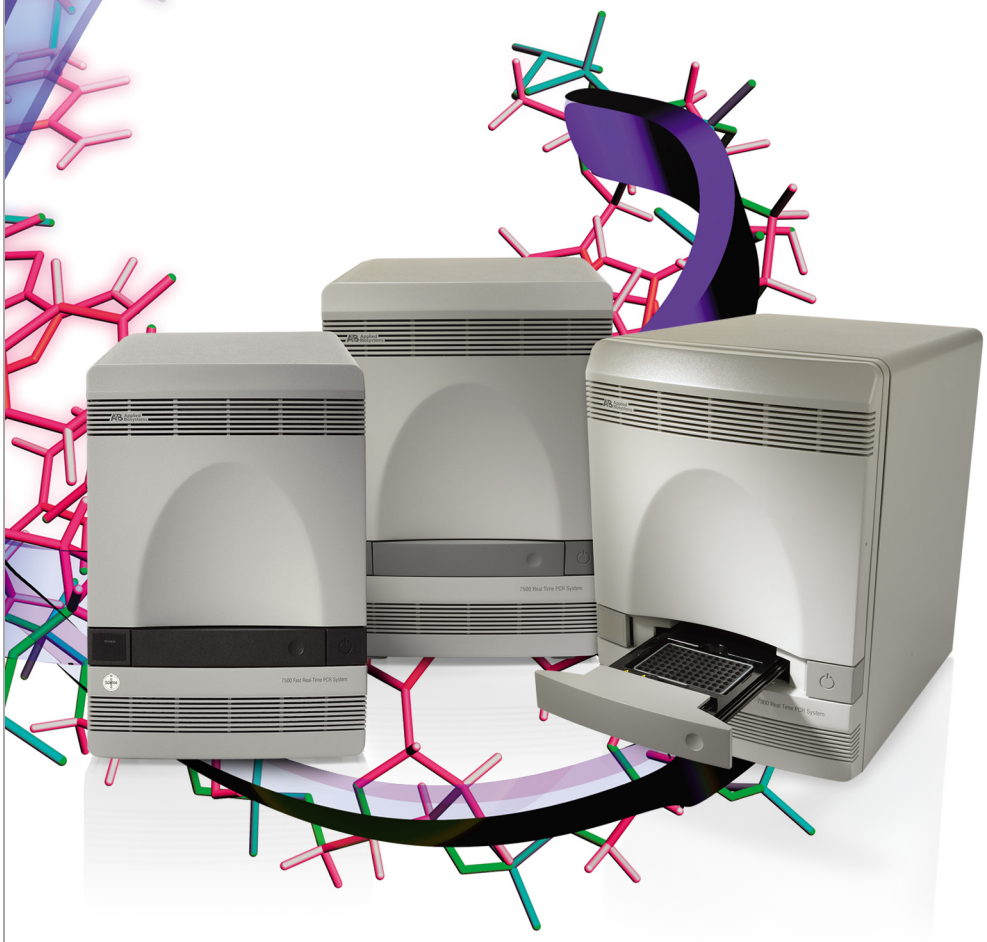
Applied Biosystems

Installation and Maintenance Guide



NOTICE TO PURCHASER:

PLEASE REFER TO THE APPLIED BIOSYSTEMS 7300/7500/7500 FAST REAL-TIME PCR SYSTEMS GETTING STARTED GUIDES (FOR ABSOLUTE QUANTIFICATION OR RELATIVE QUANTIFICATION) AND THE APPLIED BIOSYSTEMS 7300/7500/7500 FAST REAL-TIME PCR SYSTEMS GETTING STARTED GUIDES FOR ALLELIC DISCRIMINATION AND PLUS/MINUS DETECTION FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

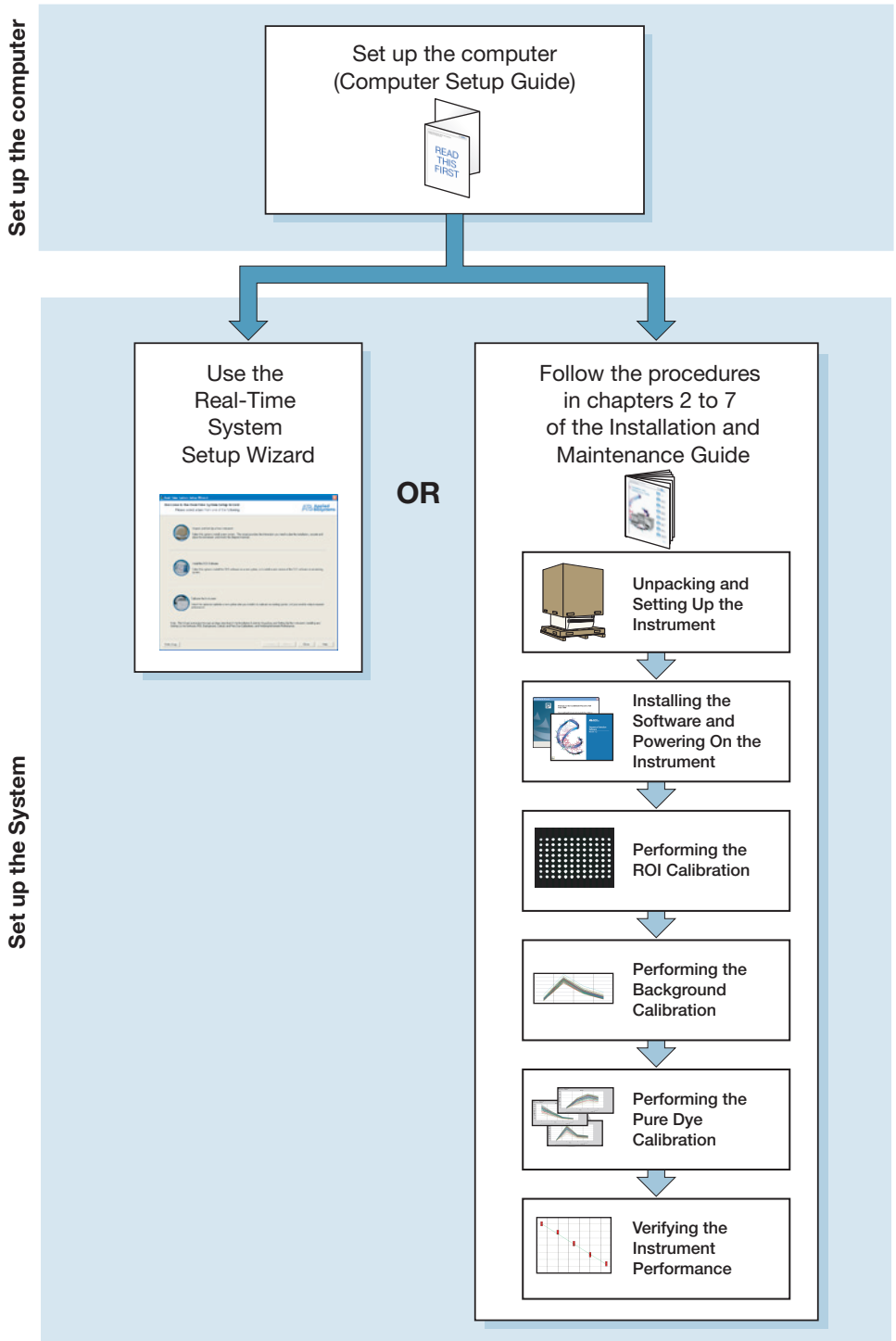
TRADEMARKS:

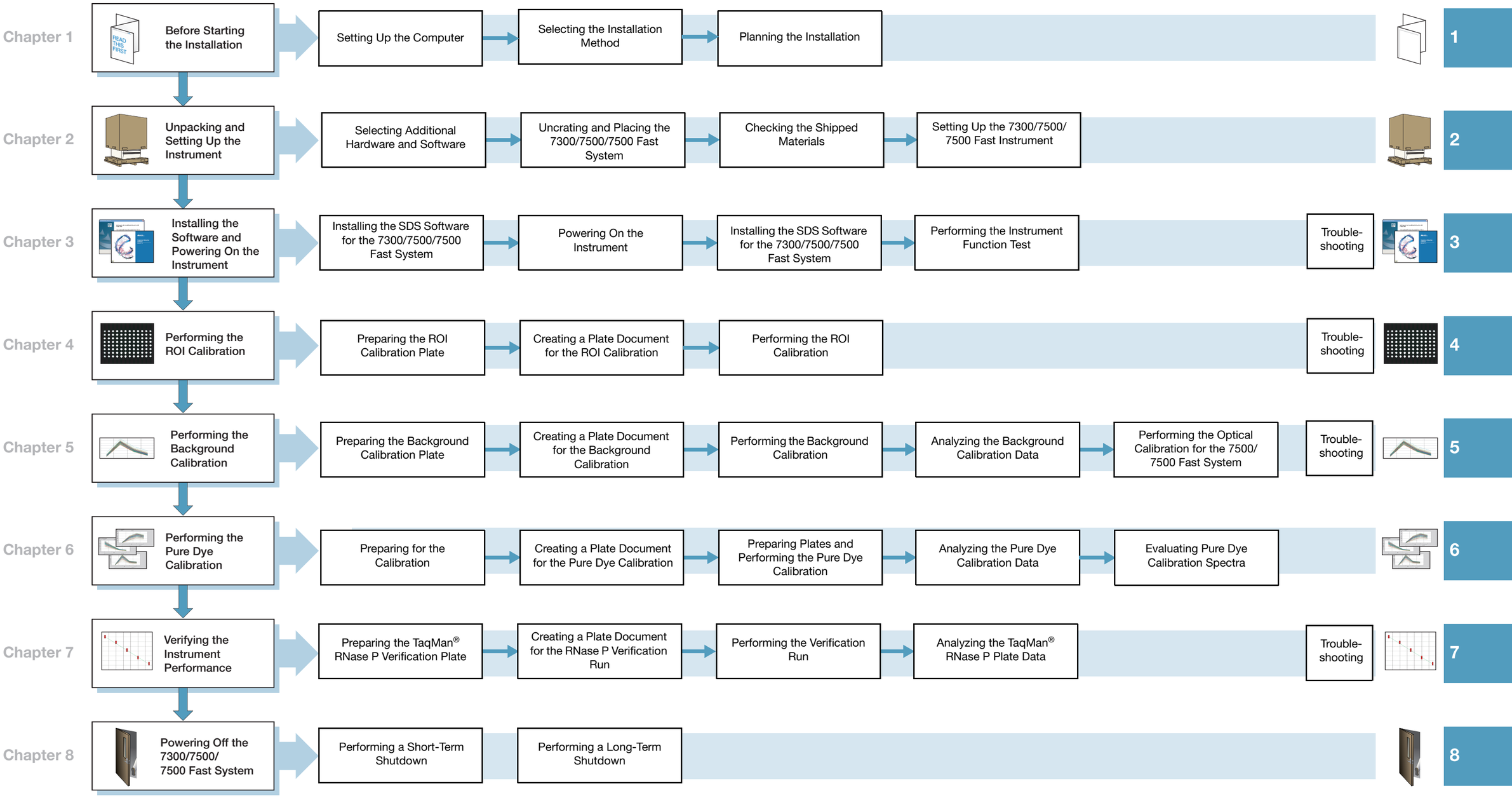
Part Number 4378657 Rev. A
09/2006

Read This First

Before you begin the installation:

To install the Real-Time PCR System:

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Preface

How to Use This Guide

Purpose of This Guide

This manual is written for principal investigators and laboratory staff responsible for installing and maintaining the Applied Biosystems 7300, 7500, and 7500 Fast Real-Time PCR Systems. This manual is designed to supplement the:

- *Applied Biosystems 7300/7500/7500 Fast System Computer Setup Guide.*
- Applied Biosystems Real-Time System Setup wizard, accessible from the Installation CD or by selecting start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.
- Applied Biosystems 7300/7500/7500 Fast System Setup and Maintenance Video CD provided with the instrument.

Audience

This guide is intended for novice and experienced Applied Biosystems 7300, 7500, and 7500 Fast Real-Time PCR System users who need to install, maintain, or troubleshoot their system.

Assumptions

This manual assumes that you:

- Are familiar with the Microsoft® Windows® XP operating system.
- Understand general techniques for preparing and handling DNA samples.
- Have a general understanding of hard drives and data storage, file transfers, and copying and pasting.

Text Conventions

This guide uses the following conventions to make text easier to understand:

- **Bold** indicates user action. For example:

  Type **0** and press **Enter** for the remaining fields.
- *Italic* text denotes new or important words and is also used for emphasis. For example:

  Before performing a run, you *must* calibrate the instrument by performing the ROI, background, and pure spectra calibrations.
- A right arrow (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select start ▸ **Programs** ▸ **Applied Biosystems** ▸ **SDS 1.3**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: Reset the Exposure Time to 2048 before performing the calibration for each filter.

IMPORTANT! Wear powder-free gloves when you handle the halogen lamp.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page xvi.

How to Obtain More Information

Related Documentation

For more information about using the 7300/7500/7500 Fast system, refer to the documents shown below.

Document Title	Online Help P/N	P/N
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Plus/Minus Detection Getting Started Guide	4347821	4378652
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Allelic Discrimination Getting Started Guide	4347822	4378653
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide	4347824	4378655
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantification Getting Started Guide	4347825	4378656
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Site Preparation Guide	4347823	4378654
Real-Time PCR Systems Chemistry Guide	4348358	4378658
Applied Biosystems 7500 FAST Real-Time PCR System, QRC	4362285	4378659
Applied Biosystems Real-Time System Computer Set Up Guide, QRC	4365367	4378660

Portable document format (PDF) versions of this guide and most of the documentation listed above are available on the SDS software installation CD.

Note: For additional documentation, see "How to Obtain Support" on page xiii.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Obtain worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

Safety and EMC Compliance Information

This section includes the following topics:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems instruments* (see "Safety Symbols" on page xvii).

Examples

The following examples show the use of safety alert words:

IMPORTANT! Wear powder-free gloves when you handle the halogen lamp.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description	Symbol	Description
	Indicates the **On** position of the main power switch.		Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates the **Off** position of the main power switch.		Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.		Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates the **On/Off** position of a push-push main power switch.		Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety *s*ymbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xviii). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Environmental Symbols on Instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **http://www.appliedbiosystems.com** for a list of customer service offices in the European Union.

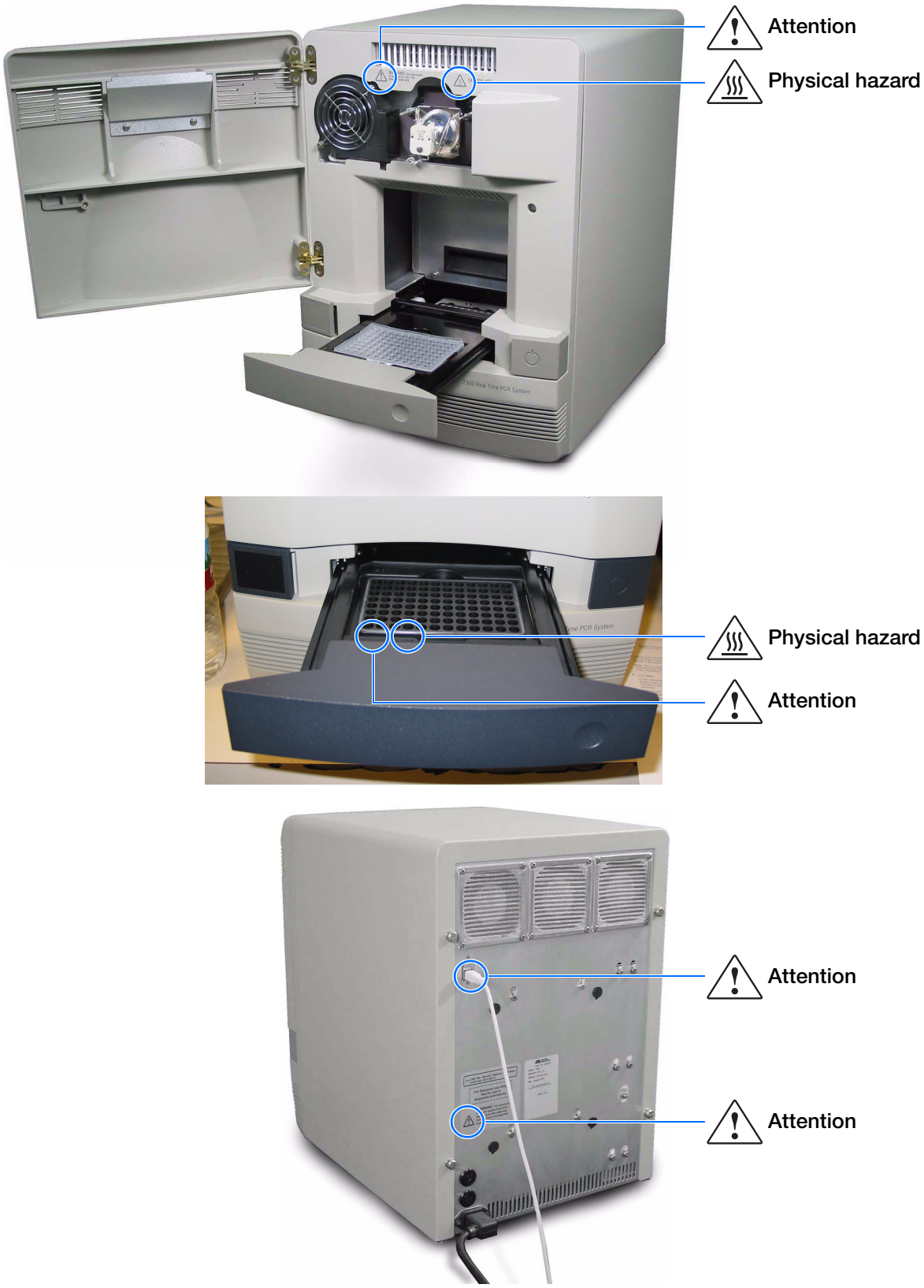
Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Français
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.
WARNING This instrument is designed for 12V, 75W Halogen lamps only.	**AVERTISSEMENT** Cet instrument est conçu pour des lampes d'halogène de 12V et 75W seulement.

Locations of Warnings

The Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System contain warnings at the locations shown below.



General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page xxi.

WARNING **PHYSICAL INJURY HAZARD.** Use this instrument as specified by Applied Biosystems. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Chemical Safety

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the **Search** field, type in the chemical name, part number, or other information that appears in the MSDS of interest.
3. Select the language of your choice, then click **Search**.
4. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page xxi.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER **ELECTRICAL HAZARD.** Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER **ELECTRICAL HAZARD.** Use properly configured and approved line cords for the voltage supply in your facility.

