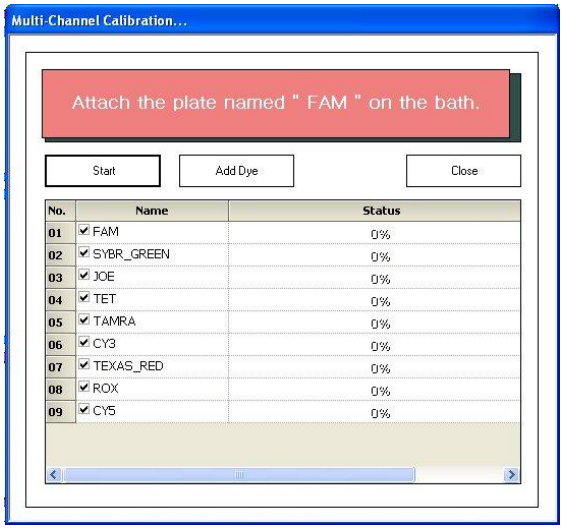
NOTE:

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

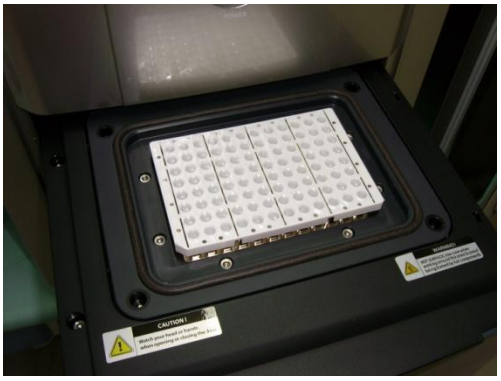
4) Click **Start**.



5) Prepare the calibration plate with a dye **X** (i.e. “FAM”) when ‘Attach the plate named ‘**X**’ (i.e. “FAM”) on the bath’ message appears.



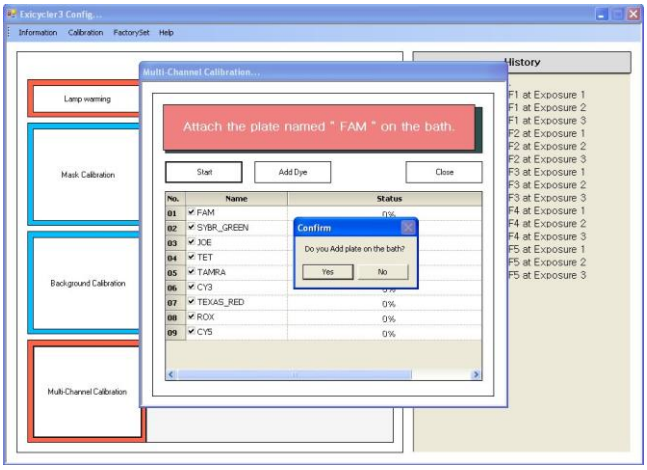
6) Place the **Multi-Channel calibration plate** with the A1 position at the top-left of the block.



IMPORTANT:

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

7) Click **Yes** in the 'Confirm' window.



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

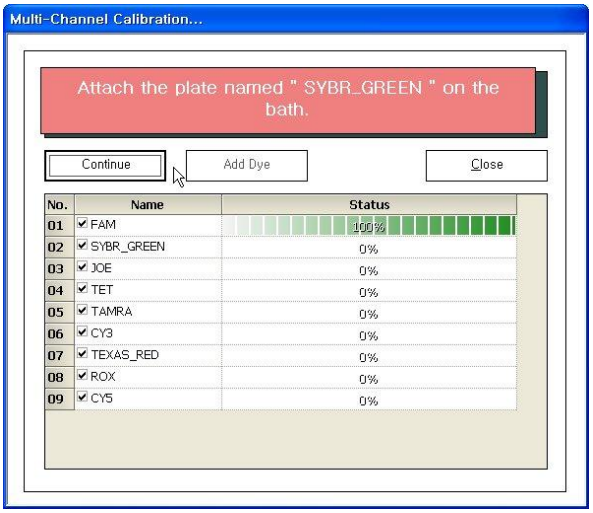
8) The door will open when the calibration is successfully complete.

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

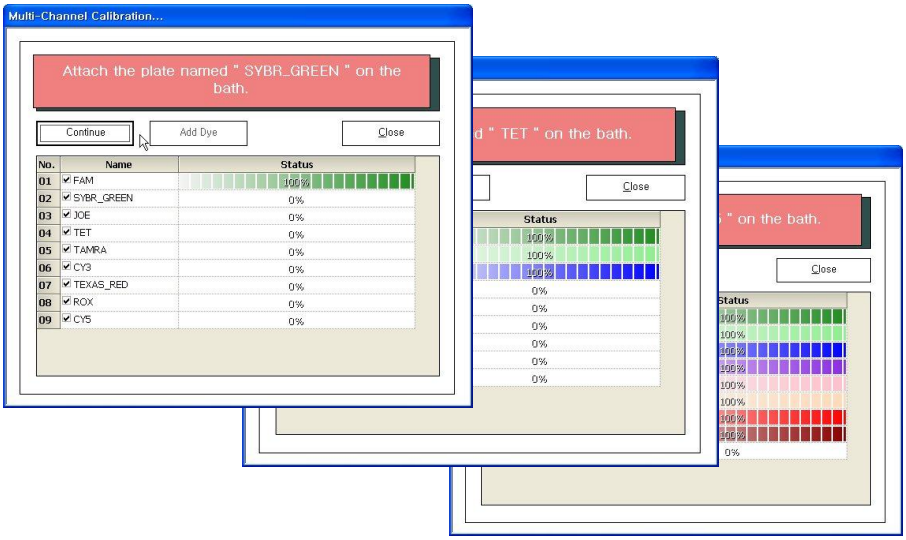
Warnings: High temperature

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

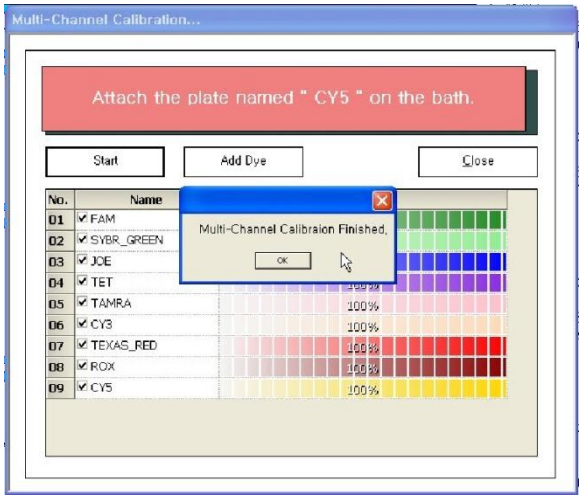
9) Prepare the calibration plate with a dye **X** (i.e. “SYBR Green”) when ‘Attach the plate named ‘**X**’ (i.e. “SYBR Green”) on the bath’ message appears.



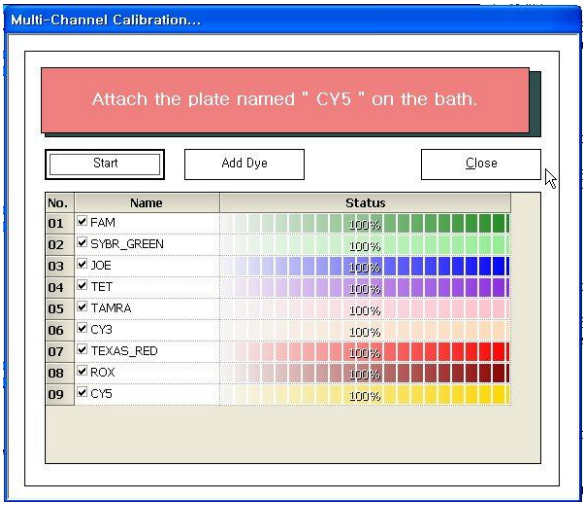
10) Click **Start** to repeat steps 4 through 8.