DANGER **ELECTRICAL HAZARD.** Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems have an installation (overvoltage) category of II, and are classified as portable equipment

Physical Hazard Safety

Moving Parts

WARNING **PHYSICAL INJURY HAZARD.** Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological Hazard Safety

General Biohazard

WARNING **BIOHAZARD.** Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 61010A-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements" and with standard UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

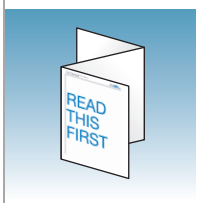
This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials," and with standard EN 61010-2-081:2002+A1:2003 "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

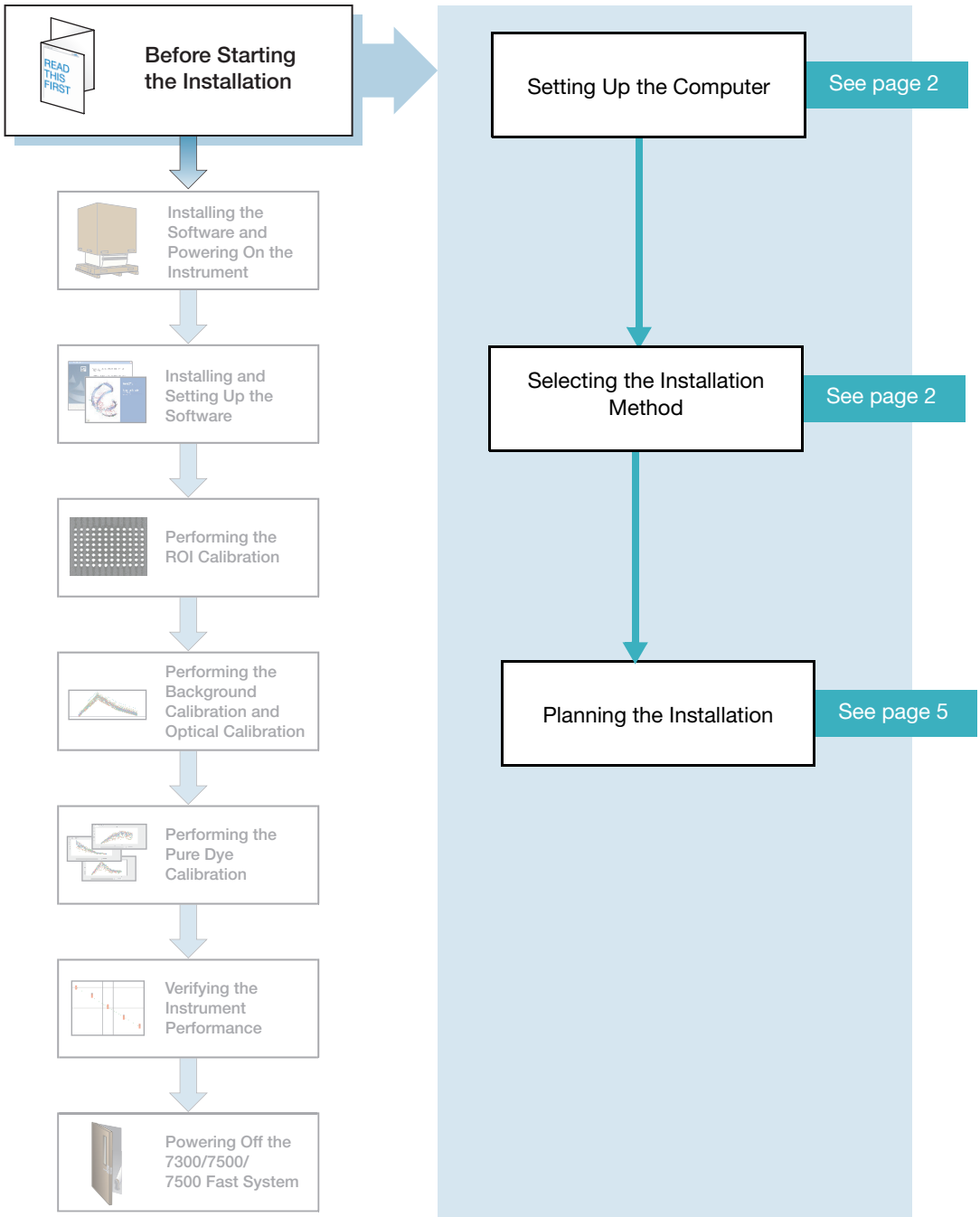
This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."



Before Starting the Installation



Notes

Setting Up the Computer

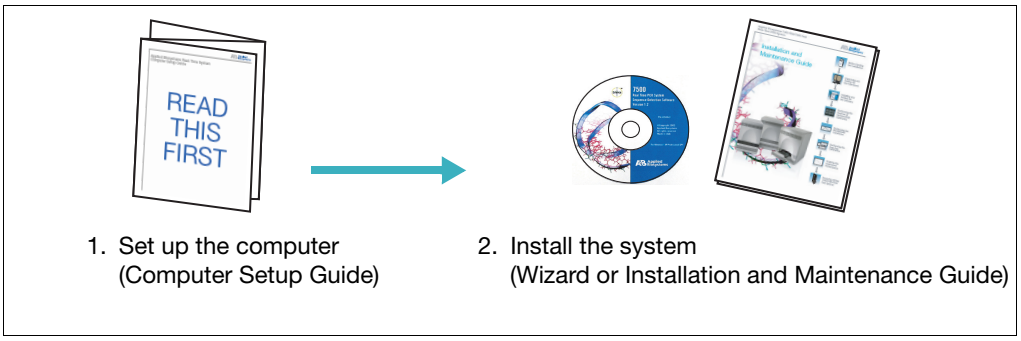
Before you install the Applied Biosystems 7300/7500/7500 Real-Time PCR System, install your computer and log in as a Windows operating system administrator, as described in the *Applied Biosystems Real-Time PCR System Computer Setup Guide*.

Selecting the Installation Method

After the computer is set up, proceed with installing the system by selecting an installation method:

- The Real-Time System Setup wizard on the Installation CD
  or
- This installation and maintenance guide

Both methods guide you through all steps for installing and calibrating the system.



Using the Real-Time System Setup Wizard

Using the Wizard to Install the System

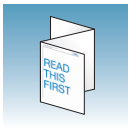
The Real-Time System Setup wizard is an online tool that you can use instead of this Installation Guide to help you install your new system.

After you set up the computer and log in as a Windows administrator as described in the *Applied Biosystems Real-Time PCR System Computer Setup Guide*, insert the Software CD in the computer CD drive. The wizard starts automatically after a short delay.

The Real-Time System Setup wizard provides step-by-step instructions for:

- Planning the installation
- Uncrating and placing the instrument
- Checking the shipped materials
- Performing an ROI calibration
- Performing a background calibration
- Performing an optical calibration (7500/7500 Fast systems only)

Notes



- Performing pure dye calibrations
- Verifying instrument performance

Using the Wizard to Recalibrate the System

You can also use the Real-Time System Setup wizard after the system is up and running, to perform the routine recalibrations and verification runs that ensure optimum performance:

Recalibration/ Verification	Purpose	When to Perform
ROI	Defines the well positions on the sample block.	• Every 6 months • After replacing the halogen lamp After an ROI recalibration, you must also perform a background, optical (7500/7500 Fast systems only), pure dye calibration, and instrument verification.
Background	Measures the level of background fluorescence in the instrument. During a run, the software removes the background fluorescence from the run data.	• Once a month • After replacing the halogen lamp After a background recalibration, you must perform an optical (7500/7500 Fast systems only).
Optical (7500/7500 Fast systems only)	Compensates for the physical effects of the additional filter present in 7500/7500 Fast instruments.	• Once a month • After performing a background calibration • After replacing the halogen lamp
Pure Dye	Characterizes each dye. During a run, the software uses the pure dye calibration spectra to distinguish the individual contribution of each dye in the collective fluorescence gathered by the instrument.	Every 6 months Before performing, you must perform a background calibration (7300 systems) or optical calibration (7500/7500 Fast systems);
RNase P	Verifies that the instrument can distinguish between 5,000 and 10,000 genome equivalents of the RNase P gene with a 99.7% confidence level.	After moving the instrument to another location or as needed to verify the function of the instrument

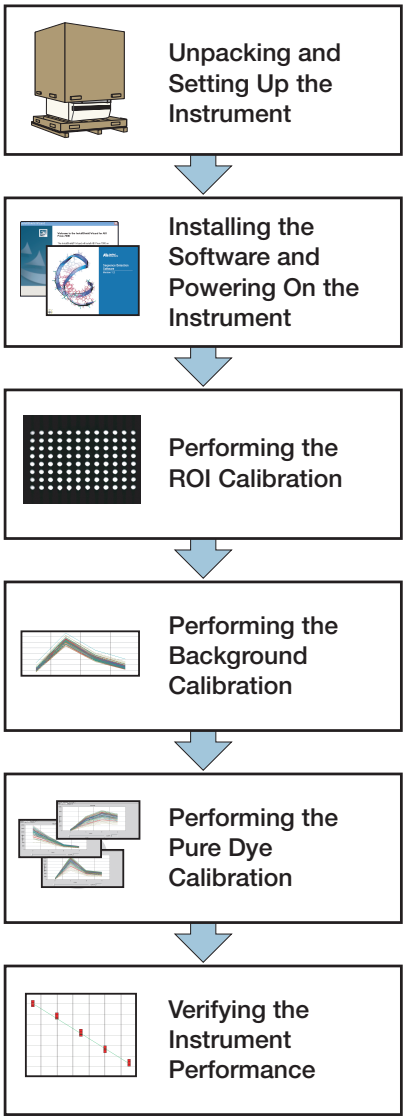
Access the wizard by selecting start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.

Notes

Using the Installation and Maintenance Guide

Using This Guide to Install the System

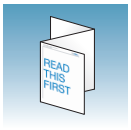
Instead of using the Real-Time System Setup wizard to install the system, you can install the system manually as described in the following chapters of this guide:



Using This Guide to Recalibrate the System

You can recalibrate the system manually after the system is up and running by following the procedures in this guide. However, the Real-Time System Setup wizard automates the recalibrations, and eliminates the need for manual inspection of pure dye spectra (Chapter 6) and manual calculation of system verification values (Chapter 7).

Notes



Planning the Installation

You can install, calibrate, and validate the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System within one 8-hour work day. The installation does not require your participation at all times. However, plan to spend most of your time working with the instrument.

Recommended Workflow

The steps you perform when you use this installation and maintenance guide, and the times associated with the steps, are listed below.

Note: The Real-Time System Setup wizard performs most of the steps listed below automatically, and requires less time for several steps. See the "Planning the Installation" page in the Real-Time System Setup wizard for steps and times for the wizard.

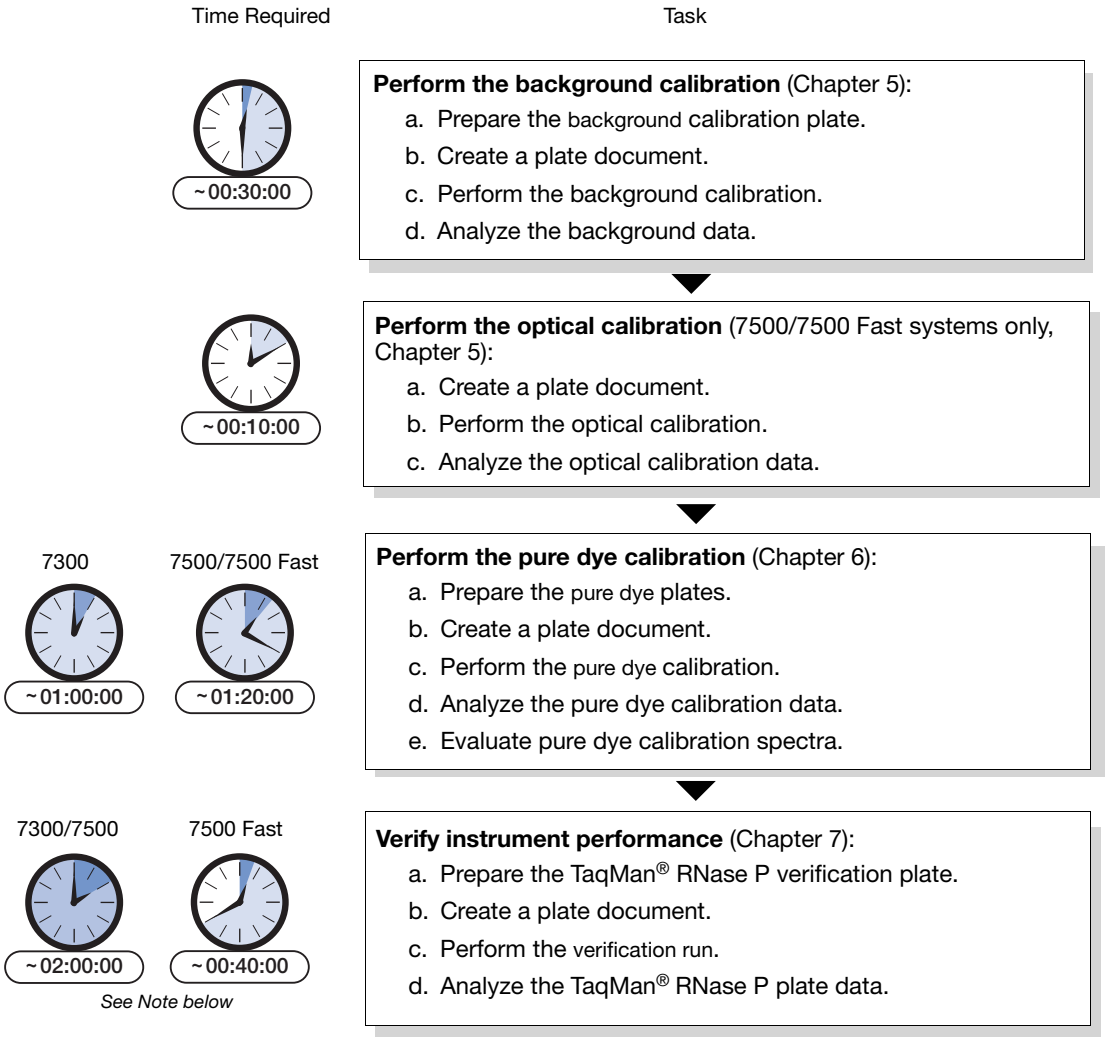
Time Required	Task
~00:10:00	**Set up the computer** (see the *Applied Biosystems Real-Time PCR System Computer Setup Guide*).
~00:45:00	**Unpack and set up the instrument** (Chapter 2): a. Select additional hardware and software. b. Uncrate and place the 7300/7500/7500 Fast system. c. Check the shipped materials. d. Set Up the 7300/7500/7500 Fast System.
~00:15:00 *See Note below*	**Install and set up the software** (Chapter 3): a. Install the SDS Software for the 7300/7500/7500 Fast System. b. Power on and install drivers, update firmware if needed. c. Perform the Instrument Function test.
~00:30:00	**Perform the ROI calibration** (Chapter 4): a. Prepare the ROI calibration plate. b. Create a plate document. c. Perform the ROI calibration.

continued on next page

Note: Some new instruments may require a firmware download. The download, which occurs automatically, takes approximately 45 minutes.You must be present only at the start and end of the download.

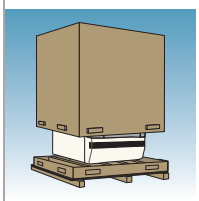
Notes

Recommended Workflow (continued)

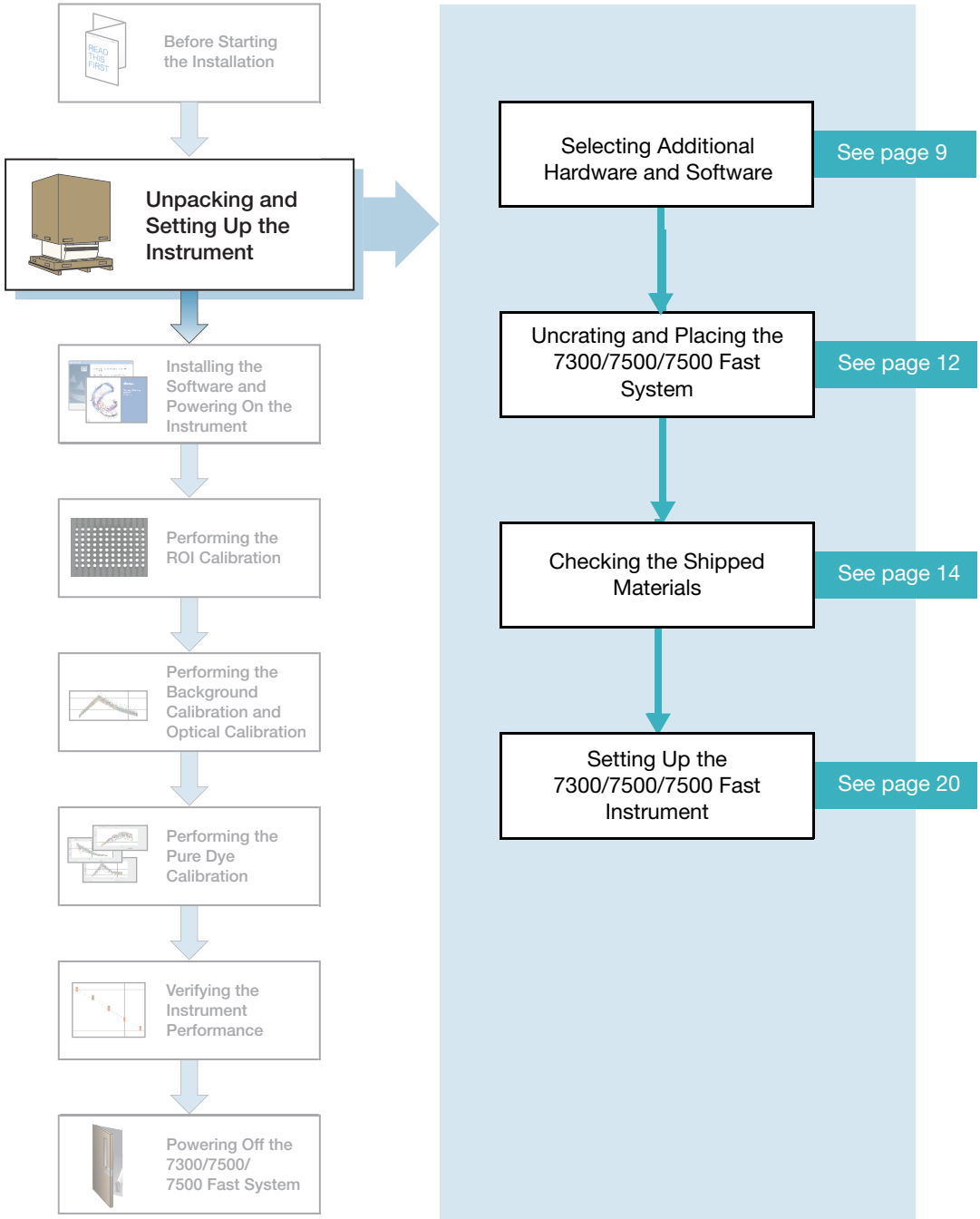


Note: You must be present at the instrument for approximately 5 minutes of the total run time.

Notes



Unpacking and Setting Up the Instrument



Notes

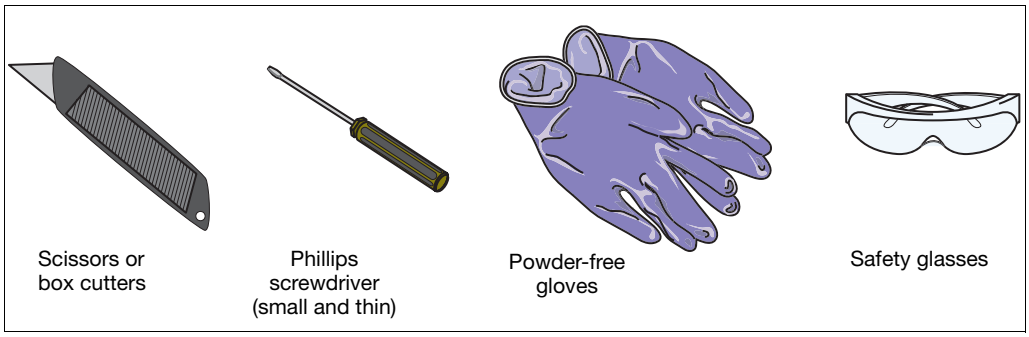
Before You Begin

Note: You can also use the Real-Time System Setup wizard to perform the procedures in this guide. To access the wizard, insert the installation CD in the CD drive of the computer. The wizard starts automatically after a short delay when you insert the CD.

Time Required

45 minutes

Materials Required

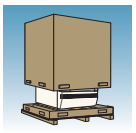


Getting Started

Before you begin installing the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System:

- Set up the computer as described in the *Applied Biosystems Real-Time PCR System Computer Setup Guide*
- Read the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Site Preparation Guide* (P/N 4378654) and complete the preinstallation checklist contained in the guide.
- Obtain the materials required for installation as shown above.
- Read "Selecting Additional Hardware and Software" on page 9 and, if necessary, obtain the additional components you want to install.

Notes



Selecting Additional Hardware and Software

Before beginning the installation, obtain additional software and hardware (if any) you want to install to the 7300/7500/7500 Fast system.

Choosing Electrical Protective Devices

Applied Biosystems recommends several protective devices to prevent loss of data and to protect the 7300/7500/7500 Fast system from damage resulting from electrical hazards.

Power Line Regulator

Applied Biosystems recommends the use of a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of ±10% of the normal voltage. Power fluctuations can adversely affect the function of the instrument and the data it produces.

Note: A power line regulator monitors the input current and adjusts the power supplied to the instrument. It does not protect against power surges or failure.

Uninterruptable Power Supply (UPS)

Applied Biosystems recommends the use of a 1.5-kVA uninterruptable power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the 7300/7500/7500 Fast system can corrupt data and possibly damage the system.

IMPORTANT! UPSs have finite battery lives, and, consequently provide power for a limited time (from 30 minutes to several hours). They are meant to delay the effects of a power outage, not to serve as replacement power sources. In the event of a power loss, perform a short-term shutdown of the 7300/7500/7500 Fast system (see page 102) unless you expect to regain power within the battery life of the UPS.