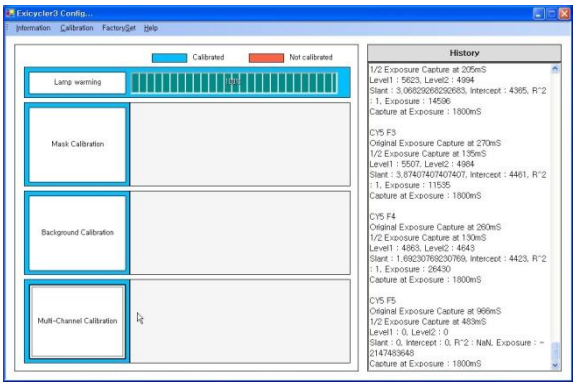
11) When the multi-channel calibration is complete, click **OK**.



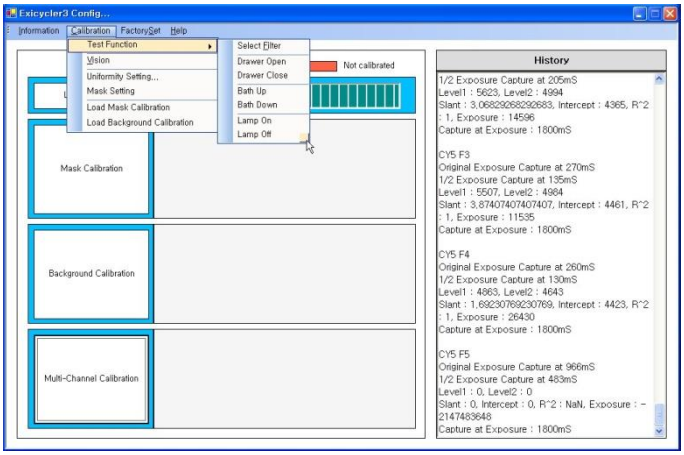
12) Click **Close** in the 'Multi-Channel Calibration' window.



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



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



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

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

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

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

Running Real-Time PCR Using Exicycle™ 96 Software

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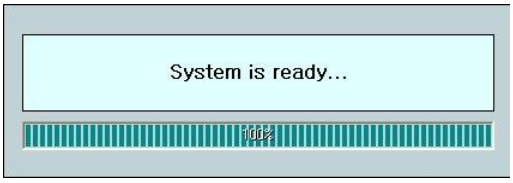
Starting 'Run Exicycler' software

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

2) **System Check** window will pop up.

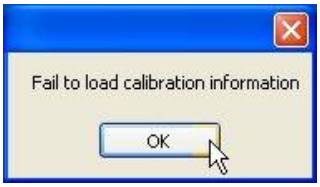


IMPORTANT:

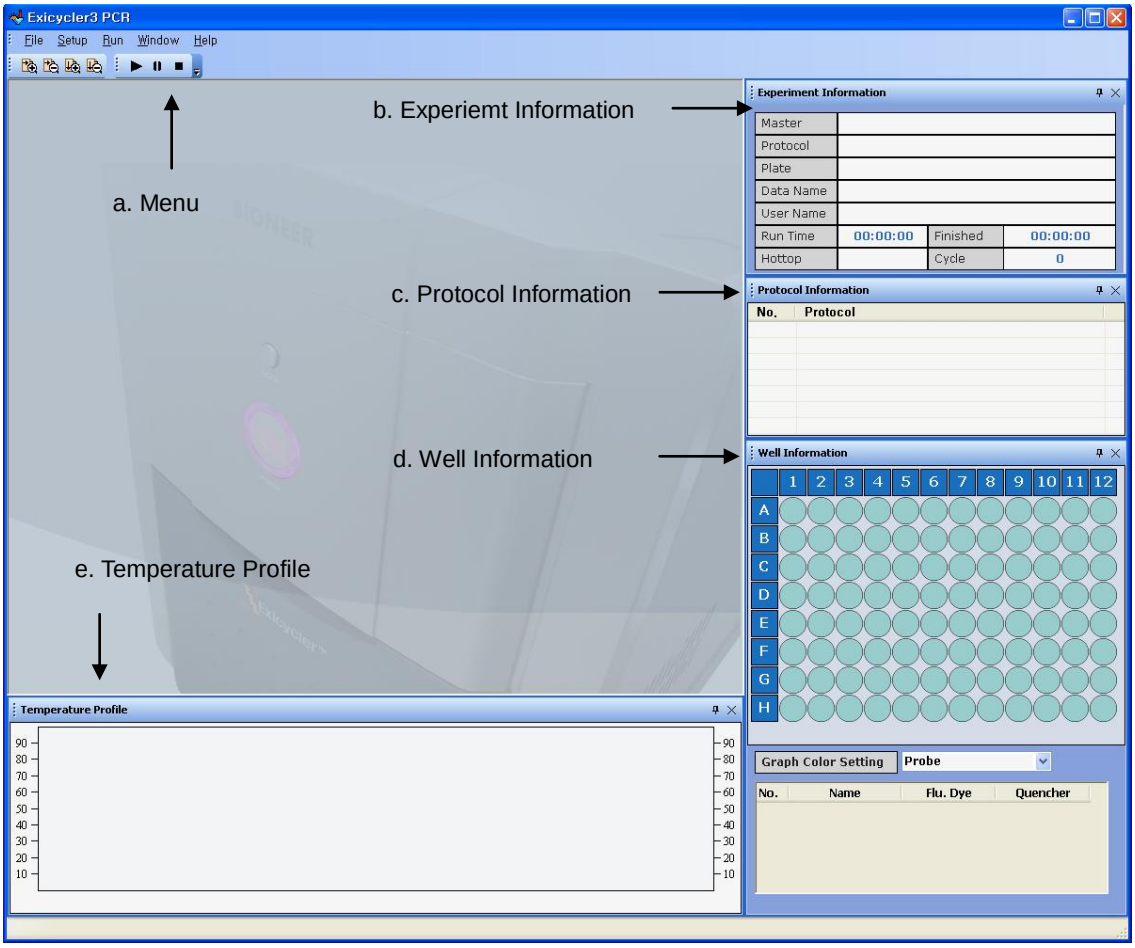
The following error message will appear if the Exicycler™ 96 is not powered on. Power on the Exicycler™ 96 by pressing the power switch in the back of the Exicycler™ 96 and double click **Run Exicycler3** on the desk top again.

IMPORTANT:

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



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



a. **Menu** consists of File, Setup, Run, Window, and Help.

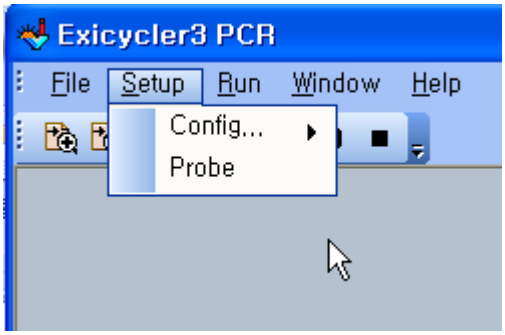
b. **Experiment Information** displays a file name, a user name, and elapsed and estimated finish time.

c. **Protocol Information** displays a cycling protocol in detail.

d. **Well information** displays sample and probe specification for each well.

e. **Temperature Profile** displays a temperature curve of the cycling protocol.

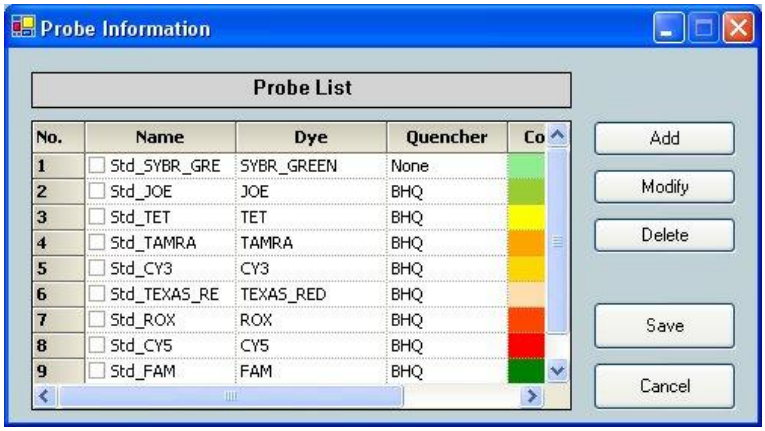
4) Go to **Setup > Probe** from the top menu.



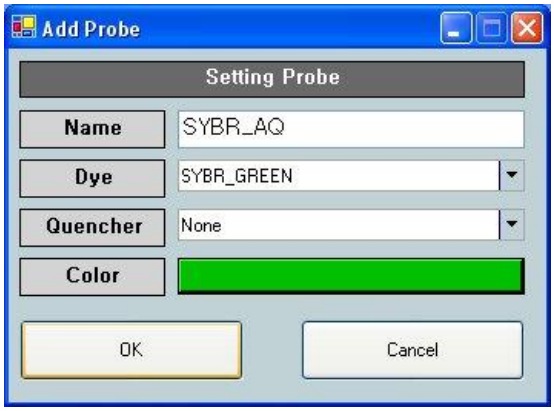
IMPORTANT:

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

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



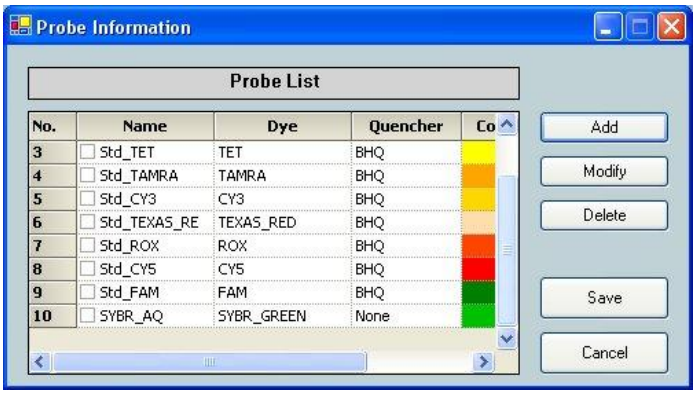
6) Enter SYBR_AQ in the **Name** field.



NOTE:

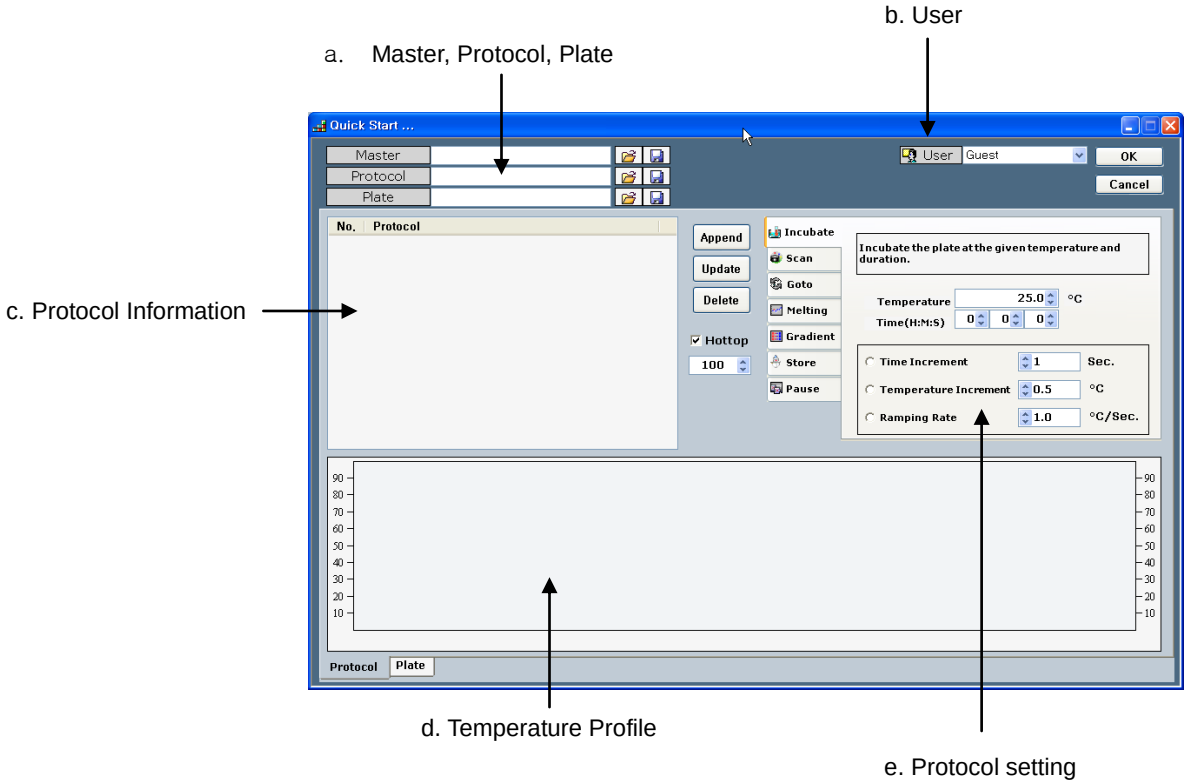
In the **Dye** drop-down list of the **Add Probe** window, select **SYBR_Green**. Select **None** in the Quencher drop-down list. Select your desirable color for display and then click **OK**.

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



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

9) **Quick Start** window will appear as follows:



a. **Master, Protocol, and Plate:** You can create, save, or open a file.

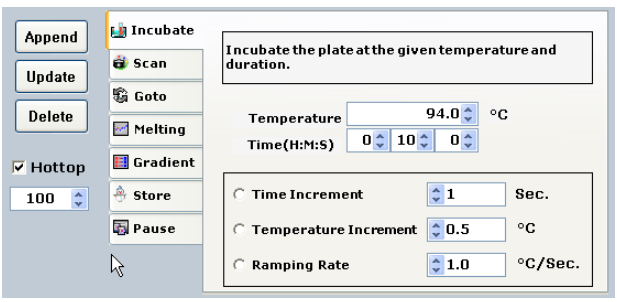
b. **User:** Select or create the user name for personal account management.

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.

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

NOTE:

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

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

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

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

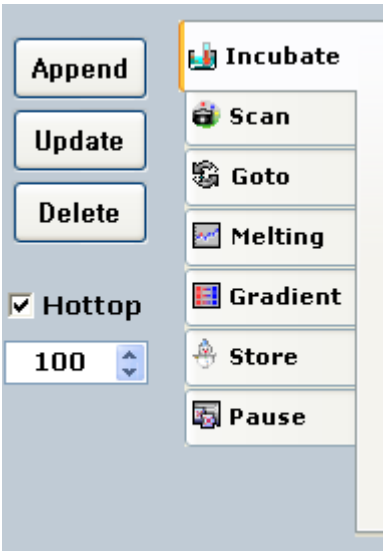
Hottop Check Box sets up a temperature for the heating lid within a range of 90°C to 110°C. The default temperature is 100°C. When the check box is unselected, the heating lid will not be heated.