Surge Protector

Applied Biosystems recommends the use of a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of and, thereby damage the components of, the computer and the 7300/7500/7500 Fast instrument.

Note: A dedicated line and ground between the instrument and the building's main electrical service can also prevent problems caused by power fluctuations.

Notes

Using the 7300/7500/7500 Fast System on a Network

The 7300/7500/7500 Fast system operates independently of the network functions of the Windows XP Professional operating system (the instrument does not require specific network protocol settings or an IP address for operation).

IMPORTANT! Do not use the 7300/7500/7500 Fast System on a wireless network. Use of a wireless network can interfere with data collection and may result in data loss.

Choosing a Backup Storage Device

Applied Biosystems recommends the use of one or more backup storage devices to prevent potential loss of data caused by unforeseen failures of the computer or its hard drive(s). If your 7300/7500/7500 Fast system includes a laptop or tower computer, then the CD-RW drive of the computer can serve as the backup storage device for your system. By saving your .sds and .sdt files to one or more writable CDs on a weekly basis, you can effectively back up the data generated by your 7300/7500/7500 Fast system. Before installing the 7300/7500/7500 Fast system, decide on a method for backing up your data.

Installing Software to the 7300/7500/7500 Fast System

If you want to install additional software to the 7300/7500/7500 Fast system computer, verify that each software application does not:

- Restrict communication through the universal serial bus (USB) ports
  or
- Interfere with the processes of the SDS software.

Note: You can verify that an application does not interfere with the processes of the SDS software by running several "dummy" plates (plates that do not contain reagents) before using the 7300/7500/7500 Fast system to run samples.

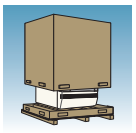
Antivirus Software

Applied Biosystems generally recommends the use of commercial antivirus software when the 7300/7500/7500 Fast system is connected to a network.

Archival or File Compression Software

Applied Biosystems recommends the use of file compression software for archiving data generated by the 7300/7500/7500 Fast system. For more information, see "Archiving and Backing Up SDS Files" on page 107.

Notes



Security Software (Firewall and Encryption Utilities)

If you plan to install a firewall or encryption utility to protect your 7300/7500/7500 Fast system on a network, confirm that the security software does not interfere with USB communication between the 7300/7500/7500 Fast instrument and the SDS software. These components may not function if the security software restricts access to USB communication.

Note: The Microsoft Windows XP Professional operating system installed on the computer shipped with the 7300/7500/7500 Fast system contains a native firewall utility. Applied Biosystems does not support the use of the Windows firewall software, and cannot provide support for problems arising through its use.

System Utilities or Performance Optimizing Software

Applied Biosystems recommends the regular use of the Windows XP Professional operating system defragmentation utility and a commercial archival utility to ensure optimal performance of the 7300/7500/7500 Fast system. (For more information, see "Cleaning Up and Defragmenting the Hard Drive" on page 114.)

Note: Before you install a different defragmentation utility or another type of performance-enhancing software, verify that the software does not interfere with the SDS software as explained in "Security Software (Firewall and Encryption Utilities)" above.

Notes

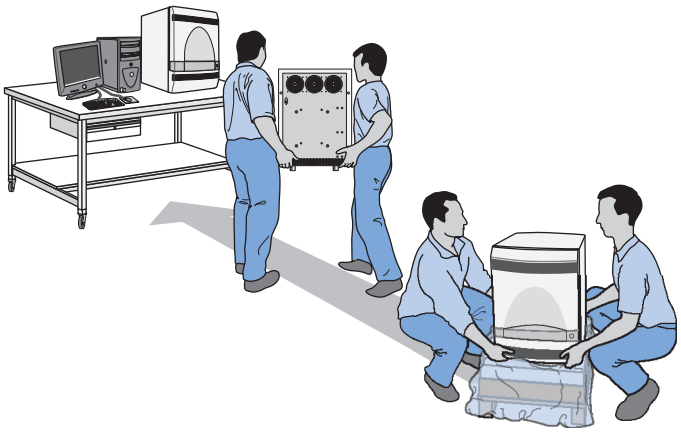
Uncrating and Placing the 7300/7500/7500 Fast System

WARNING **PHYSICAL INJURY HAZARD**. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the 7300/7500/7500 Fast instrument.

Preinstallation Checklists

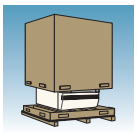
Before assembling your 7300/7500/7500 Fast system, review and complete the preinstallation checklists in the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Site Preparation Guide.* The guide is shipped to you before the instrument arrives and contains important environmental and electrical requirements for the 7300/7500/7500 Fast system.

Guidelines for Lifting and Moving



- Verify that the surface you will be placing the instrument on supports at least 54.5 kg (120 lbs).
- Verify that the pathway to the final position of the instrument is clear of obstructions.
- Keep your spine in a good neutral position.
- Bend at the knees and lift with your legs.
- Do not lift an object and twist your torso at the same time.
- Coordinate your intentions with your assistant before lifting and carrying.

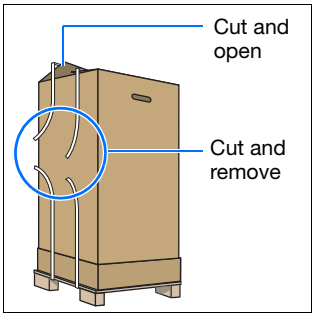
Notes

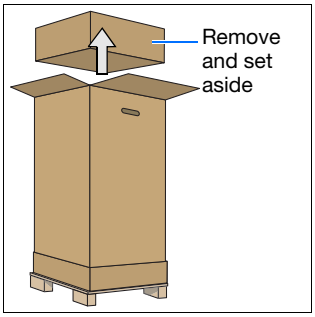


Placing the System Components

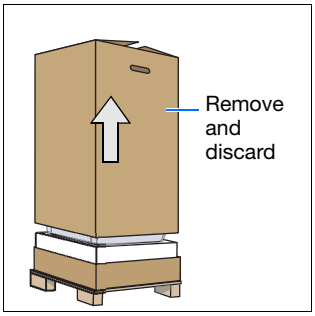
1. On the outside of the instrument crate, examine these indicators:
   - Tilt indicator (lower portion of crate), which indicates if the crate has been severely tilted
   - Shock indicator (upper portion of crate), which indicates if the crate has been subjected to excessive force

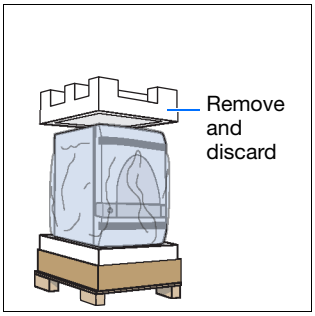
   If either of these devices indicate tilt or shock effects, perform the remaining steps in this procedure, then visually inspect all contents of the crate:
   - If any items are damaged, contact Applied Biosystems.
   - If no items are damaged, continue with the installation.



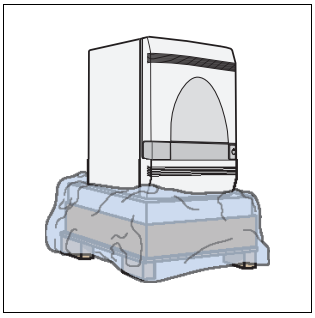


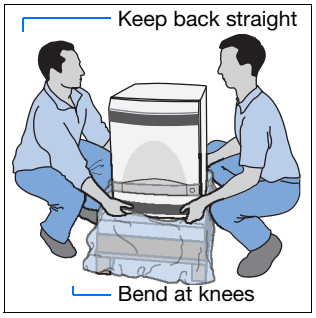
2. Cut the straps securing the instrument crate.
3. Cut the tape securing the top flaps of the instrument crate and open them.
4. Remove the Packing Kit from the instrument and set it aside.
5. Lift and remove the lid from the instrument crate.





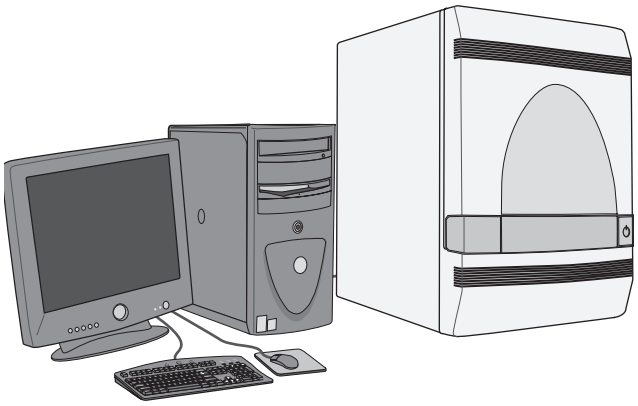
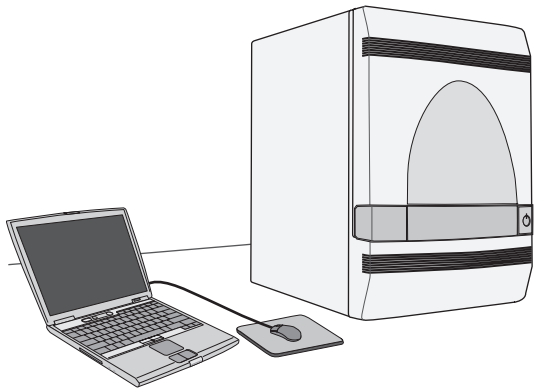
6. Remove the protective cover from the 7300/7500/7500 Fast instrument.
7. Position yourselves on either side of the instrument and grasp it firmly at the corners.
8. Keeping your back straight, lift with your legs and place the instrument onto the bench. Place the instrument on the bench next to the computer as shown below.





Notes

IMPORTANT! Do not connect the computer to the instrument at this time.

7300/7500/7500 Fast System Tower Computer Setup	7300/7500/7500 Fast System Laptop Computer Setup
	

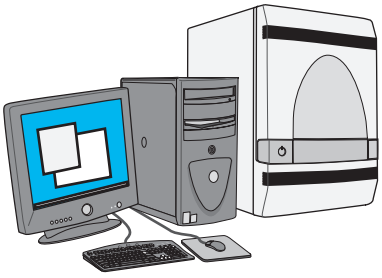
Checking the Shipped Materials

Before you begin the installation, verify that you have received the components shipped with the purchase of a 7300/7500/7500 Fast system.

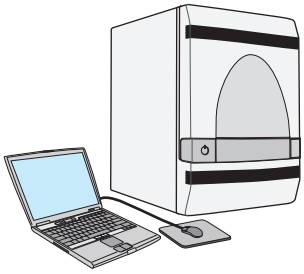
Checking the Materials

1. Verify that you received one of the following.

✔	Instrument and Computer
	• 7300/7500/7500 Fast instrument • Dell® Tower Computer • Dell Flat Screen Monitor
	• 7300/7500/7500 Fast instrument • Dell® Laptop Computer

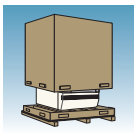


7300/7500/7500 Fast instrument with Dell® tower computer and flat-screen monitor



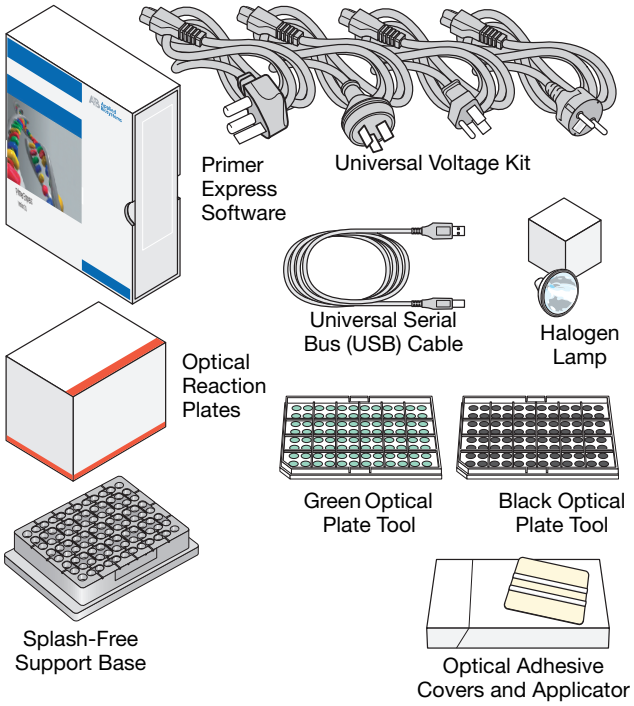
7300/7500/7500 Fast instrument with Dell® laptop computer

Notes



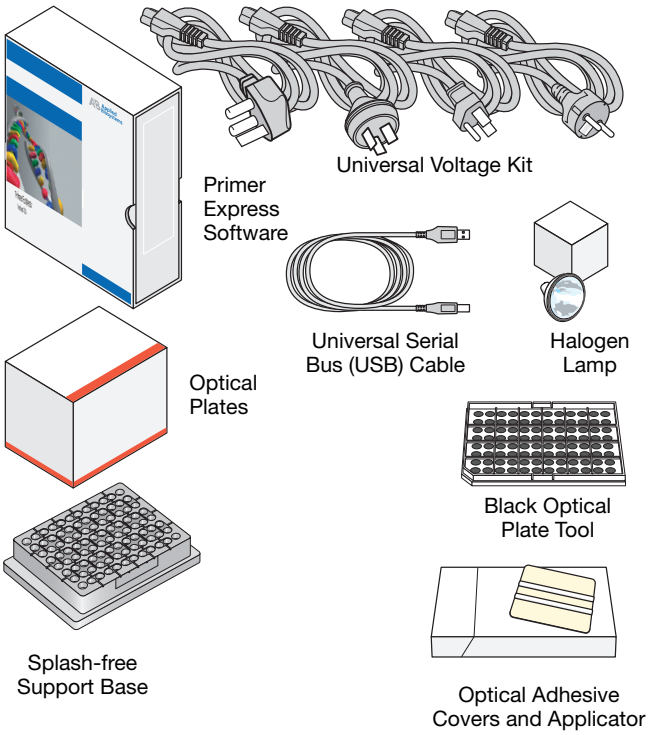
2. Open the Packing kit and verify that it contains the following, depending on your instrument:

✔	Packing Kit	P/N
	7300/7500 Packing kit	4349804
	• Primer Express® Software Kit	4361938
	• Universal Voltage Kit (Australian, British, European, North American, and Japanese Power Cords)	603615
	• Optical Reaction Plates, 96-well, with bar codes (100 plates)	4306737
	• Universal Serial Bus (USB) Cable	4328260
	• Halogen Lamp (12V, 75W)	4345287
	• Green Optical Plate Tool	4306819
	• Black Optical Plate Tool	4305872
	• Splash-Free Support Base	4312063
	• Optical Adhesive Covers and Applicator	4311971 4348209
	Miscellaneous items (business card holder, reply card for installation quality, warranty card, release notes)	—

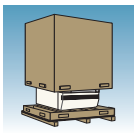


Notes

✔	Packing Kit	P/N
	7500 Fast Packing kit	4361854
	• Primer Express® Software Kit Version 3.0	4361938
	• Universal Voltage Kit (Australian, British, European, North American, and Japanese Power Cords)	603615
	• Universal Serial Bus (USB) Cable	4328260
	• Halogen Lamp (12V 75W)	4345287
	• Optical 96-Well Fast Thermal Cycling Plate with Barcode (code 128) (20 plates)	4346906
	• Black Optical Plate Tool	4305872
	• Splash-free Support Base	4312603
	• Optical Adhesive Covers and Applicator	4311971 4348209
	• Paper, Fluorescent Green (not shown)	4323077
	Miscellaneous items (business card holder, Reply card for installation quality, warranty card, release notes)	—



Notes

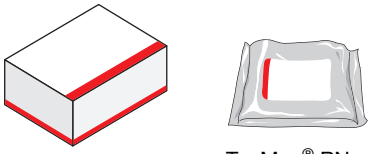


3. Verify that you received the following kits:

✔	Chemistry/Calibration Kits	P/N
	TaqMan® RNase P Chemistry Verification Plate (7300/7500 systems)	4350584
	TaqMan® RNase P Chemistry Fast Verification Plate (7500 Fast systems)	4351979
	The kit(s) appropriate for your system: • Applied Biosystems 7300 Real-Time PCR System Spectral Calibration Kit, containing: – Background Plate – Pure Dye Plates (FAM™, JOE™, NED™, ROX™, SYBR® Green, TAMRA™, and VIC® dyes) – ROI Calibration Plate • Applied Biosystems 7500 Real-Time PCR System Spectral Calibration Kits I and II, containing: – Background Plate – Pure Dye Plates (CY3, CY5, FAM, JOE, NED, ROX, SYBR Green, TAMRA, TEXAS RED®, and VIC dyes) – ROI Calibration Plate • Applied Biosystems 7500 Fast Real-Time PCR System Spectral Calibration Kits I and II, containing: – Background Plate – Pure Dye Plates (CY3, CY5, FAM, JOE, NED, ROX, SYBR Green, TAMRA, TEXAS RED, and VIC dyes) – ROI Calibration Plate	4349182 4349180 (Kit I) 4351151 (Kit II) 4360788 (Kit I) 4362201 (Kit II)

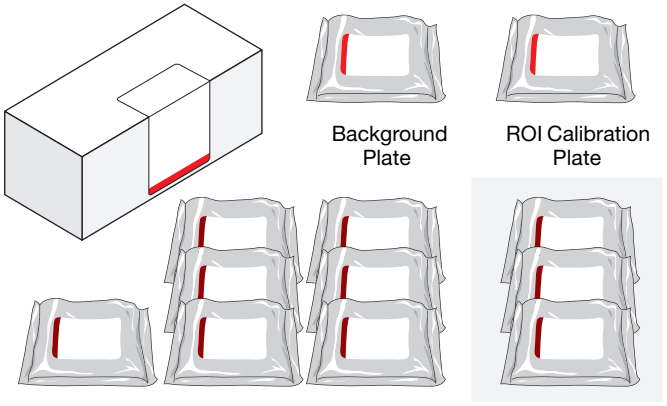
continued on next page

TaqMan® RNase P Instrument Verification Plate



TaqMan® RNase P Instrument Verification Plate

Applied Biosystems 7300 or 7500/7500 Fast Real-Time PCR System Spectral Calibration Kit



Background Plate

ROI Calibration Plate

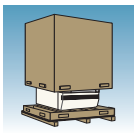
7300/7500/7500 Fast Pure Dye Plates (FAM™, JOE™, NED™, ROX™, SYBR® Green, TAMRA,™ and VIC® dyes)

7500/7500 Fast Systems Only (CY3, CY5, and TEXAS RED® dyes)

Notes

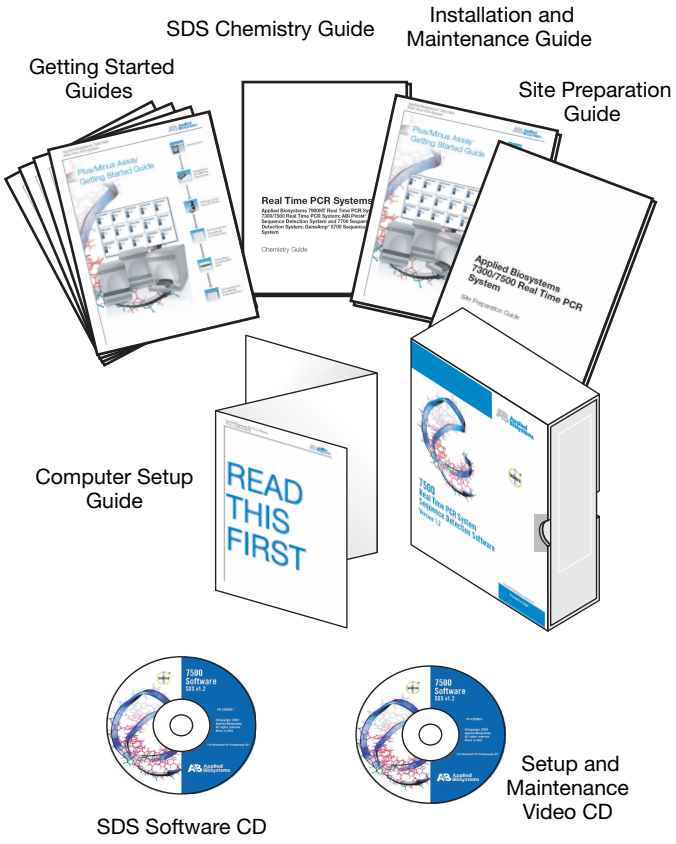
✔	Chemistry/Calibration Kits	P/N
	TaqMan® Reagent Starter Kit (7300/7500 systems, not shown): • TaqMan® Universal PCR Master Mix (2X), No Amperase® UNG (1 mL) • TaqMan® Gene Expression Assay, Eukaryotic 18S rRNA (0.3 mL) • Human Raji cDNA, (25 ng/µL)	4352405
	TaqMan® Fast Reagent Starter Kit (7500 Fast systems, not shown): • TaqMan® Fast Universal PCR Master Mix(2X), No Amperase® UNG (1.25 mL) • TaqMan® Gene Expression Assay, Eukaryotic 18S rRNA (0.3 mL) • Human Raji cDNA, (25 ng/µL)	4352407
	Genomic Assays Catalog CD (not shown)	4362363

Notes



4. Unpack the software and documentation kit and verify that you received the following:

✔	Software and Documentation	P/N
	Applied Biosystems Real-Time PCR System Computer Setup Guide	4378660
	Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide (this document)	4378657
	Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Getting Started Guides:	
	• *Absolute Quantification*	4378656
	• *Relative Quantification*	4378655
	• *Allelic Discrimination*	4378653
	• *Plus/Minus*	4378652
	Applied Biosystems Real-Time PCR Systems Chemistry Guide	4378658
	Applied Biosystems 7500 Fast Real-Time PCR System Quick Reference Card (not shown)	4378659
	Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Site Preparation Guide	4378654
	CD, SDS Software, v1.3.1:	
	• 7300 System	4350809
	• 7300 System RT PCR RQ Study	4350814
	• 7500 System	4350819
	• 7500 Fast System	4363619
	Miscellaneous items (Setup and Maintenance Video CD, Mouse Pad, Customer Letter, Registration Card)	—



Notes

Setting Up the 7300/7500/7500 Fast Instrument

CAUTION *Do not* connect the USB cable to the 7300/7500/7500 Fast instrument at this time.