11) Click **Append** to add the new step into the protocol.

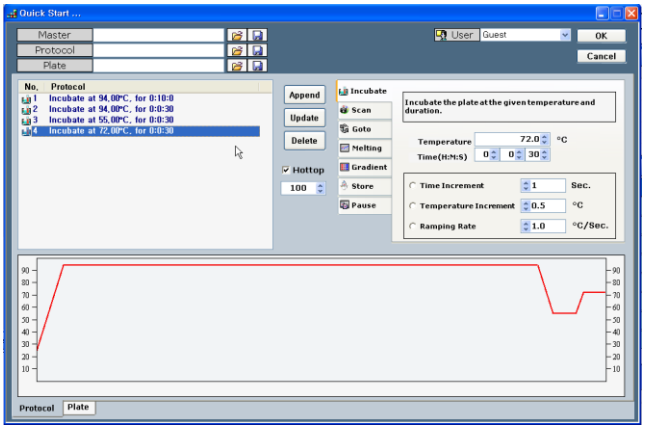
a. **Append** button is used when you want to add new steps into the protocol.

b. **Update** button is used when you want to make changes on steps.

c. **Delete** button is used when you want to delete steps from the protocol.



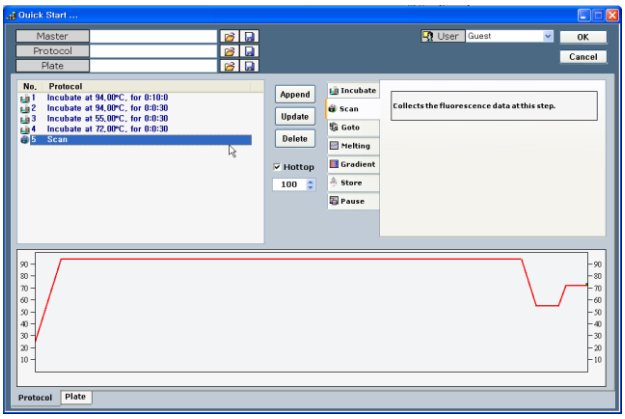
12) Click **Incubate** tab to add another Incubation step and then click **Append**. Repeat steps 2 through 4 if needed. (i.e. 94°C 30 sec / 55°C 30 sec / 72°C 30 sec).



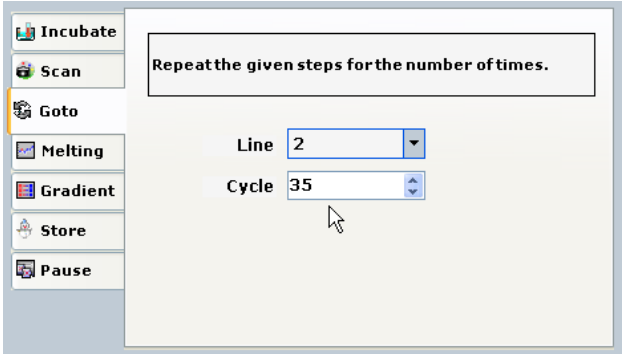
NOTE:

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

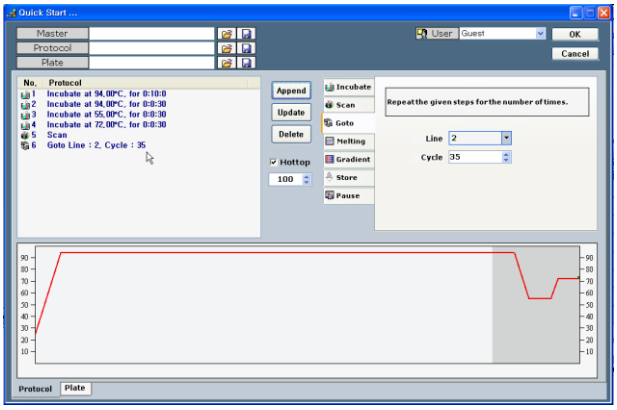
13) Click **Scan** tab and then **Append**.



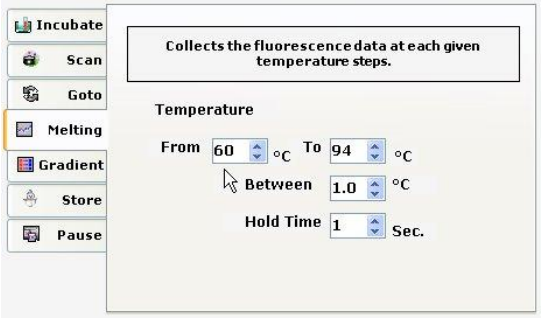
14) Click **Goto** tab and select a starting step (i.e. “2”) in the **Line** drop-down list. Enter a number of cycles (i.e. “35”) in the **Cycle** field, and then click **Append**.



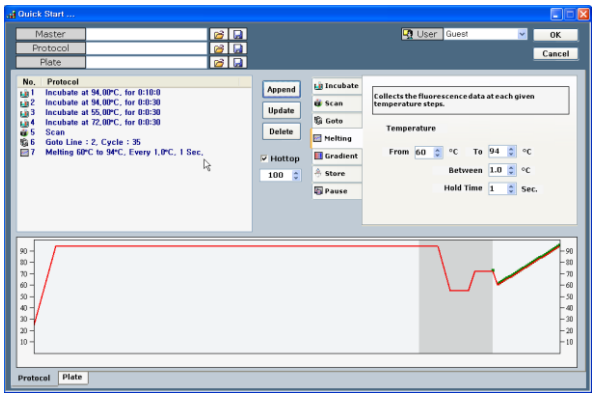
15) The protocol is created and saved. The **Temperature Profile** window displays an estimated temperature curve as follows:



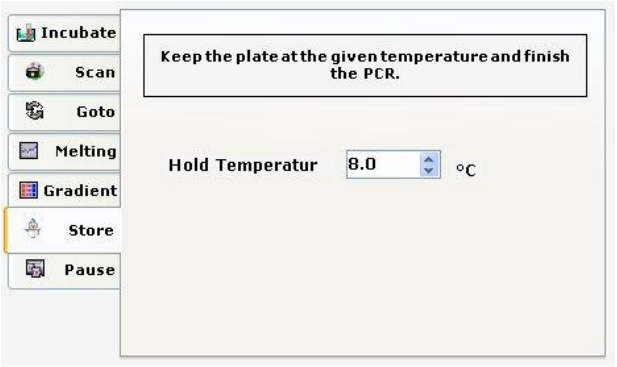
16) Click **Melting** tab to perform melting analysis. Enter the starting temperature in the **From** filed (i.e. “60”), the ending temperature in the **To** field (i.e. “94”), a temperature interval in the **Between** field (i.e. “1”), and a hold time in the **Hold Time** field (i.e. “1”).



17) Click **Append**.