This section describes how to connect the 7300/7500/7500 Fast system.

Materials Required

- Phillips screwdriver (small and thin)
- Power cord (from packing kit)

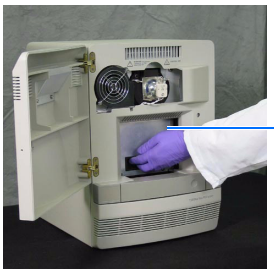
Preparing the Site

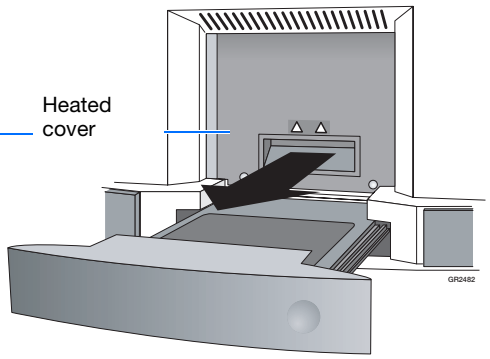
Prepare the installation site as described in the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Site Preparation Guide*. Refer to this guide for weights, dimensions, and electrical requirements.

Setting Up the System

1. Open the access door to the 7300/7500/7500 Fast system.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.

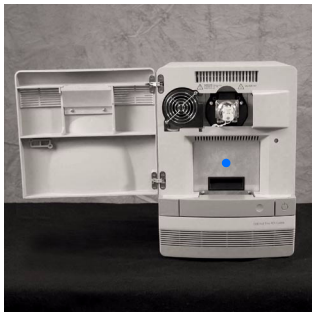
2. Verify that the heated cover assembly is pulled fully toward the front of the instrument.



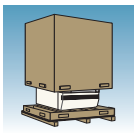


3. Check the instrument for damage caused by the transportation of the 7300/7500/7500 Fast system.

   If the instrument is damaged, note the location and appearance of the damage, then contact Applied Biosystems technical support or your service representative for assistance (see "How to Obtain Support" on page xiii).



Notes



4. Close the access door of the instrument.

5. Connect the power cord to the 7300/7500/7500 Fast instrument, then to the receptacle wall circuit.

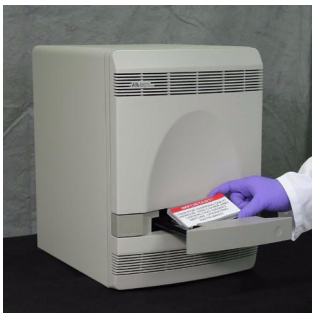
   Note: Power cords for different voltages are provided in the packing kit. Connect the cord with the receptacle appropriate for your voltage, then discard remaining cables.

6. Press the power button at the lower right front panel, then wait for the 7300/7500/7500 Fast system to boot (about 30 seconds).

7. When the Power status light on the lower left front panel lights, press the tray to open it.

8. Remove the packaging plate from the tray and set it aside.

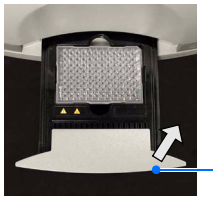
   Note: Do not discard the packaging plate. Use it for long-term shutdown of the system (see page 104).



Notes

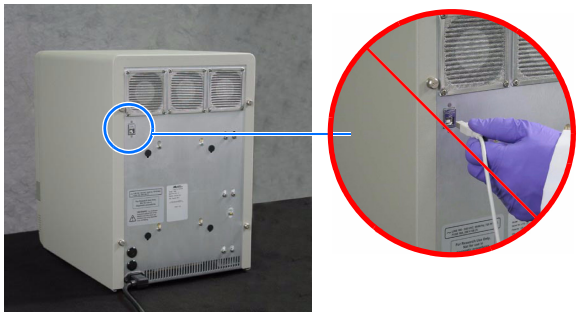
9. Close the tray door, then press the power button again to power off the instrument.

Note: When closing the instrument tray door, apply pressure to the right side of the tray and at an angle.



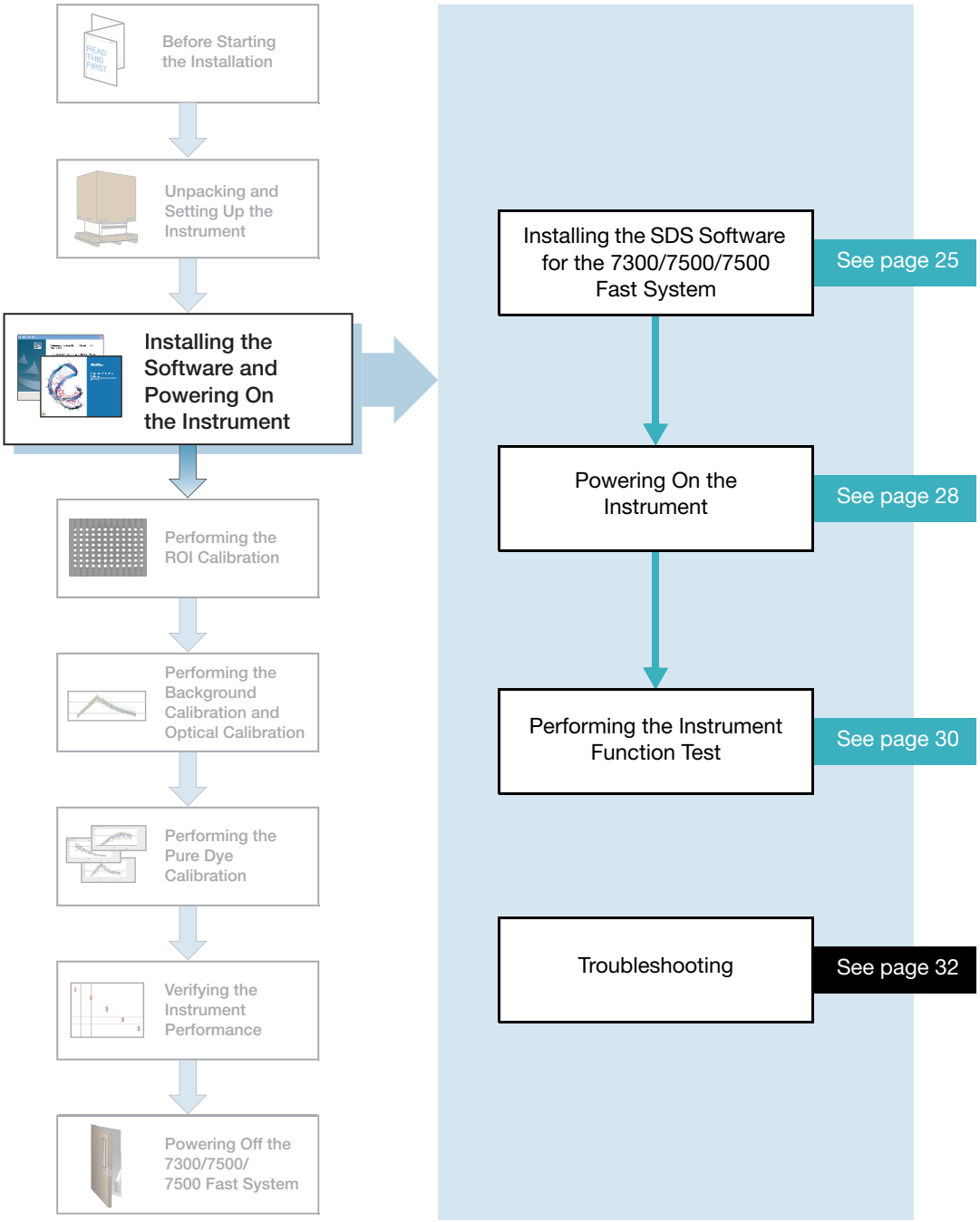
If you have additional hardware that you want to install (see page 9), do so now.

CAUTION Do *not* connect the USB cable to the 7300/7500/7500 Fast instrument or computer at this time.



Notes

Installing the Software and Powering On the Instrument



Notes

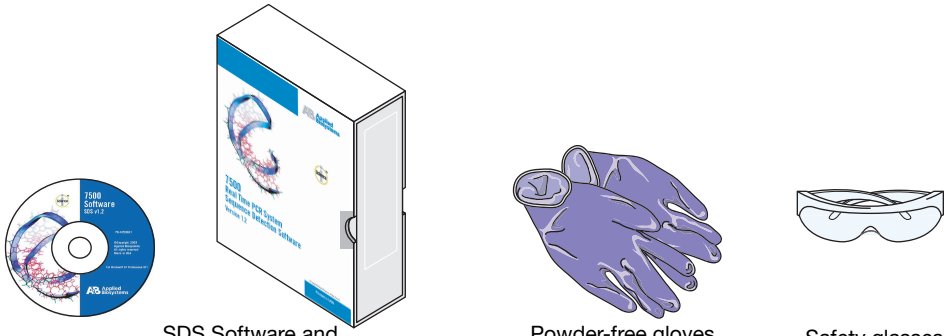
Overview

Note: You can also use the Real-Time System Setup wizard to perform the procedures in this guide. To access the wizard, insert the installation CD in the CD drive of the computer. The Real-Time System Setup wizard starts automatically after a slight delay when you insert the CD.

Time Required

45 minutes

Materials Required



IMPORTANT! If you plan to configure the 7300/7500/7500 Fast system computer with additional software (see page 10), complete the installation of the 7300/7500/7500 Fast system as described in this guide before installing other software. Installing third-party software to the 7300/7500/7500 Fast system computer before completing the procedures in this manual can complicate the installation.

Notes

Installing the SDS Software for the 7300/7500/7500 Fast System

IMPORTANT! You must be logged in with Windows administrator privileges to install the SDS Software.

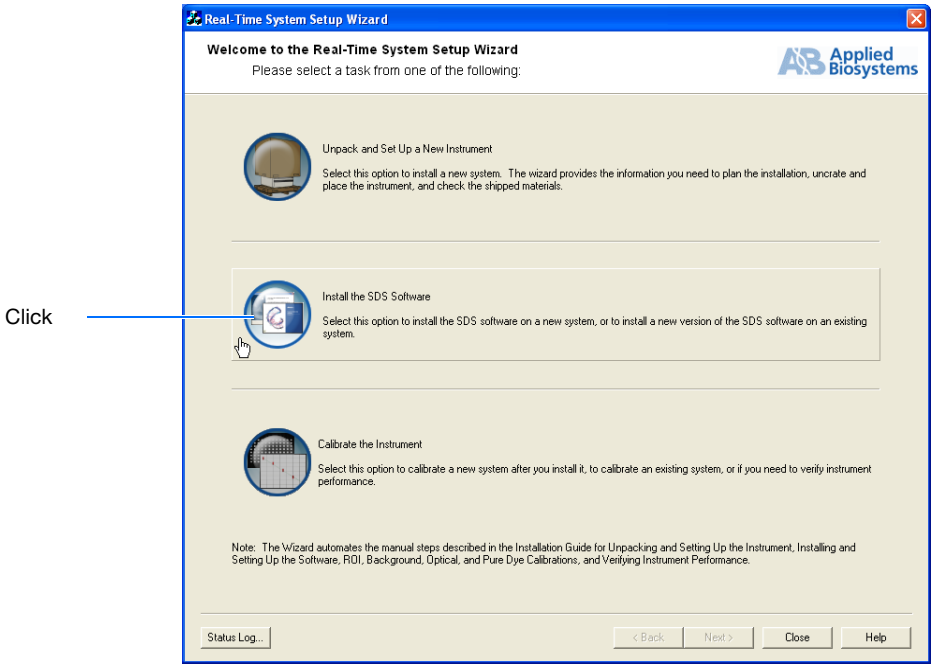
1. Obtain the Software CD and Documentation CD from the Software and Documentation kit.

2. Power on the computer, then log in as a Windows operating system administrator, as described in the *Applied Biosystems Real-Time PCR System Computer Setup Guide*.

3. Insert the SDS Software CD into the CD drive of the computer. The Real-Time System Setup wizard starts automatically after a short delay.

   If the wizard does not automatically start, double-click (**My Computer**), navigate to the CD drive, then double-click **SystemSetupWizard.exe**.

4. Click **Install the SDS Software**.



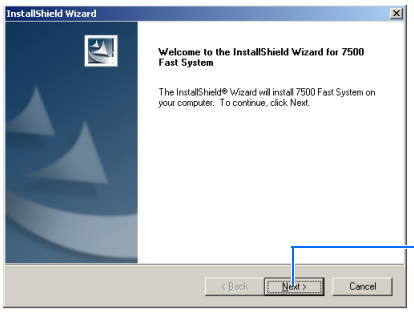
5. In the Software Installation Materials Required page, select the **I have obtained the materials listed above check** box, then click Next >.

Notes

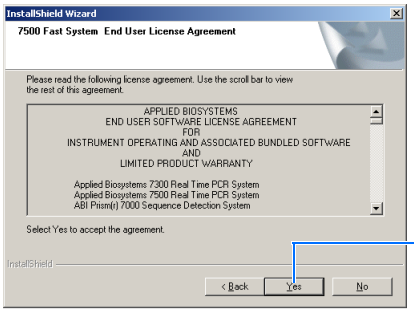
6. In the Software Installation Materials Install the Software page, click Install / Upgrade SDS Software.

   The InstallShield® Wizard opens.

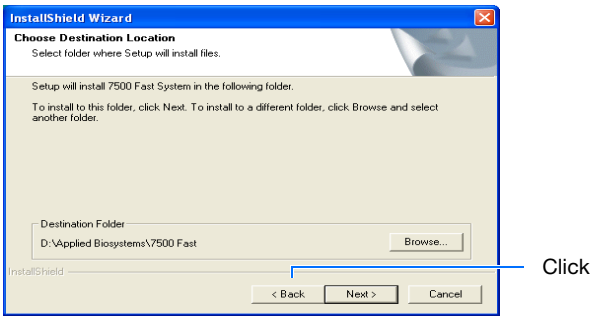
7. In the Welcome page, click Next >.



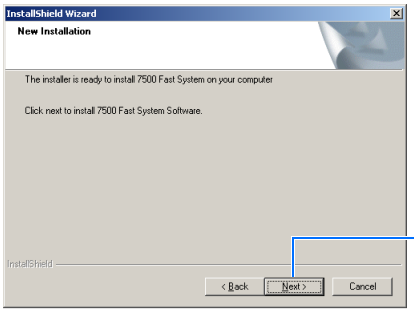
8. In the End User License Agreement page, click Yes to accept the agreement.



9. In the Choose Destination Location page, click Next > to accept the default location.



10. In the New Installation page, click Next > to start installing the software.



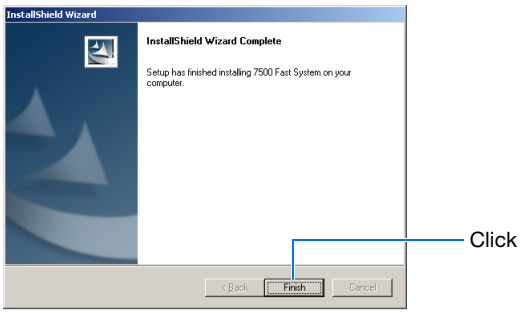
Notes

11. When the software completes the installation, click Finish.

7300 systems without RQ software: Skip to step 13.

7300 with RQ, 7500/7500 Fast systems:

- After a short delay, the Product Registration dialog box is displayed.
- Continue with step 12.



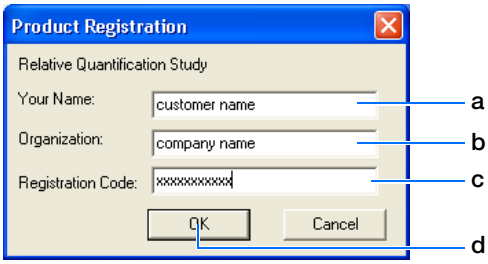
12. Register the product (the Registration dialog box is displayed only for the 7300 system RQ Study and 7500/7500 Fast systems):

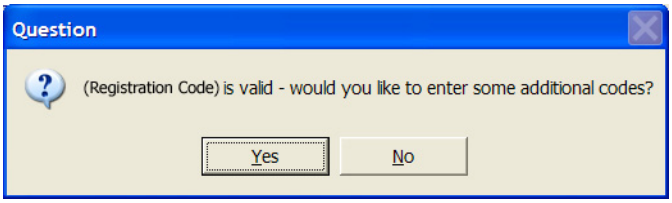
a. In the Your Name field, enter your name.

b. In the Organization field, enter the name of your business or organization.

c. In the Registration Code field, enter the registration code located on the CD case.

d. Click OK.

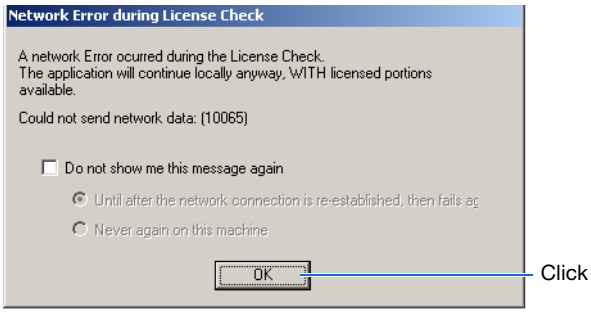




13. If a Network communications error is displayed, click OK. The installation will complete successfully even if this error is displayed.

Note: This message is displayed if your computer is not connected to a network. If you do not plan to connect this computer to a network, select **Do not show me this message again**, then select **Never again on this machine**.

A message is displayed prompting you to enter additional registration codes.



14. If you have additional registration codes, click Yes, then repeat step 12. Otherwise, click No to continue.

15. In the Real-Time System Setup wizard Software Installation page, click Close.

16. In the Real-Time System Setup wizard Welcome page, click Close.

Continue with "Powering On the Instrument" on page 28.