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



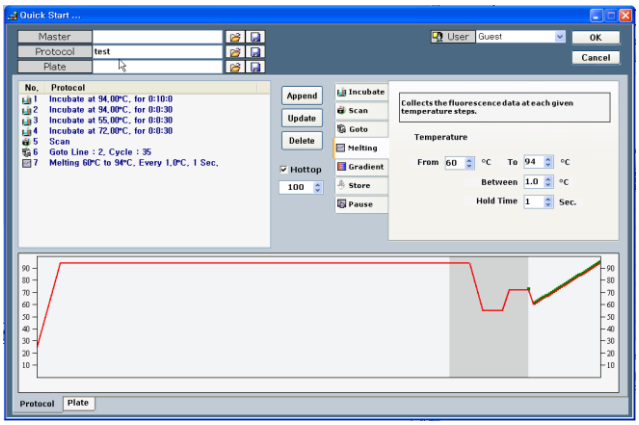
NOTE:

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

IMPORTANT:

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

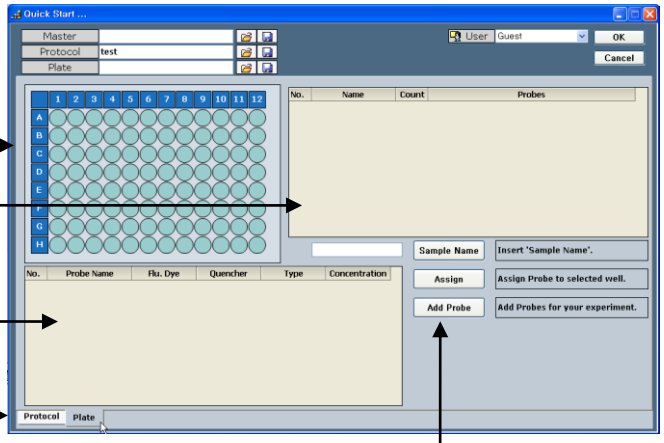
19) Enter a protocol file name in the **Protocol** field at the top left (i.e. “Test”) and then click  button to save the protocol.



NOTE:

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

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



a. **96-well plate position** specifies well locations for samples.

b. **Name/Count/Probe** displays a name, probe information for each sample.

c. **Probe Name/Flu. Dye/Quencher/Type/Concentration** specifies a name, a fluorescence dye, a quencher dye, a type, and a concentration for each sample.

d. **Protocol/Plate tab** switches between the **Protocol Information** and the **Plate Information** windows.

e. **Sample Name** enters a sample name.

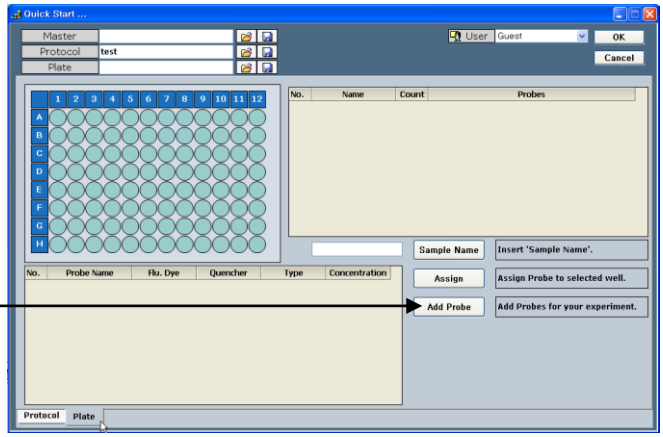
Assign specifies the information of each well such as probe and type.

Add Probe adds the probe to use in experiment.

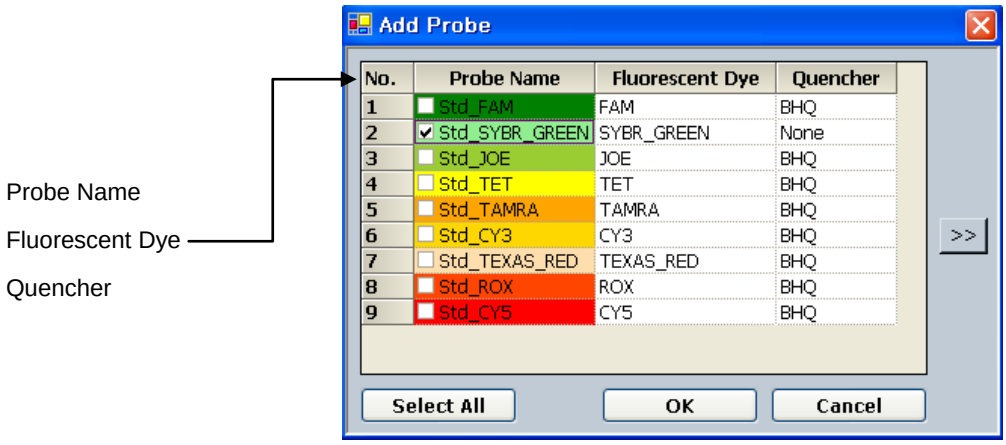
NOTE:

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

21) Click **Add Probe**, the Add Probe window will appear.



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

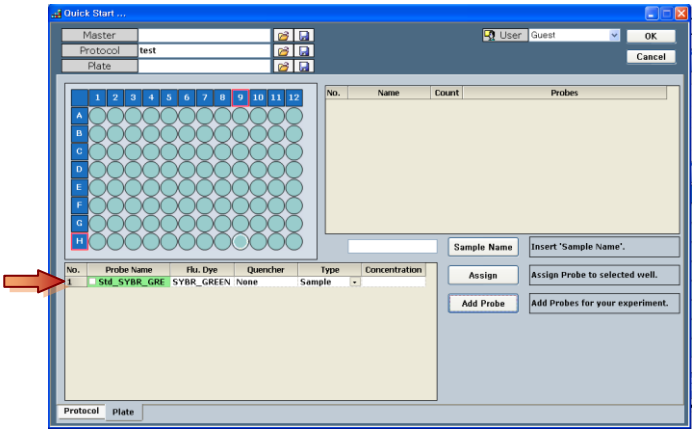


a. Probe Name: displays the name assigned by the user.

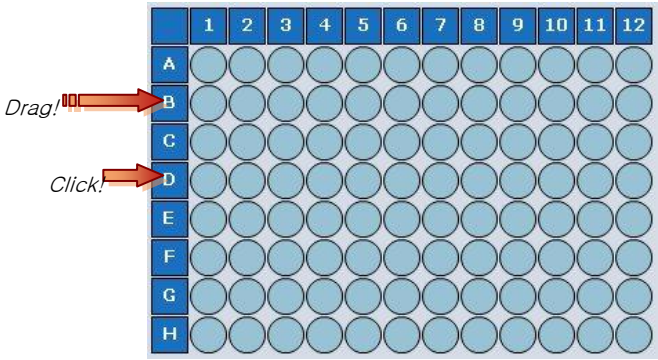
b. Fluorescent Dye: displays a name of fluorescence dye assigned by the user.

c. Quencher: displays a name of a quencher assigned by the user.

23) The probe is loaded and shown as follows:



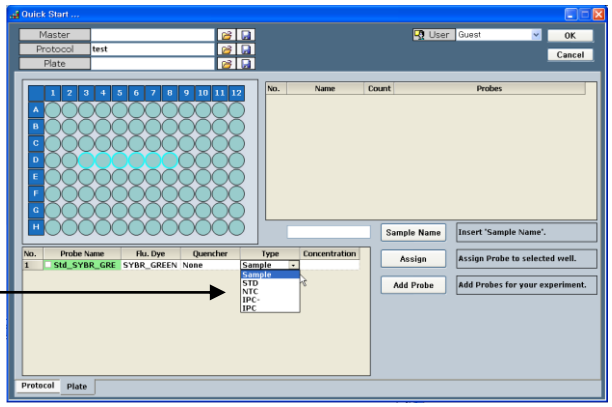
24) Select wells from the 96-well plate diagram.