Notes

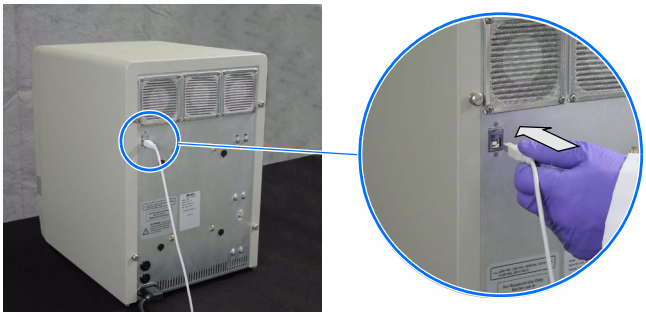
Powering On the Instrument

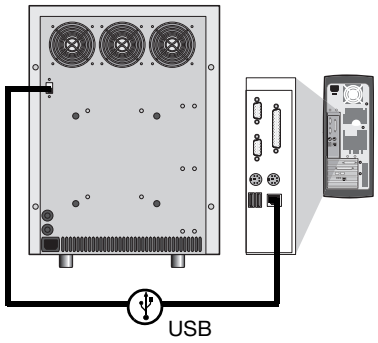
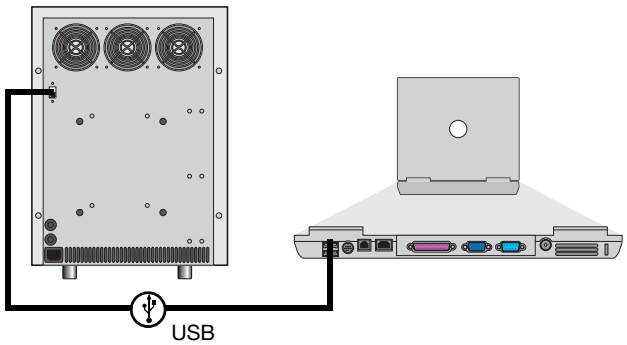
IMPORTANT! Do not power on the instrument unless you have installed the SDS software. If you power on the instrument before you install the SDS software, the Windows operating system installs a generic driver for the instrument, which does not allow the instrument to communicate with the SDS software.

IMPORTANT! Make sure that the computer Hibernate power setting is disabled. If the Hibernate setting is enabled, data collection stops when the computer goes into Hibernate mode. For more information, see "Setting the Display Settings and Power Options" on page 137.

Powering On

1. Connect the Universal Serial Bus (USB) cable between the:
   - USB connector on the back left of the instrument
     and
   - Either USB port on the computer



Tower Computer USB Connection	Laptop Computer USB Connection
	

Notes

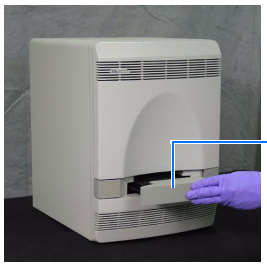
2. Press the power button on the 7300/7500/7500 Fast instrument. The following occur:

- The indicator lights on the lower left of the front panel cycle through a power on sequence.
  - If the green Power On indicator is flashing, make sure the tray is closed.
  - If the red Error indicator is lit, see "Troubleshooting – Front Panel Indicators" on page 32.

Power button

Indicator lights

Power On
(flashing)



- When the green Power indicator is lit (not flashing):
  - Communication is established between the computer and the instrument.
  - The Windows XP operating system recognizes the instrument as new hardware.
  - The Windows XP operating system automatically installs the drivers needed to control the instrument.

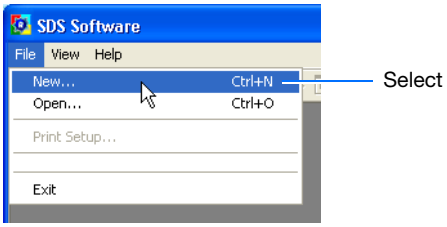
- When the installation is complete, the Found New Hardware message box indicates that the hardware is ready to use.

Notes

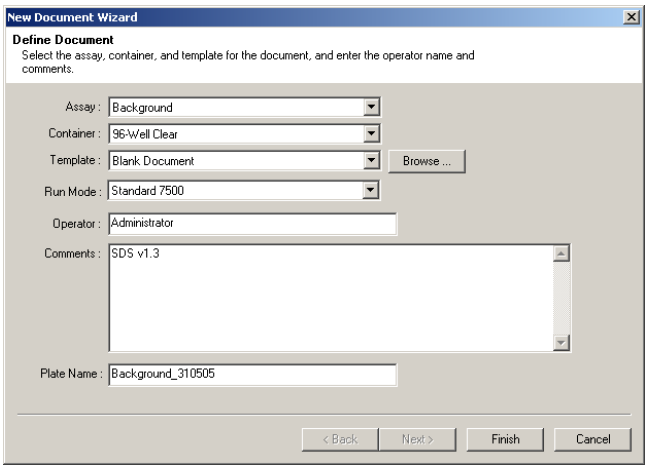
Performing the Instrument Function Test

1. If the SDS software is not running, in the desktop, double-click the icon for the SDS software ( ).

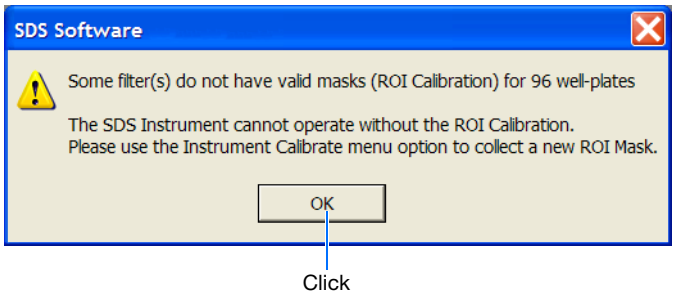
2. In the SDS software, click (or select **File ▸ New**).



3. In the New Document wizard, click Finish to accept the default parameters.

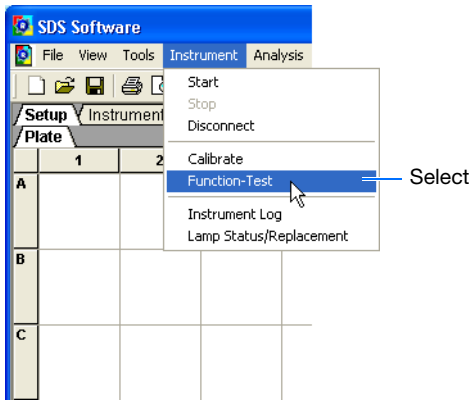


4. If an ROI error is displayed, click OK. You perform the ROI calibration after you perform the Function test.

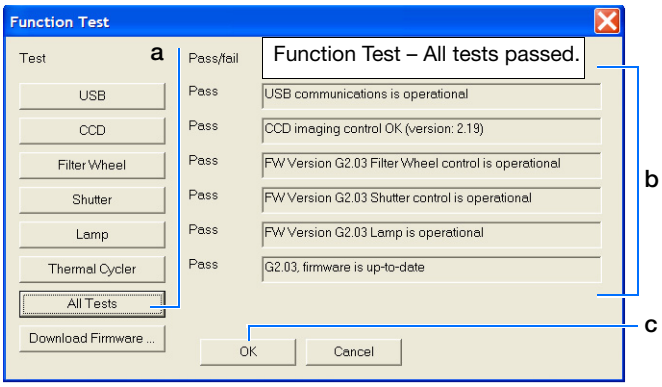


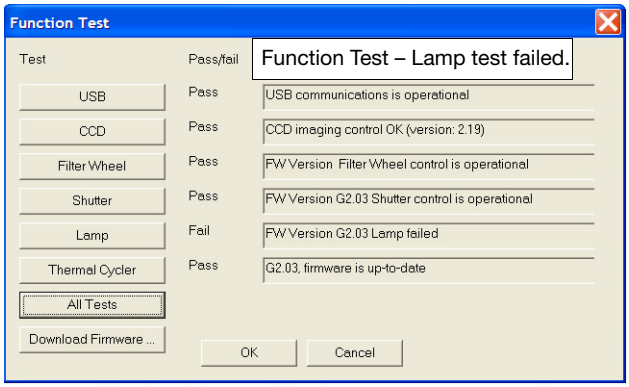
Notes

5. Select **Instrument ▸ Function Test**.



6. In the Function Test dialog box:
   a. Click All Tests.
   b. Examine the Pass/Fail column:
      - If all the function tests Pass, go to step 7.
      - If any of the function tests Fail, see "Troubleshooting – Function Tests" on page 33.
   c. Click OK.

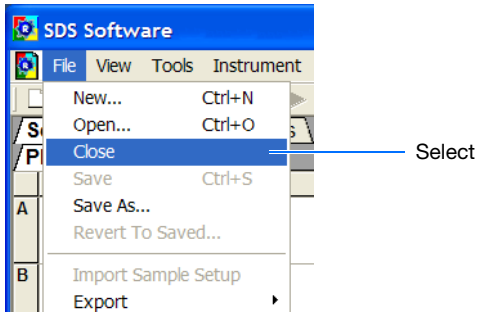




7. Select **File ▸ Close** to close the plate document.

8. When the software prompts you to save the plate document, click No.

9. In the SDS software, select **File ▸ Exit**.



Continue with "Performing the Regions of Interest (ROI) Calibration" on page 35.

Notes

Troubleshooting

This section contains:

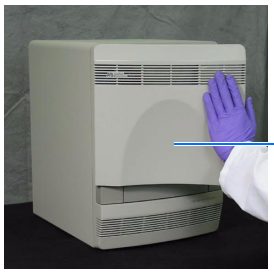
Troubleshooting – Front Panel Indicators

Troubleshooting – Front Panel Indicators

Condition: The red Error indicator is lit.

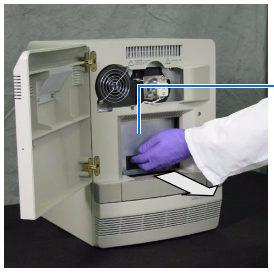
1. Press on the instrument door to ensure that it is closed.

   If the green Power On indicator lights up, the open instrument door caused the error, and installation continues.



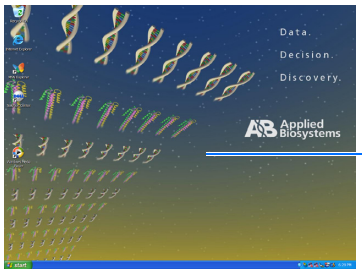
2. If the red Error indicator remains lit:
   a. Open the instrument door.
   b. Pull the heated cover door to verify that it is closed.
   c. Close the instrument door.

   If the green Power On indicator lights up, the open heated cover door caused the error, and installation continues.



3. If the red Error indicator remains lit, verify that the Windows desktop is displayed on the computer. If the Windows desktop is not displayed:
   a. Power off the 7300/7500/7500 Fast instrument.
   b. Restart the computer.
   c. Wait until the Windows desktop appears.
   d. Power on the 7300/7500/7500 Fast instrument.

   If the green Power On indicator lights up, installation continues.



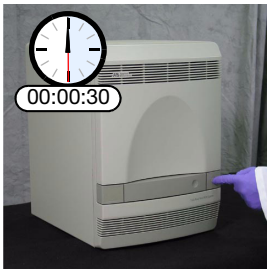
Notes

Troubleshooting – Front Panel Indicators *(continued)*

4. If the red Error indicator remains lit:
   a. Verify that the USB cable is connected to the back of the instrument.
   b. Verify that the other end of the USB cable is connected to the computer.

   If the green Power On indicator lights up, it indicates that the USB cable was not connected, and installation continues.

5. If the red Error indicator remains lit:
   a. Power off the 7300/7500/7500 Fast instrument.
   b. Wait for 30 seconds.
   c. Power on the 7300/7500/7500 Fast instrument.



6. If the red Error indicator remains lit, contact Applied Biosystems technical support (see page xiii) or your service representative.

Troubleshooting – Function Tests

Troubleshooting – Function Tests

Condition: USB Test Failure

1. Power off the instrument, wait 30 seconds, then power on the instrument.
2. Perform the Instrument Function Test (see page 30).
3. If the test fails again, check that the USB connections to the instrument and computer are secure.
4. Perform the Instrument Function Test (see page 30).
5. If the test fails again, contact Applied Biosystems technical support (see page xiii) or your service representative.

(continued on next page)

Notes

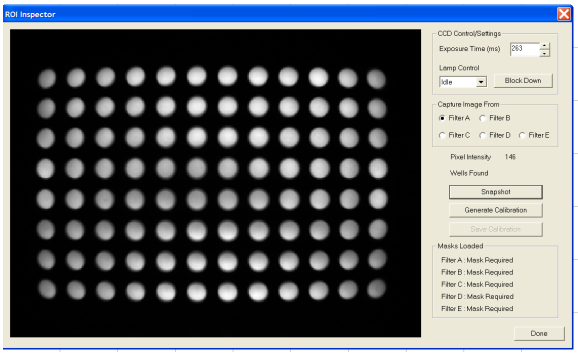
Troubleshooting – Function Tests *(continued)*

Condition: CCD, Filter Wheel, or Shutter Test Failures

1. Power off the instrument, wait 30 seconds, then power on the instrument.
2. Perform the Instrument Function Test (see page 30).
3. If the test fails again, perform the ROI calibration as explained in "Performing the Regions of Interest (ROI) Calibration" on page 35.
4. Determine if the ROI image appears in the ROI Inspector.

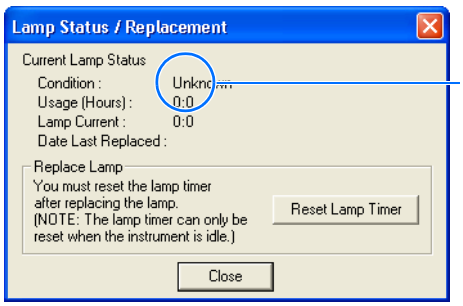
   Yes – Continue with the installation.

   No – Contact Applied Biosystems technical support (see page xiii) or your service representative.



Condition: Lamp Test Failure

1. Power off the instrument, wait 30 seconds, then power on the instrument.
2. Perform the Instrument Function Test (see page 30).
3. If the test fails again, select **Instrument ▸ Lamp Status/Replacement**.
4. If the Lamp Status/Replacement dialog box reports the lamp status as Failed, replace the halogen lamp (see "Replacing the Halogen Lamp" on page 118).
5. Perform the Instrument Function Test (see page 30).
6. If the test fails again, contact Applied Biosystems technical support (see page xiii) or your service representative.

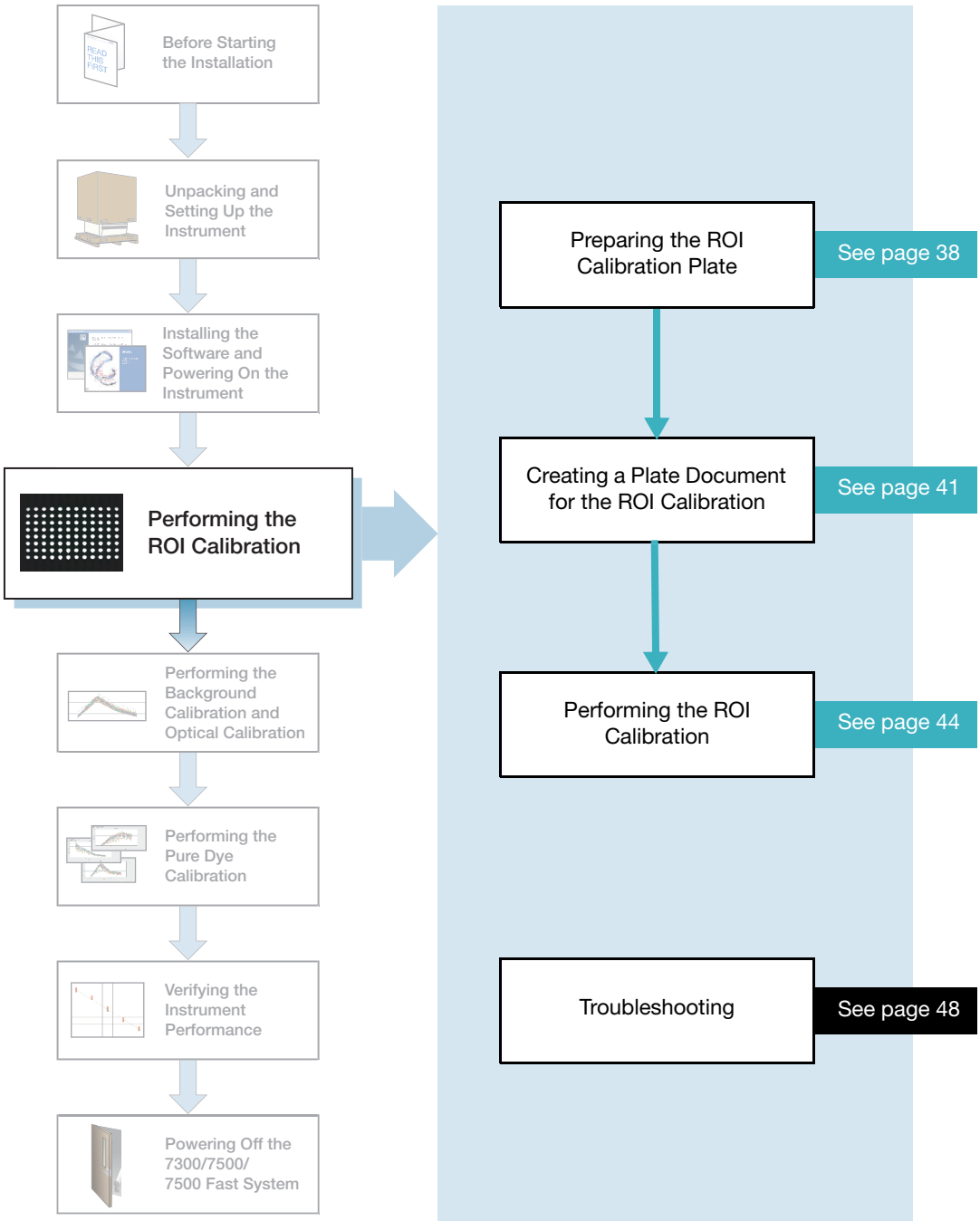


Condition: Thermal Cycler Test Failure

1. Power off the instrument, wait 30 seconds, then power on the instrument.
2. Perform the Instrument Function Test (see page 30).
3. If the test fails again, contact Applied Biosystems technical support (see page xiii) or your service representative.

Notes

Performing the Regions of Interest (ROI) Calibration



Notes

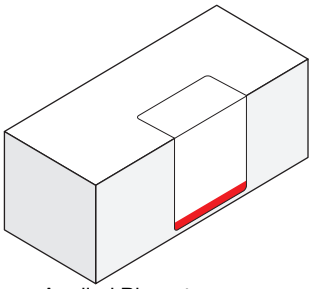
Overview

Note: You can also use the Real-Time System Setup wizard to perform the procedure in this chapter. To access the wizard, select start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.

Time Required

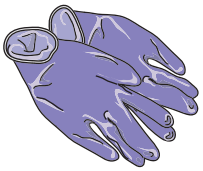
30 minutes

Materials Required



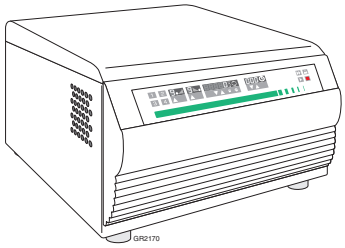
Applied Biosystems Real-Time PCR System Spectral Calibration Kit

ROI Calibration Plate



Powder-free Gloves

Safety Goggles



Centrifuge with plate adapter

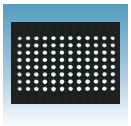
When to Perform the ROI Calibration

Perform a regions of interest (ROI) calibration:

- When installing the system (you must perform all calibrations in this order: ROI ▸ background ▸ optical (7500/7500 Fast systems only) ▸ pure dye ▸ instrument verification).
- Every 6 months, or as often as necessary, depending on instrument use.
- After replacing the lamp.

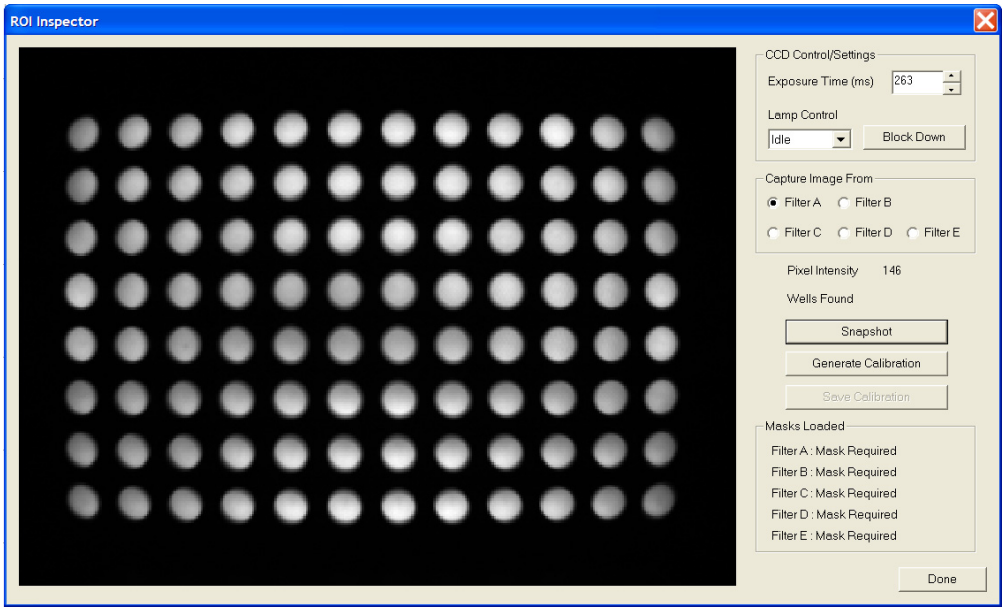
IMPORTANT! After every ROI calibration, you must perform a background calibration, optical calibration (7500/7500 Fast systems only), pure dye calibration, and instrument verification.

Notes



Purpose of the ROI Calibration

A regions of interest (ROI) calibration maps the positions of the wells on the sample block so that the software can associate increases in fluorescence during a run with specific wells of the plate. Because the instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during runs, you must generate a calibration image for each individual filter to account for minor differences in the optical path.



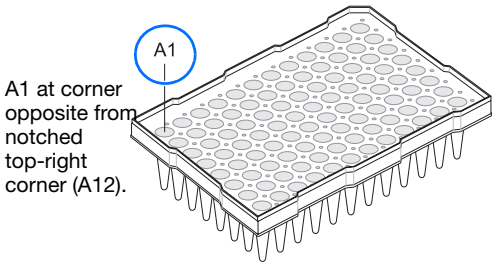
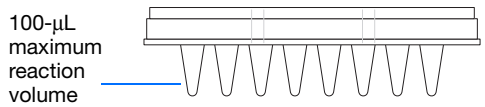
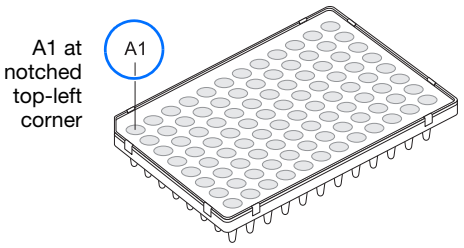
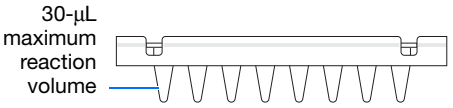
ROI calibration image

Notes

Preparing the ROI Calibration Plate

Standard Plates versus Fast Plates

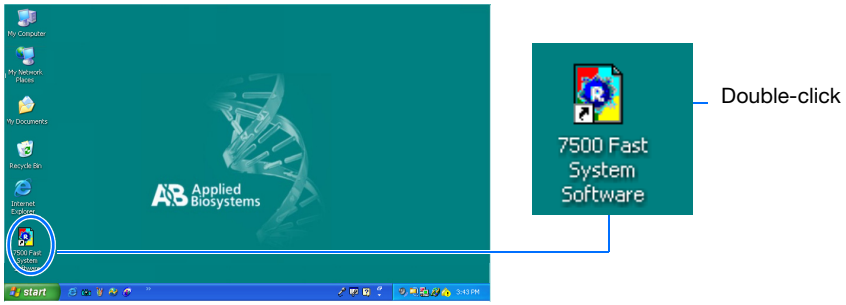
Use the plate appropriate for your system.

Standard Plates – 7300 and 7500 Systems	Fast Plates – 7500 Fast Systems
Notched corner at top right   **Vortex** standard plates to ensure complete mixing, then **centrifuge** to ensure that all reagents are contained in the bottom of the well. **Note:** Optical 96-Well Fast Plates do not fit correctly into the standard block.	Notched corner at top left   **Centrifuge** Fast plates to ensure that all reagents are in the bottom of the well. **IMPORTANT! Do not vortex** Fast plates. Vortexing can cause loss of smaller reaction volumes. **Note:** Standard plates are not compatible with the 7500 Fast system and may be crushed by the 96-Well Fast Block.

Preparing the Plate

IMPORTANT! Wear powder-free gloves when you handle the ROI calibration plate.

1. In the desktop, double-click the icon for the SDS software ( ) to start the software.



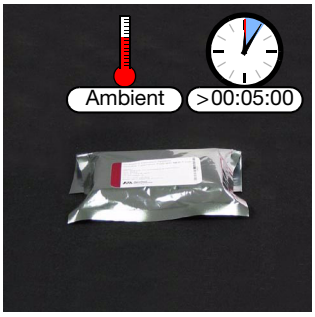
Notes

2. Retrieve the spectral calibration kit from the freezer, then remove the prepared ROI calibration plate.

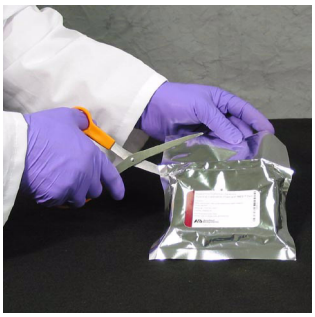
3. Return the spectral calibration kit to the freezer.

4. Allow the ROI calibration plate to warm to room temperature (approximately 5 minutes).

   IMPORTANT! Do not remove an ROI calibration plate from its packaging until you are ready to run it. The fluorescent dye in the wells of the plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.



5. Remove the ROI calibration plate from its packaging. Leave the optical film on the plate.



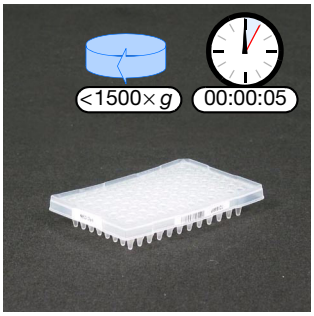
Do not discard the packaging for the ROI calibration plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

1. **Standard plates only**: Vortex the plate for 5 seconds. Do not vortex Fast plates.

   (Remaining steps apply to both standard and Fast plates.)

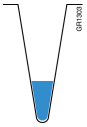
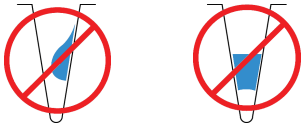
2. Briefly centrifuge the ROI calibration plate in a centrifuge with a plate adapter (<1500x*g*).

   IMPORTANT! The plate must be well mixed and centrifuged.



Notes

3. Verify that the liquid in each well of the ROI plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

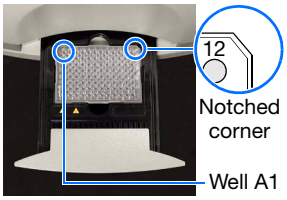
Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

Loading the Plate

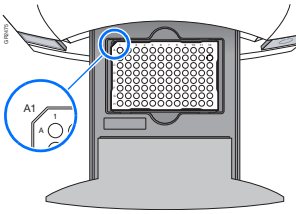
1. Press the tray door to open it.

2. Load the ROI calibration plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.

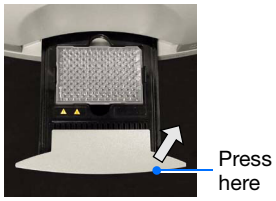
Load standard plates (7300/7500 system) with the notched A12 position at the top-right of the tray.



Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.



3. Close the tray. Apply pressure to the right side of the tray and at an angle.

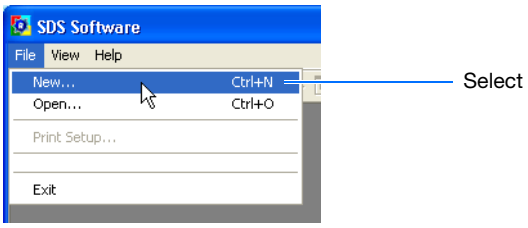


Continue with "Creating a Plate Document for the ROI Calibration" on page 41.

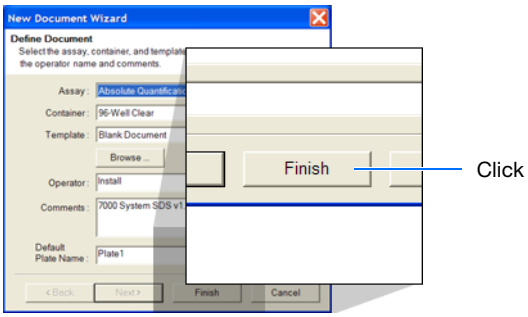
Notes

Creating a Plate Document for the ROI Calibration

1. Click (or select **File** ▸ **New**).

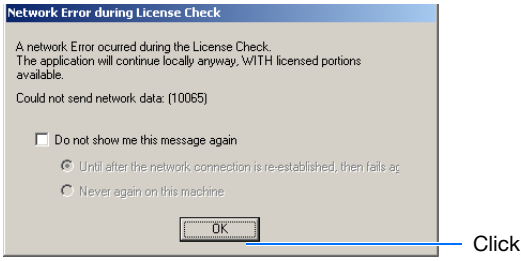


2. In the New Document wizard, click Finish to accept the default parameters.



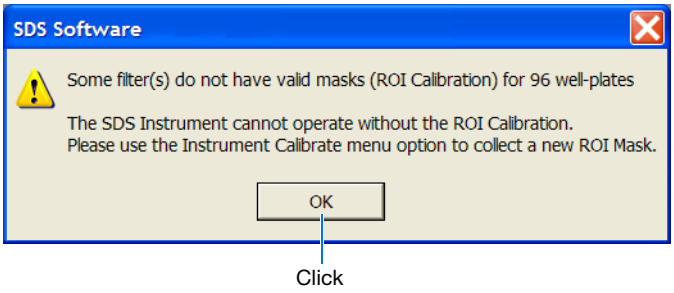
Note: It is not necessary to name or save the plate document. The SDS software automatically saves the ROI data to a set of calibration files on the computer hard drive.

If a Network communications error is displayed, click OK. You can perform the ROI calibration even if this error is displayed.



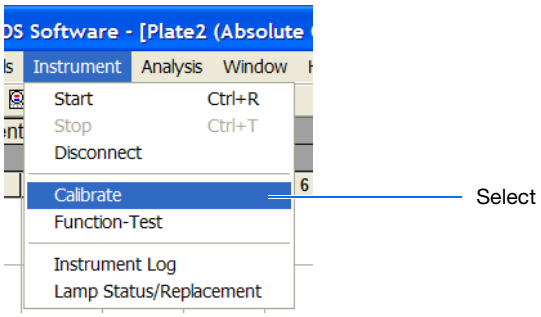
Note: This message is displayed if your computer is not connected to a network. If you do not plan to connect this computer to a network, select **Do not show me this message again**, then select **Never again on this machine**.

If an ROI error is displayed, click OK to proceed with ROI calibration.



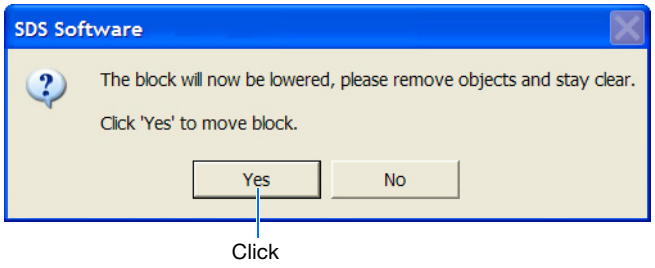
Notes

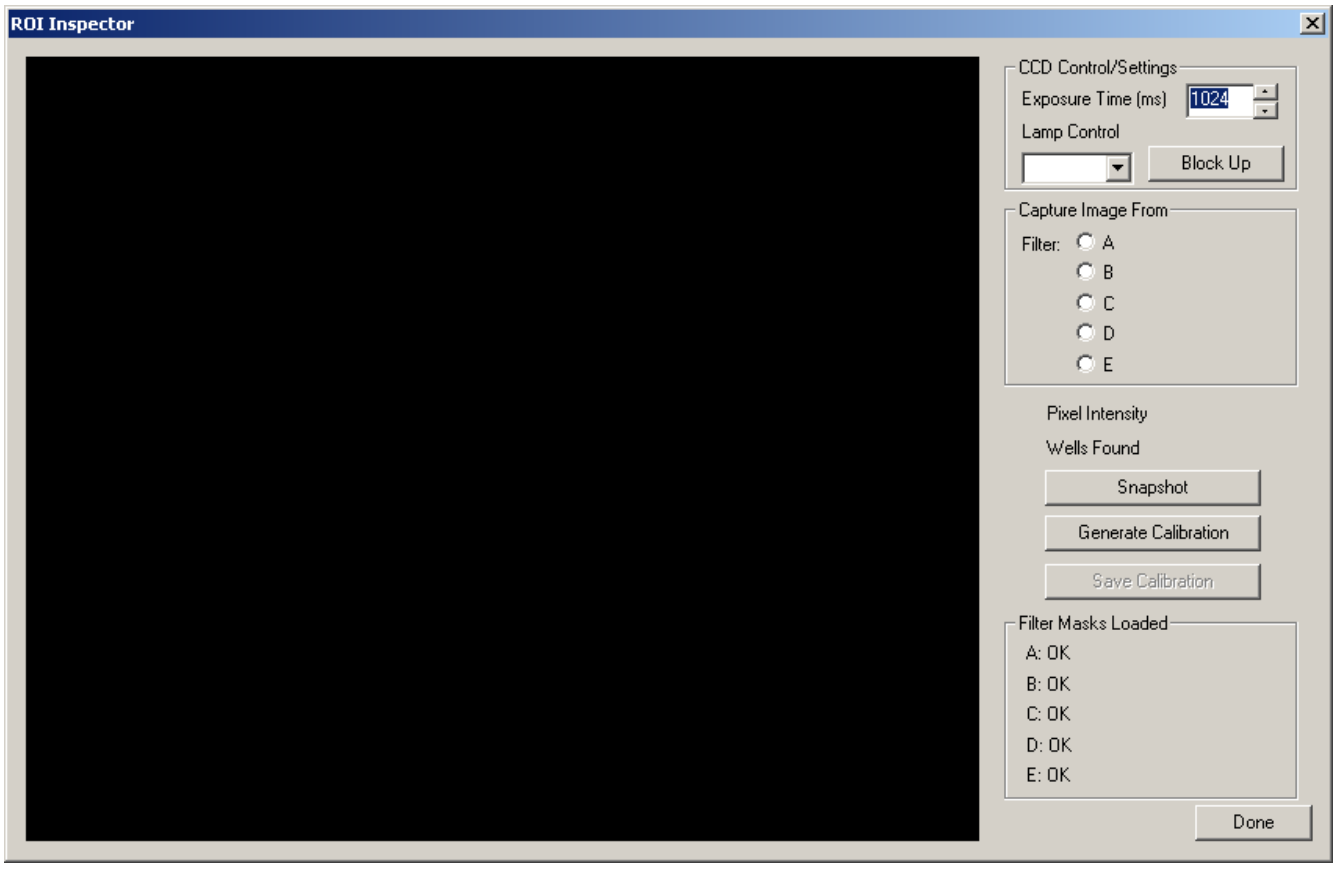
3. In the SDS software, select **Instrument ▸ Calibrate**.



4. In the warning dialog box, click Yes to lower the sample block.

   The ROI Inspector dialog box opens.

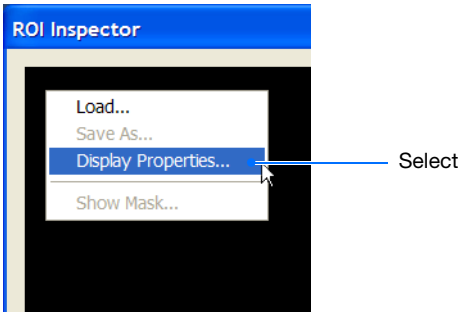




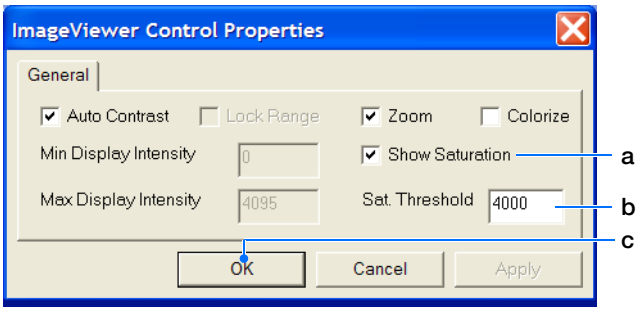
ROI Inspector

Notes

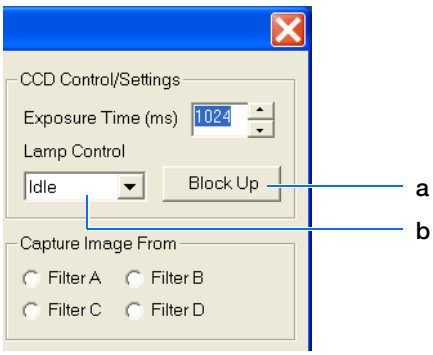
5. In the ROI Inspector dialog box, right-click the black area of the window, then select **Display Properties**.



6. In the Image Viewer Control Properties dialog box:
   a. Select **Show Saturation**.
   b. In the Sat Threshold field, enter **4000**.
   c. Click OK.



7. In the ROI Inspector dialog box, set the lamp control:
   a. Click Block Up.
   b. Select **Idle** from the Lamp Control drop-down list (sets the lamp to lower voltage for ROI calibration).

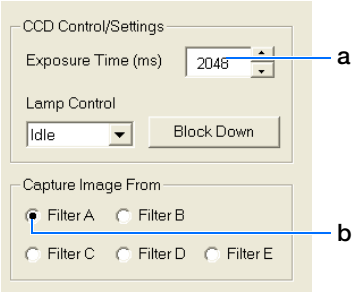


Continue with "Performing the ROI Calibration" on page 44.

Notes

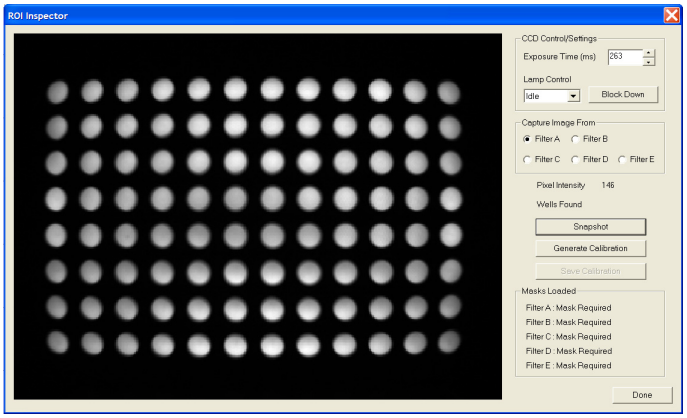
Performing the ROI Calibration

1. In the ROI Inspector dialog box:
   a. In the Exposure Time field, enter **2048**.
   b. Select **Filter A**.

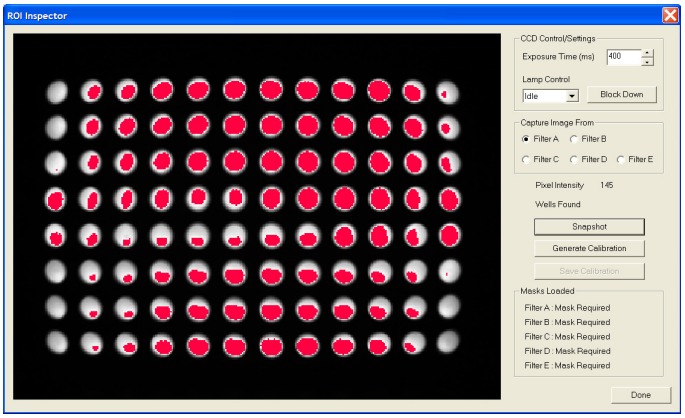


2. Click Snapshot to generate an ROI image.

3. Determine if your ROI image is acceptable (the figures to the right show an unsaturated image and an oversaturated image). Wells in an acceptable image:
   - Must be as bright as possible without oversaturating. (When you generate the ROI calibration on page 45, a warning is displayed if wells are oversaturated).
   - Can contain some, but do not have to contain any, red pixels, which represent saturation.