NOTE:

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

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



a. **Sample** represents unknown samples.

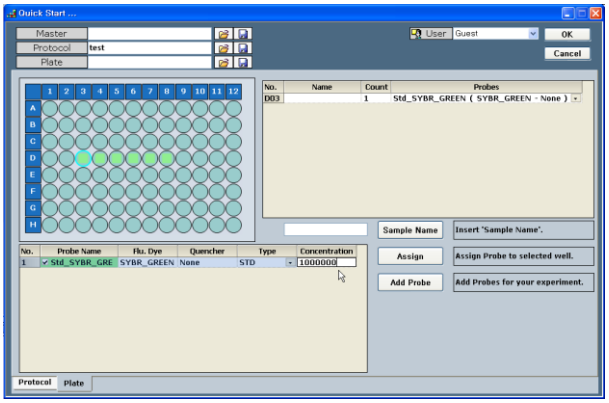
b. **STD** stands for a standard sample of a known concentration. Absolute Quantification

c. **NTC** stands for **N**o **T**emplate **C**ontrol and is a sample without a template.

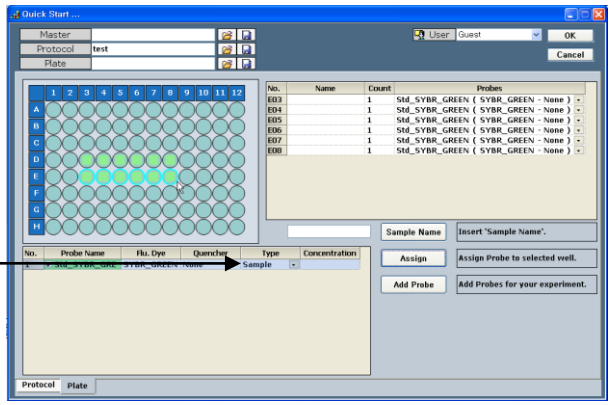
d. **IPC** stands for **I**nternal **P**ositive **C**ontrol and is a sample that monitors the PCR run during Existence / Nonexistence reaction. It will also diagnose the cause of the negative result of PCR.

e. **IPC-** is a sample that is used as a template and contains a reagent to prevent the IPC reaction during Existence / Nonexistence reaction. IPC- is not amplified during the Real-Time PCR.

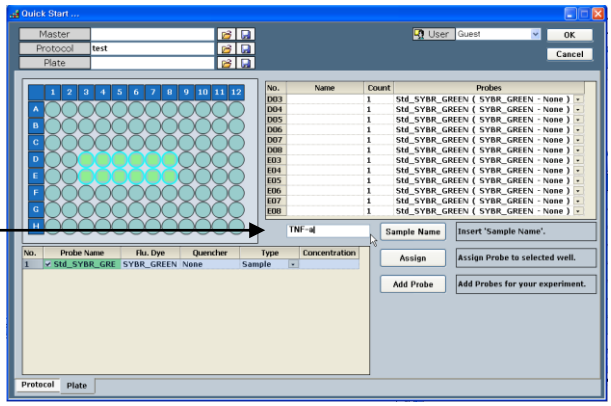
26) Click the well D3 and enter concentration. For example, enter '1000000' for a concentration of 10^6-copy and then click **Assign**. Repeat this for wells D4 through D8 with concentrations of serial diluted standards.



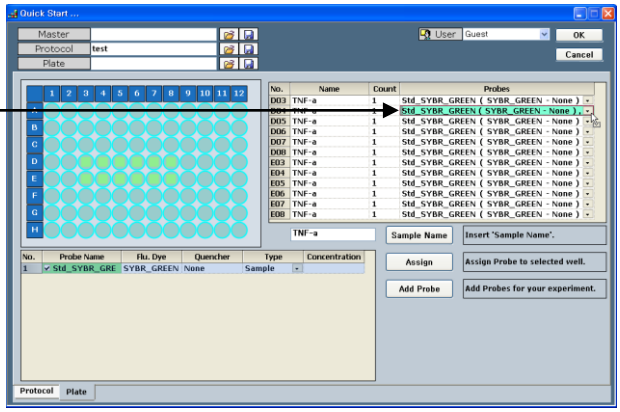
27) Select wells E3 through E8 in the plate diagram. Select **Sample** in the **Type** drop-down list, and the click **Assign**.



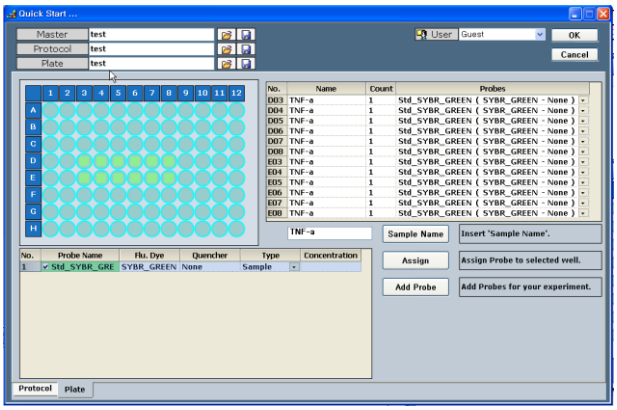
28) Select cells D3 through E8 and enter a sample name in the **Sample Name** filed. Click **Sample Name** to save the sample name.



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



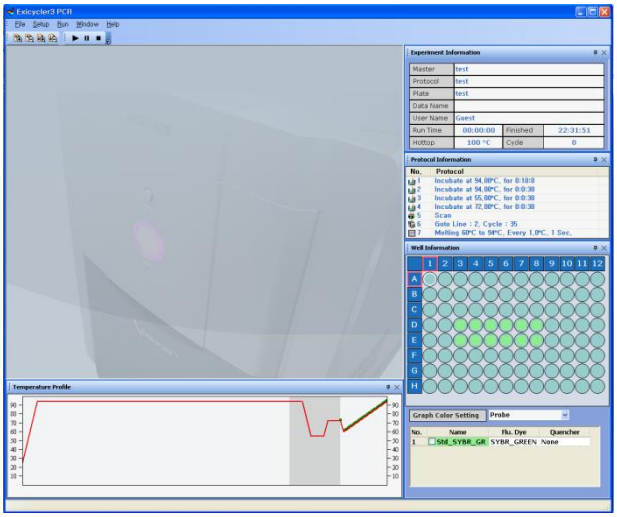
30) Enter a plate name in the **Plate** field (i.e. Test) and then click button to save the plate file.



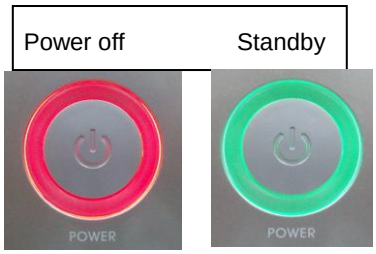
31) The protocol file and plate file are saved. Enter a master file name in the **Master** field (i.e. Test) and then click button to save the master file.

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

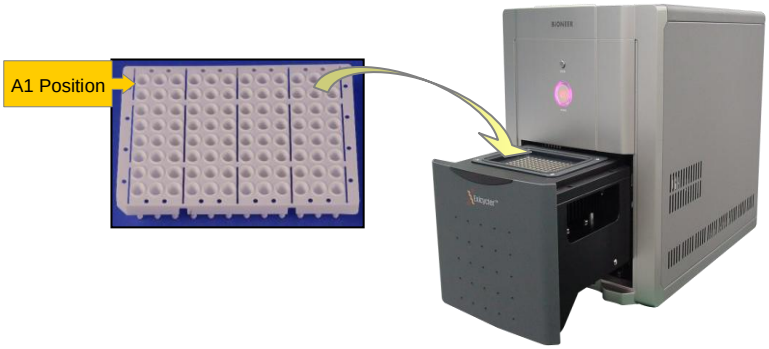
32) Click **OK** at the top right to display the go back to the main window.