Unsaturated ROI image



Over-saturated ROI image

Unsaturated	Normal Saturation	Over Saturated
		
Good	Acceptable	Bad

4. If your ROI image is acceptable, skip to step 5.

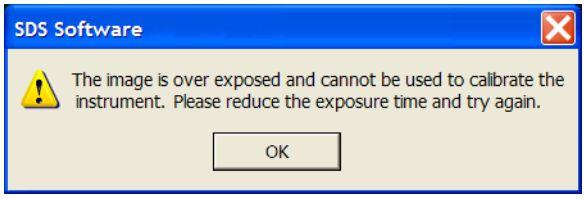
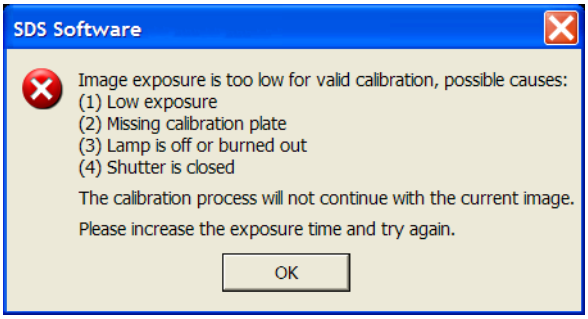
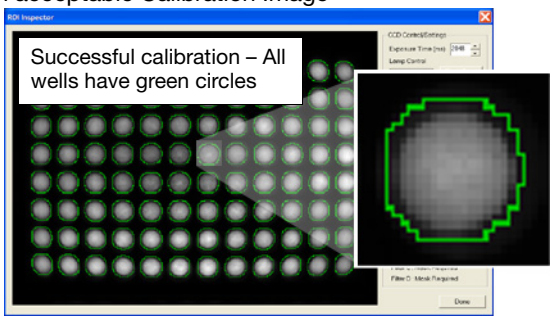
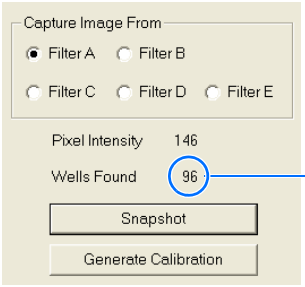
   If your ROI image is oversaturated, decrease the Exposure Time by half, then click Snapshot. Repeat until you obtain an acceptable ROI image.

   If you cannot obtain an acceptable image, see "Troubleshooting" on page 48.

Notes

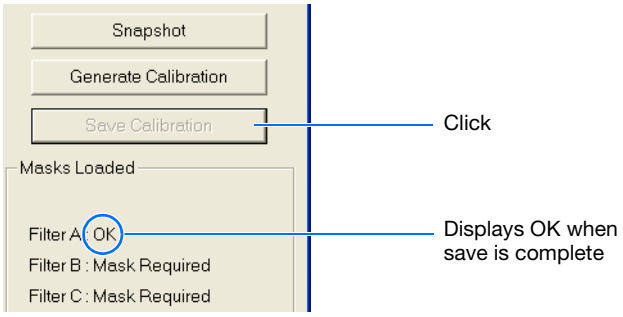
5. Click Generate Calibration.

The software takes a snapshot, then displays a message dialog box or an ROI image:

If the software displays:	Do this:
 *or* 	a. Click OK. b. Decrease the value in the Exposure Time field (see step 1a) by half. c. Repeat steps 2 and 5.
A very faint ROI image or you cannot generate a successful calibration.	See "Troubleshooting" on page 48.
An acceptable Calibration Image  **Note:** A green circle indicates that the region of interest for the well position is bright enough.	Verify that the: • Wells Found value is 96.  • ROI Image displays a green circle around each well area.

6. Click Save Calibration.

The software saves the newly generated ROI calibration for Filter A. "OK" appears next to Filter A in the Masks Loaded section of the ROI Inspector window.

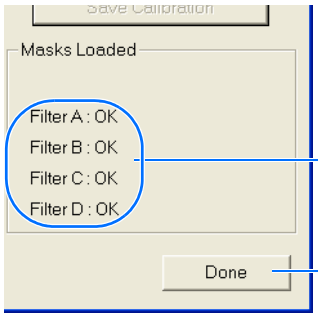


Notes

7. Repeat steps 1 through 5 for the remaining filters: Filter B, Filter C, and Filter D (and Filter E for 7500 and 7500 Fast instruments).

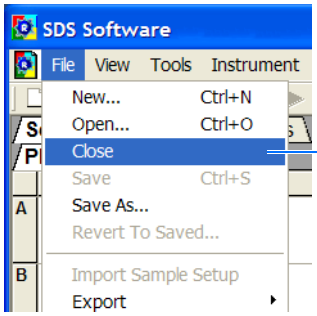
   Reset the Exposure Time to **2048** before performing the calibration for each filter.

   The ROI calibration is complete when Masks Loaded for all the Filters displays OK.



8. Click Done to close the ROI Inspector.

9. In the SDS software, select **File ▸ Close**.

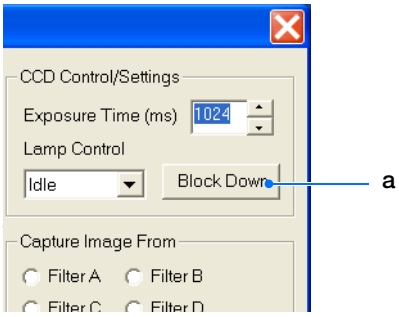


10. When prompted to save the plate document, click No.

Unloading the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. In the ROI Inspector dialog box, click Block Down.



2. Press the tray door to open it.

Note: If you cannot open the tray door, the sample block may be in its raised position, locking the tray position. To lower the block, click Block Down.

Notes

3. Press the tray door to move the tray into the instrument.

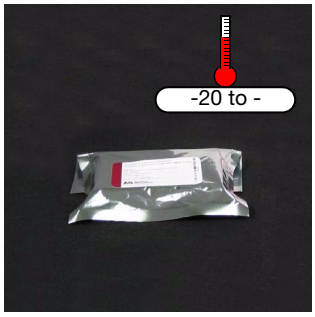
4. Place the calibration plate inside its packaging sleeve.

5. **7300 systems:** Return the packaged plate to the spectral calibration kit in the freezer.

   7500/7500 Fast systems:

   – If you will perform background and optical calibrations (described in the next chapter) within the next 8 hours, keep the ROI calibration plate at room temperature in the packaging. The optical calibration you perform after background calibration uses the ROI calibration plate.

   – If you be perform background and optical calibrations on another day, return the packaged plate to the spectral calibration kit in the freezer.



Note: Do not discard the ROI calibration plate. If you store the plate in its packaging sleeve at –20 to –25 °C, you can use the plate up to three times after you open it.

Continue with "Performing the Background Calibration and Optical Calibration" on page 49.

IMPORTANT! After you perform an ROI calibration, you must also perform a background calibration (see page 49), an optical calibration (7500 systems only, see page 59), and pure dye calibrations (see page 67), and instrument verification (see page 87).

Notes

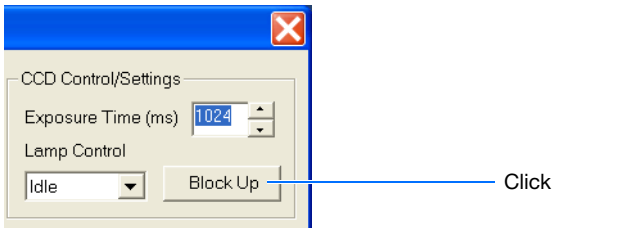
Troubleshooting

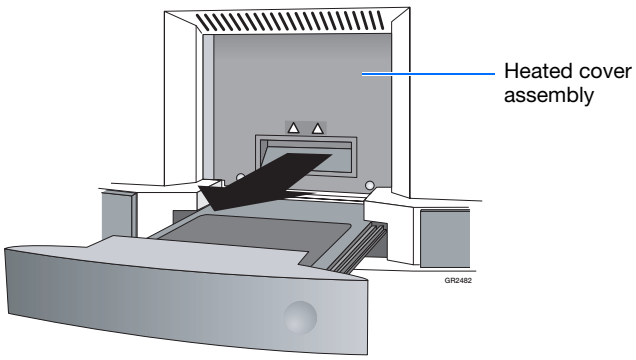
Troubleshooting – ROI Image

Condition: The ROI image is faint or you cannot generate a successful calibration.

The sample block may be in its lowered position.

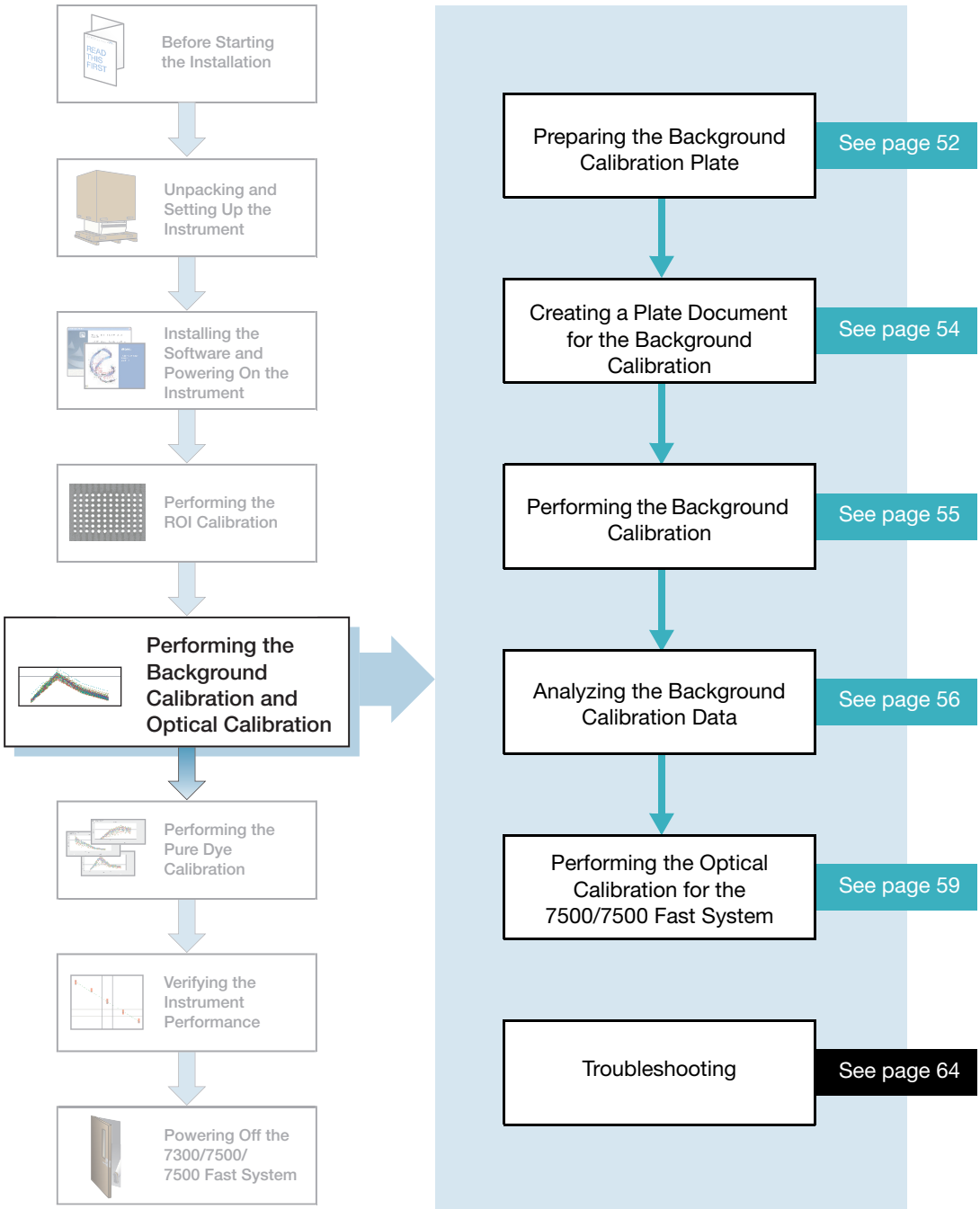
- If the CCD Control Settings in the ROI Inspector displays Block Up, click Block Up to raise the sample block.
- Check that the heated cover assembly is pulled all the way forward to ensure that the tray can be pushed in properly.





Notes

Performing the Background Calibration and Optical Calibration



Notes

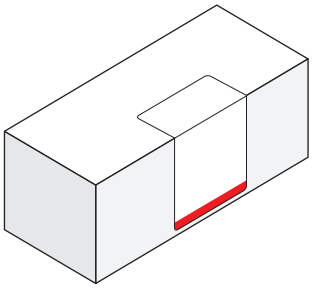
Overview

Note: You can also use the Real-Time System Setup wizard to perform the procedure in this chapter. To access the wizard, select start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.

Time Required

- **7300 systems:** Background calibration: 30 minutes
- **7500 systems:**
  - Background calibration: 30 minutes
  - Optical calibration: 10 minutes

Materials Required

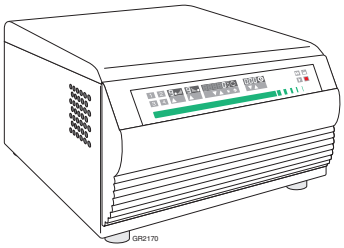


Applied BiosystemsReal-Time PCR System Spectral Calibration Kit

Background Plate

Powder-free Gloves

Safety Goggles



Centrifuge with plate adapter

When to Perform a Background Calibration

Perform a background calibration:

- When installing the system (you must perform all calibrations in this order: ROI ▸ background ▸ optical (7500/7500 Fast systems only) ▸ pure dye ▸ instrument verification).
- Monthly or as often as necessary, depending on instrument use.
- After replacing the lamp.

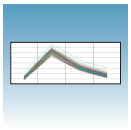
IMPORTANT! 7500/7500 Fast systems only: You must perform an optical calibration after every background calibration.

Purpose of the Background Calibration

A background calibration measures the level of background fluorescence in the instrument. During a background calibration run, the system:

- Performs continuous reads of a background plate containing PCR buffer for 10 minutes at 60 °C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

Notes



The software then uses the calibration file during subsequent runs to remove the background fluorescence from the run data.

Background Fluorescence

Fluorescence data collected by the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System includes a fluorescent signal inherent to the system, commonly referred to as background fluorescence. Background fluorescence is a composite signal found in all spectral data. This signal consists of fluorescence from several sources, including:

- Background electronic signal
- Contaminants in the sample block
- The plastic consumable (plates and caps)

Guidelines for Calibration

For a new instrument:

- Always start with a new calibration kit.
- Make sure the centrifuge you use is clean. Before centrifuging, wipe down the bucket using a tissue.
- Handle the calibration plates with care to prevent contamination. Do not place plates on a lab bench, which may contaminate the plate. Always put calibration plates back into their original bags.

Notes

Preparing the Background Calibration Plate

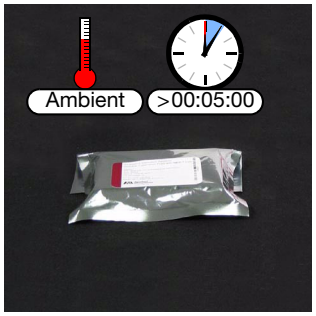
IMPORTANT! Wear powder-free gloves when you handle the background plate.

1. Retrieve the spectral calibration kit from the freezer, then remove the prepared background plate.

   Alternatively, create a background calibration plate as described in Appendix B, "Creating a Background Plate."

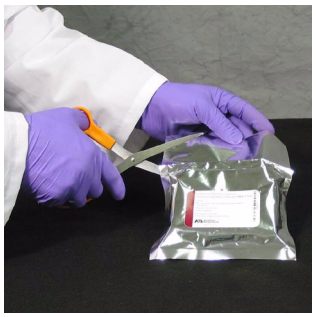
2. Return the spectral calibration kit to the freezer.

3. Allow the background plate to warm to room temperature (at least 5 minutes).



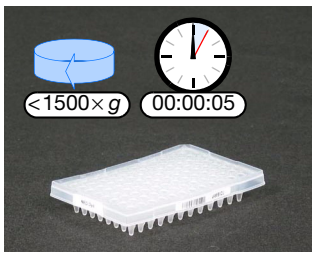
4. Remove the background plate from its packaging.

   IMPORTANT! Do not discard the packaging for the background plate. The background plate can be used up to three times if it is stored in its original packaging sleeve.

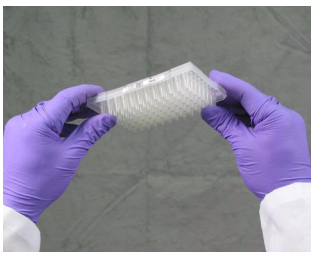


5. **Standard plates only**: Vortex the plate for 5 seconds. Do not vortex Fast plates.

   (Remaining steps apply to both standard and Fast plates.)

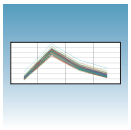


6. Briefly centrifuge the background plate in a centrifuge with a plate adapter (<1500x*g*).



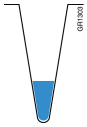
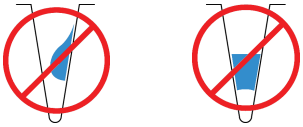
   IMPORTANT! The plate must be well mixed and centrifuged.

Notes



7. Verify that the liquid in each well of the background plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

   IMPORTANT! Do not allow the bottom of the background plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block and cause an abnormally high background signal.

Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

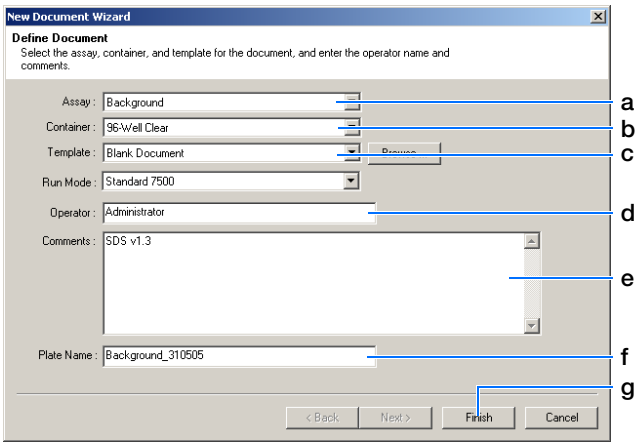
Continue with "Creating a Plate Document for the Background Calibration" on page 54.

Notes

Creating a Plate Document for the Background Calibration

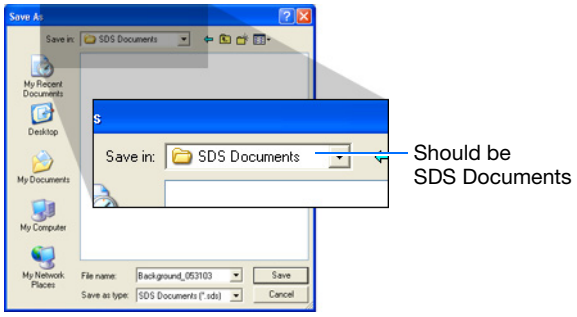
1. In the SDS software, click (or select **File ▸ New**).

2. Configure the New Document dialog box:
   a. Select **Assay ▸ Background**.
   b. Select **Container ▸ 96-Well Clear**.
   c. Select **Template ▸ Blank Document**.
   d. In the Operator field, enter your name.
   e. In the Comments field, enter any additional information that you want to save to the file (such as the plate bar code).
   f. In the Plate Name field, enter:
      Background_*<date in DDMMYY format>*
      For example, the name for a plate run on May 31, 2005 would be: Background_310505.
   g. Click Finish.



3. In the SDS software, select **File ▸ Save As**.

4. In the Save As dialog box:
   a. If the Save in field does not display SDS Documents, navigate to **D: drive ▸ Applied Biosystems ▸ SDS Documents**.
   b. Click Save.



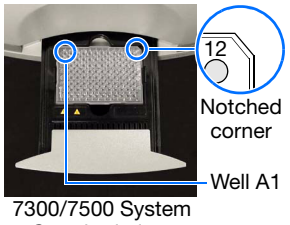
Continue with "Performing the Background Calibration" on page 55.

Notes

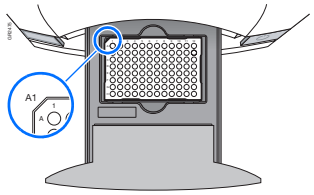
Performing the Background Calibration

1. Load the plate in the instrument as described in "Loading the Plate" on page 40.

   Note: If you cannot open the tray, the sample block may be in its raised position, locking the tray position. To lower the block, select **Instrument ▸ Calibrate**, then exit the ROI Inspector.