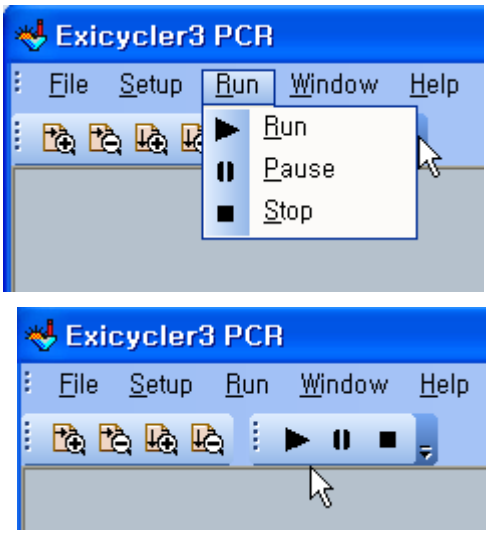
33) Ensure the **POWER** button in the front of the Exicycler™ 96 is blinking in green.



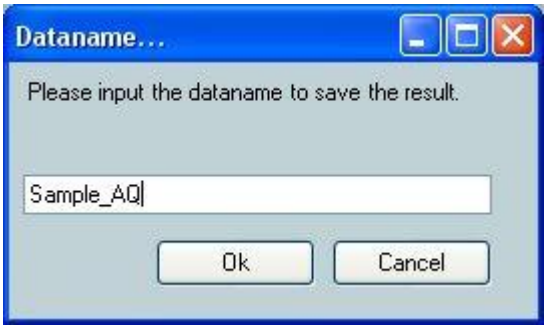
34) Press the **DOOR** button for a full second to open the door. Load the 96-well plate with samples with the A1 position at the top-left of the block.



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

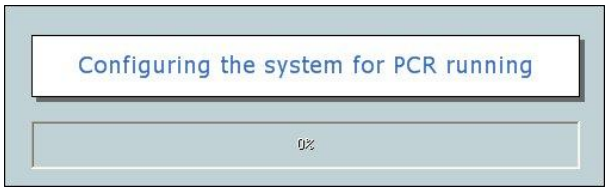


36) Enter a data name (i.e.Sample_AQ) in the **Dataname** window and click **Ok**. If you do not enter the data name, default name will be used.

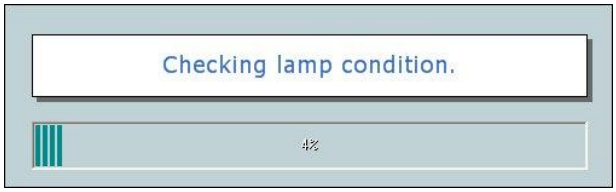


37) The following three message boxes will appear in the following order.

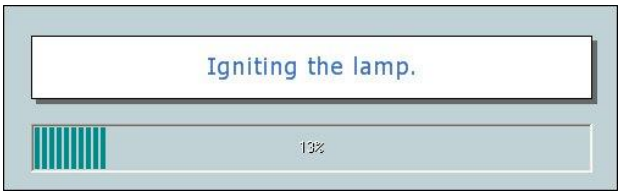
a. The message will appear when closing the door of the Exicycler™ 96 if the door was open.



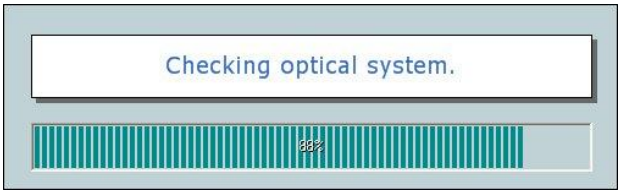
b. The message will appear when checking the lamp condition.



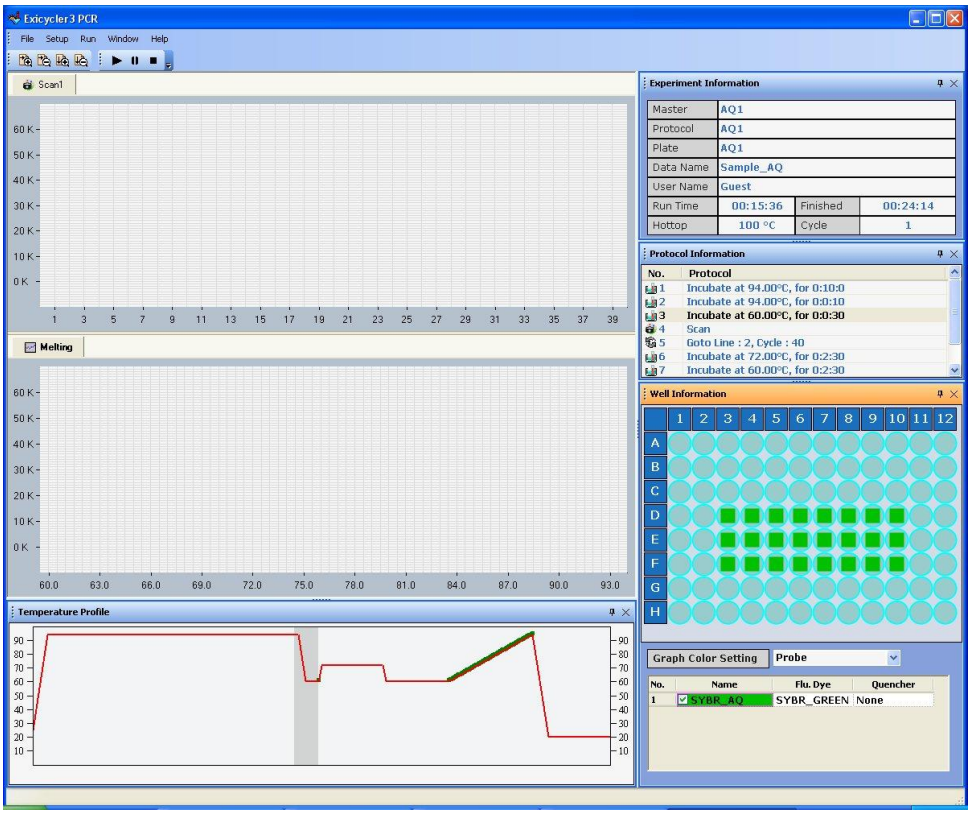
c. The message will appear while the lamp is turning on.



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



38) 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.

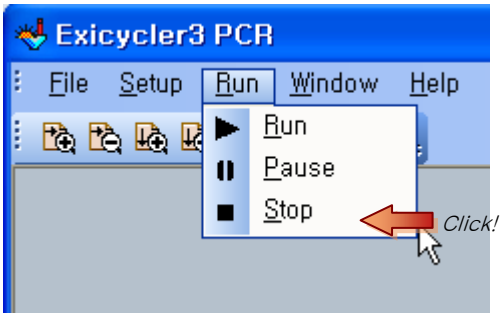
c. **Temperature Profile** displays a temperature profile and in real time

d. **Experiment Information** displays information for the experiment currently in progress.

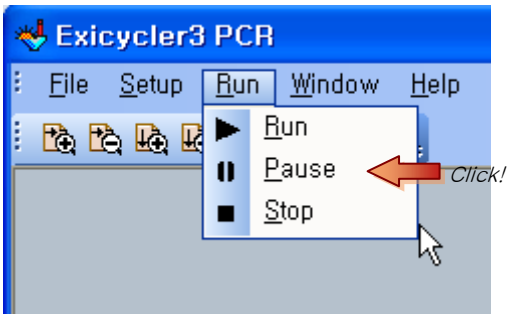
e. **Protocol Information** highlights the step currently running.

f. **Well Information** displays set up details for each well when selecting either **Probe** or **Type** in the **Graph Color Setting** drop-down list. To display the curve of amplification, select well from the 96-well plate diagram and click the check box from the probe option.

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



40) Go to **Run > Pause** to pause the Exicycler™ 96 or click the **Pause** button.



IMPORTANT:

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

IMPORTANT:

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

NOTE:

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

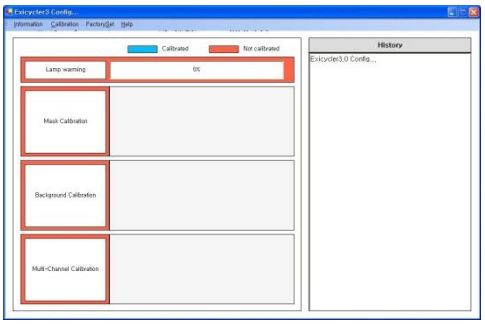
NOTE:

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

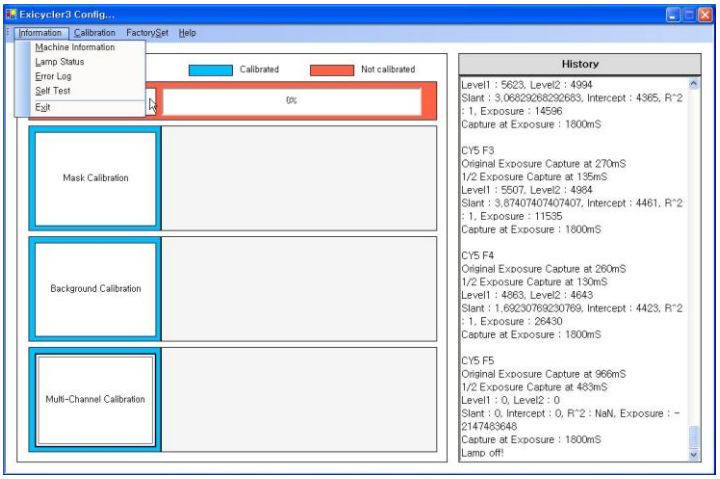
Performing System Diagnosis using ExiCfg

1) Power on the Exicycler™ 96 by pressing the **POWER** button.

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



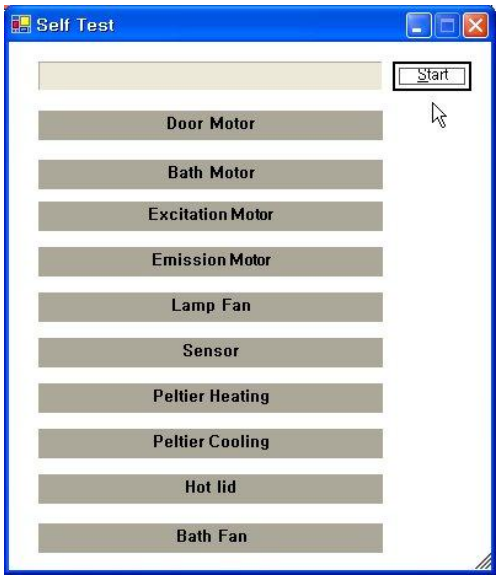
3) Go to **Information > Self Test**.



NOTE:

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

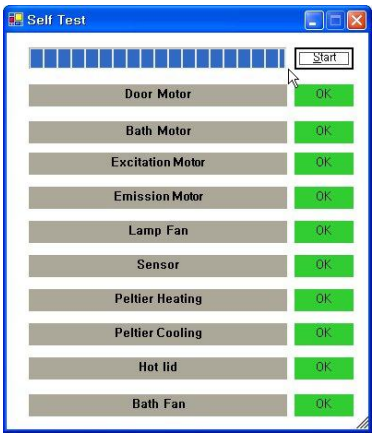
4) Click **Start** in the **Self Test** window.



IMPORTANT:

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

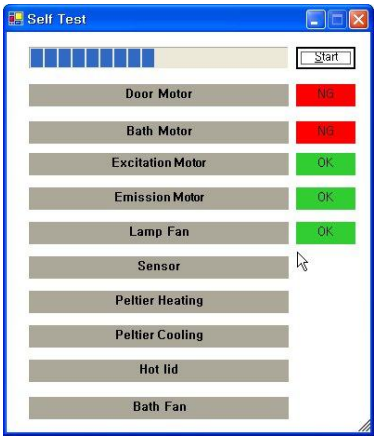
5) All of the self-diagnosis tests pass completely without any problems, **OK** signs will show up in green.



NOTE:

The self-diagnosis is complete with two short beeps.

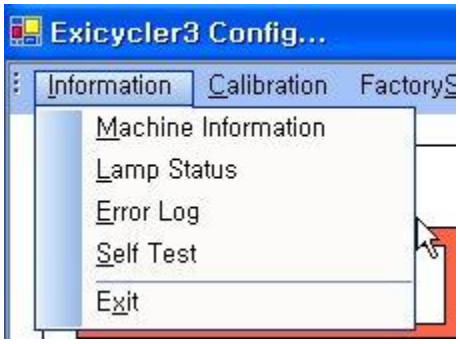
6) When any of the self-diagnosis tests fail, **NG** signs will appear in red.

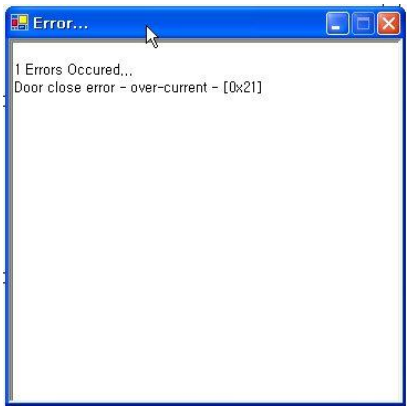


NOTE:

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.





Troubleshooting

Troubleshooting

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

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

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

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

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

Ordering Information and Warranty

Ordering Information and Warranty

Product	
A-2060	Exicycler™ 96 Real Time Quantitative Thermal Block
Kit	
K-6200	*AccuPower®* Greenstar™ qPCR PreMix, Exicycler 8-well Strip, 12 strips / 96 tests, 50㎕ reaction
K-6203	*AccuPower®* Greenstar™ qPCR PreMix, Exicycler 96-well Plate, 1 plate / 96 tests, 50㎕ reaction
K-6110	*AccuPower®* DualStar™ qPCR PreMix, Exicycler 8-well Strip, 12 strips / 96 tests, 50㎕ reaction
K-6113	*AccuPower®* DualStar™ qPCR PreMix, Exicycler 96-well Plate, 1 plate / 96 tests, 50㎕ reaction
A-2060-A1	Exicycler™ 96 Calibration Kit
Tube & Sealing Tape	
3111-50	0.2ml 8-strip PCR Tube for Real-Time PCR (opaque white)
3111-52	96-well Semi-skirted PCR Plate for Real-Time PCR (opaque white)
3111-41AB	Sealing Film for Real-Time PCR Plate

Warranty

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

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

1. How to request warranty service

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

2. Repairs under warranty

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

3. Exclusion from warranty

The product is excluded from warranty if:

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

Warranty Service Request Form

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

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