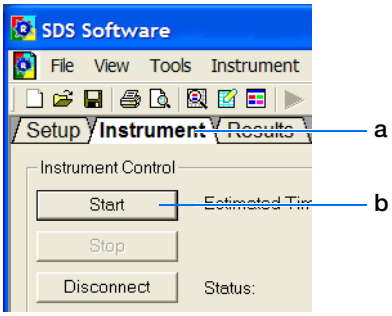
7300/7500 System
Standard plate



7500 Fast System
Fast plate

2. In the SDS software:
   a. Select the **Instrument** tab.
   b. Click Start.

   The instrument begins the background calibration run.



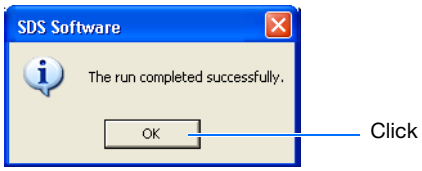
   Note: Before starting the run, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the correct temperature.

Continue with "Analyzing the Background Calibration Data" on page 56.

Notes

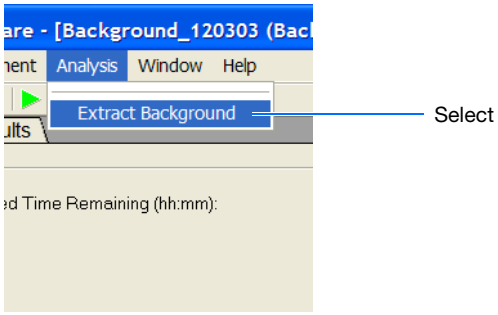
Analyzing the Background Calibration Data

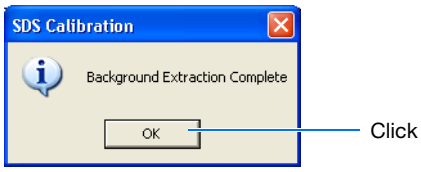
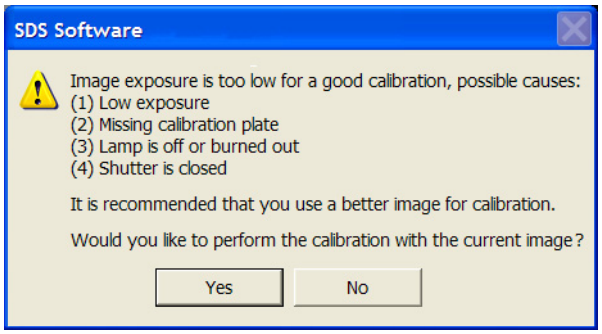
1. When the run is complete, click OK.



2. Click ▶ (or select **Analysis ▸ Extract Background**).

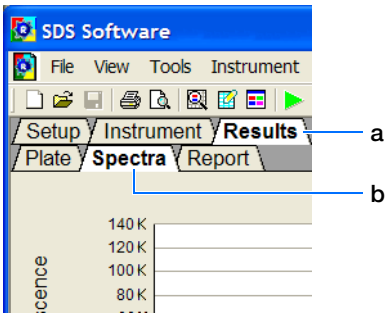
   The software extracts the background signal, then displays one of the following messages:



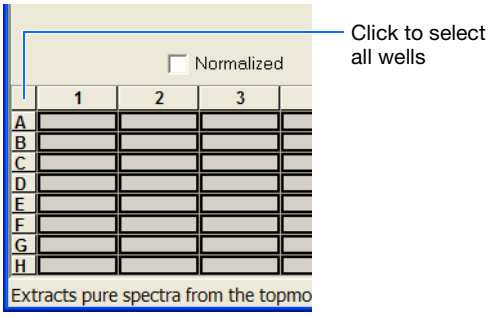
If the software displays:	Do this:
Background Extraction Complete – The analysis is successful. 	Click OK, then go to step 4.
Image exposure is too low… – The software stopped the extraction because several raw spectra are at or below the detectable threshold for the calibration. 	a. Click No. b. Verify that the instrument contains the background plate. c. Test the lamp. See "Monitoring Lamp Status" on page 117. d. Run the background plate again. e. If the SDS software continues to display the Image exposure is too low… dialog box, click Yes, then go to step 4.
Image exposure is too high… – The run is unsuccessful. The software stopped the extraction because one or more raw spectra exceed the maximum limit for the 7300/7500/7500 Fast system.	Click No, then troubleshoot the failed run. See "Troubleshooting" on page 64.

Notes

4. In the plate document, select the **Results** tab, then select the **Spectra** tab.

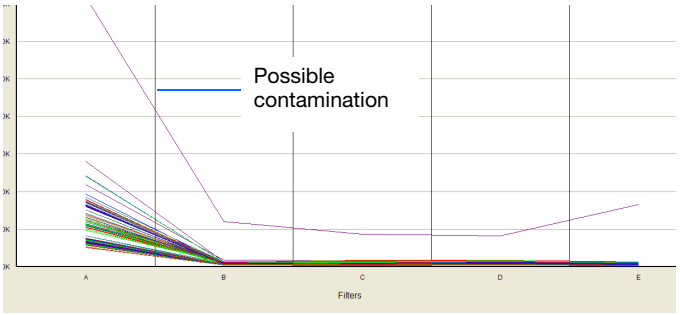


5. Select all wells of the plate document.



6. Inspect the raw data for irregular spectral peaks that exceed the following fluorescent standard units (FSU):

Filter	FSU
A, B, C, D (7300/7500)	>72,000
E (7500)	>90,000



If one or more wells produce raw spectra that exceed the specified FSU, the background plate or the sample block could contain a fluorescent contaminant. Determine the source of the contamination. See "Troubleshooting" on page 64.

7. Select **File** ▸ **Close**.

Notes

Unloading the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. When the run is complete, remove the background plate:
   a. Press the tray to open it.
   b. Remove the background plate.
   c. Press the tray to move it into the instrument.

2. Place the background plate inside its packaging sleeve, then return it to the spectral calibration kit in the freezer.

IMPORTANT! Do not discard the background plate. If you store the background plate in its original packaging sleeve, you can use the plate up to three times after you open it.

Continue with:

- **7300 system** – "Performing the Pure Dye Calibration" on page 67.
- **7500/7500 Fast system** – "Performing the Optical Calibration for the 7500/7500 Fast System" on page 59.

Notes

Performing the Optical Calibration for the 7500/7500 Fast System

Time Required 10 minutes

Materials Required ROI calibration plate

Purpose of the 7500/7500 Fast System Optical Calibration The optical calibration compensates for the physical effects of the additional filter present in 7500/7500 Fast instruments that is not present in the 7300 instrument.

When to Perform the Optical Calibration Perform an optical calibration:

- When installing the system (you must perform all calibrations in this order: ROI ▸ background ▸ optical (7500/7500 Fast systems only) ▸ pure dye ▸ instrument verification).
- After every 7500/7500 Fast system background calibration.

Preparing the ROI Calibration Plate

If you kept your ROI calibration plate at room temperature after performing an ROI calibration (described in Chapter 4, "Performing the Regions of Interest (ROI) Calibration") skip to step 6 below to spin down any condensation that may have formed when the plate was at room temperature.

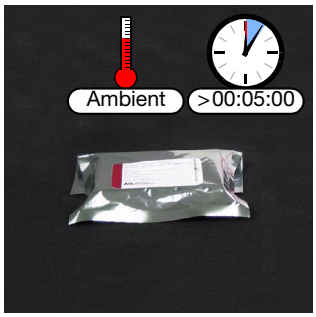
IMPORTANT! Wear powder-free gloves when you handle the ROI calibration plate.

1. Retrieve the spectral calibration kit from the freezer and remove the prepared ROI calibration plate.
2. Return the spectral calibration kit to the freezer.

5

Notes

3. Allow the ROI calibration plate to warm to room temperature (approximately 5 minutes).



IMPORTANT! Do not remove an ROI calibration plate from its packaging until you are ready to run it. The fluorescent dye in the wells of the plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.

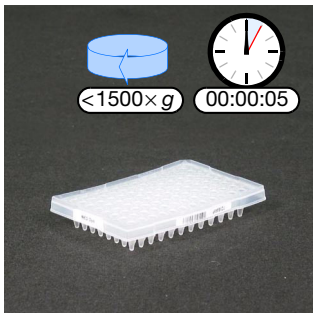
4. Remove the ROI calibration plate from its packaging.

IMPORTANT! Do not discard the packaging for the ROI calibration plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

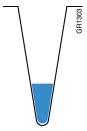
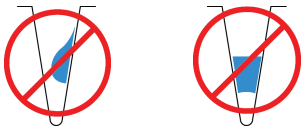
5. **Standard plates only**: Vortex the plate for 5 seconds. Do not vortex Fast plates.

   (Remaining steps apply to both standard and Fast plates.)

6. Briefly centrifuge the ROI calibration plate in a centrifuge with a plate adapter (<1500 x *g*).



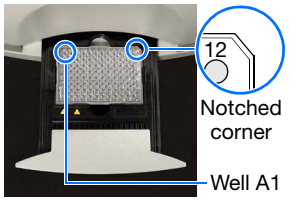
7. Verify that the liquid in each well of the background plate is positioned at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Correct	Incorrect
 Liquid is positioned at bottom of well.	 • Not centrifuged with enough force, or • Not centrifuged for enough time

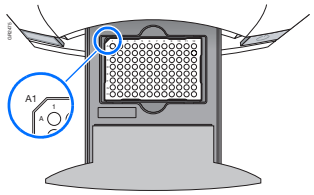
Notes

8. Load the plate in the instrument as described in "Loading the Plate" on page 40.

IMPORTANT! Wear powder-free gloves when you handle the ROI calibration plate.



7300/7500 System
Standard plate

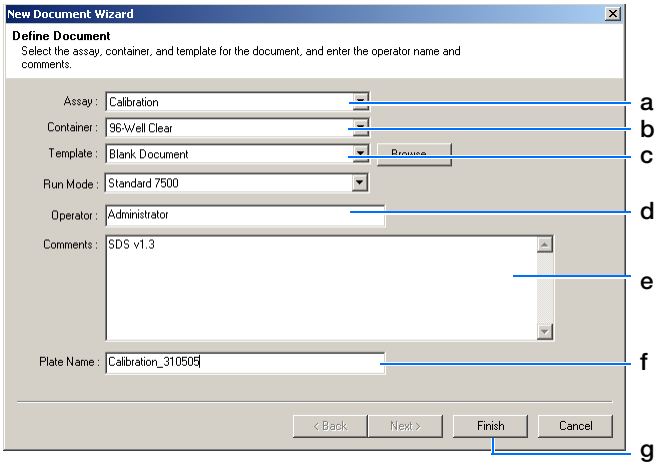


7500 Fast System
Fast plate

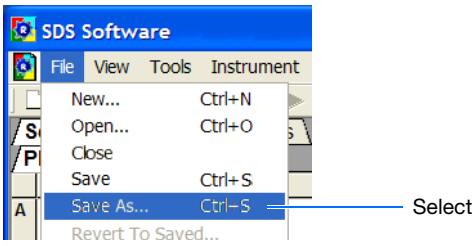
Creating a Plate Document for the Optical Calibration Run

1. Click (or select **File ▸ New**).

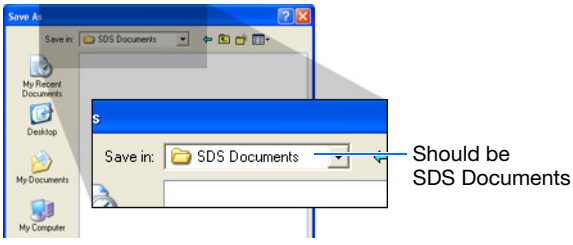
2. Configure the New Document dialog box:
   a. Select **Assay ▸ Calibration**.
   b. Select **Container ▸ 96-Well Clear**.
   c. Select **Template ▸ Blank Document**.
   d. In the Operator field, enter your name.
   e. In the Comments field, enter any additional information that you want to save to the file (such as the plate bar code).
   f. In the Plate Name field, enter:
   Calibration_*<date in DDMMYY format>*
   For example, the name for a calibration performed on May 31, 2005 would be: Calibration_310505.
   g. Click Finish.



3. In the SDS software, select **File ▸ Save As**.



4. In the Save As dialog box:
   a. If the Save in field does not display SDS Documents, navigate to **D: drive ▸ Applied Biosystems ▸ SDS Documents**.
   b. Click Save.



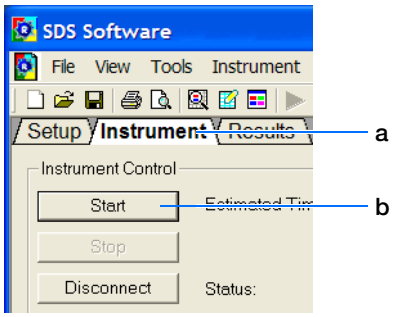
Continue with "Performing the Optical Calibration" on page 62.

Notes

Performing the Optical Calibration

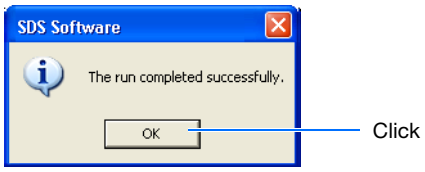
1. In the SDS software:
   a. Select the **Instrument** tab.
   b. Click Start.

   The instrument begins the calibration.

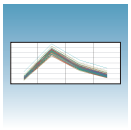


Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the correct temperature.

2. When the run is complete, click OK.



Notes



Analyzing the Optical Calibration Data

1. In the SDS software, click ▶ (or select **Analysis** ▸ **Extract**).

   The software extracts the optical calibration, then displays a message indicating the extraction is complete.

2. Click OK.

3. In the SDS software, select **File** ▸ **Close**.

Unloading the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the ROI calibration plate:
   a. Press the tray to open it.
   b. Remove the ROI calibration plate.
   c. Press the tray to move it into the instrument.

2. Place the ROI calibration plate inside its packaging sleeve, then return it to the spectral calibration kit in the freezer.

IMPORTANT! Do not discard the ROI calibration plate. If you store the plate in its original packaging sleeve, you can use the plate up to three times after you open it.

Continue with "Performing the Pure Dye Calibration" on page 67.

Notes

Troubleshooting

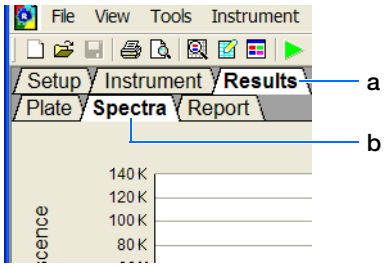
Troubleshooting – Background Calibration

Condition: Cannot extract the data (background calibration failed)

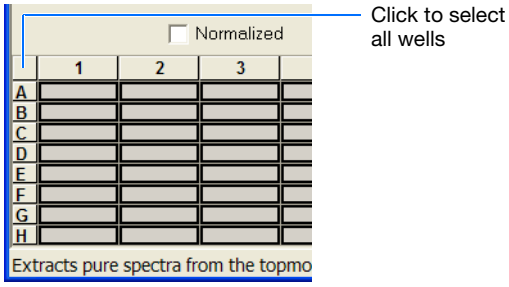
Signals that exceed 72,000 fluorescent standard units (FSU) are considered beyond the limit of normal background fluorescence for a 7300/7500/7500 Fast instrument. Such signals may indicate that either the background or the sample block contains fluorescent contaminants. Common contaminants include: ink residue from permanent pens, powder from disposable gloves, and dust.

To determine the source and location of the contamination:

1. In the plate document for the calibration:
   a. Select the **Results** tab.
   b. Select the **Spectra** tab.

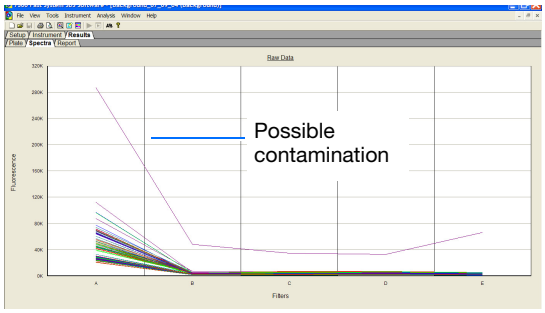


2. Select all wells of the plate document.



3. Inspect the raw background data for an irregular spectral peak or peaks.

   Wells producing raw spectra that exceed 72,000 FSU are considered irregular and could be contaminated.



Notes

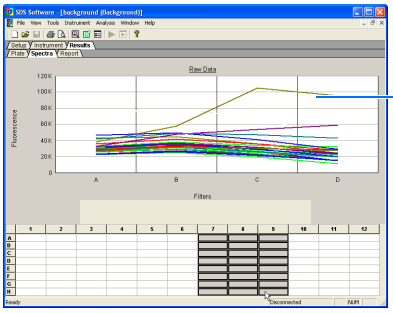
Troubleshooting – Background Calibration

4. Locate the contaminated well position(s) by selecting successively fewer wells in the plate document.

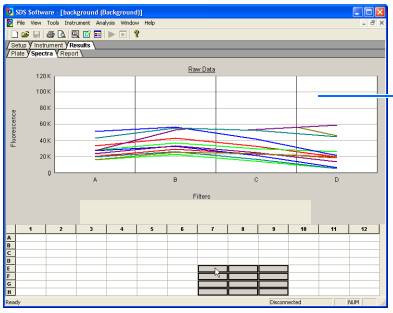
   The items that follow show an example of how to determine the location of a contaminated well.

   a. In the Spectra tab, select columns 1-6. The raw data from the selected wells does not include the irregular peak. Therefore, the contaminated well must be in columns 7-12.

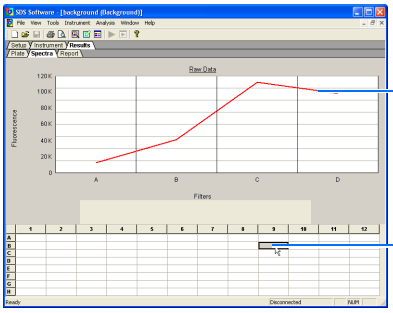
   b. Select columns 7-9. The raw data from the selected wells includes the irregular peak. The contaminated well must be in columns 7-9.



   c. Select wells in row E and below in columns 7-9. The raw data from the selected wells does not include the irregular peak. The contaminated well must be in the first four wells of columns 7-9.



   d. Finally, by selecting each of the wells from the first four wells of columns 7-9, you can determine the location of the contaminated well (B9).



5. Repeat step 4 until you identify the location of each contaminated well.

Notes

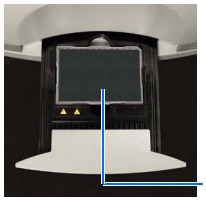
Troubleshooting – Background Calibration

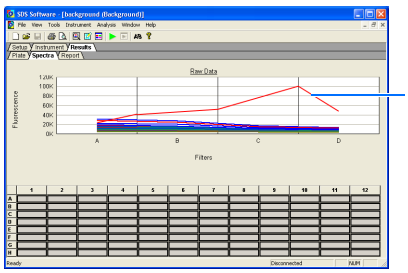
6. Create a new background plate (see Appendix B, “Creating a Background Plate” on page 125.)
7. Perform a background calibration (see “Performing the Background Calibration” on page 55).
8. Click (or select **Analysis** ▸ **Extract Background**).
9. Repeat step 4 on page 65 to examine the contaminated well position(s).

   If the contaminated well positions with the new background plate are:
   - In the same location as you saw in step 4, then the sample block is contaminated. Decontaminate the sample block (see “Decontaminating the Sample Block” on page 108).
   - No longer present, the original background plate was contaminated. You can inspect the original background plate Make sure there is no particulate matter on the bottom of the plate or on the cover.

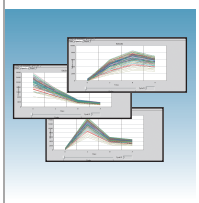
10. If the calibration fails after you use a new background plate or decontaminate the sample block, perform the following test:
    a. Press the tray to open it.
    b. Load the black plate tool from the packing kit (or a plate containing a piece of black paper) into the plate holder.
    c. Push the tray back into the instrument.
11. Perform a background calibration (see “Performing the Background Calibration” on page 55).
    a. Click (or select **Analysis** ▸ **Extract Background**).
    b. Select the **Results** tab, then select the **Spectra** tab.
    c. Select all wells of the plate document.
12. View the Spectral plot for the peak(s) and choose from the following:

    If the contaminated well is:
    - Present, then the optics of your 7300/7500/7500 Fast system may be contaminated. Contact Applied Biosystems technical support or your service representative for further assistance.
    - Absent, then the sample block is contaminated. Decontaminate the sample block (see “Decontaminating the Sample Block” on page 108).

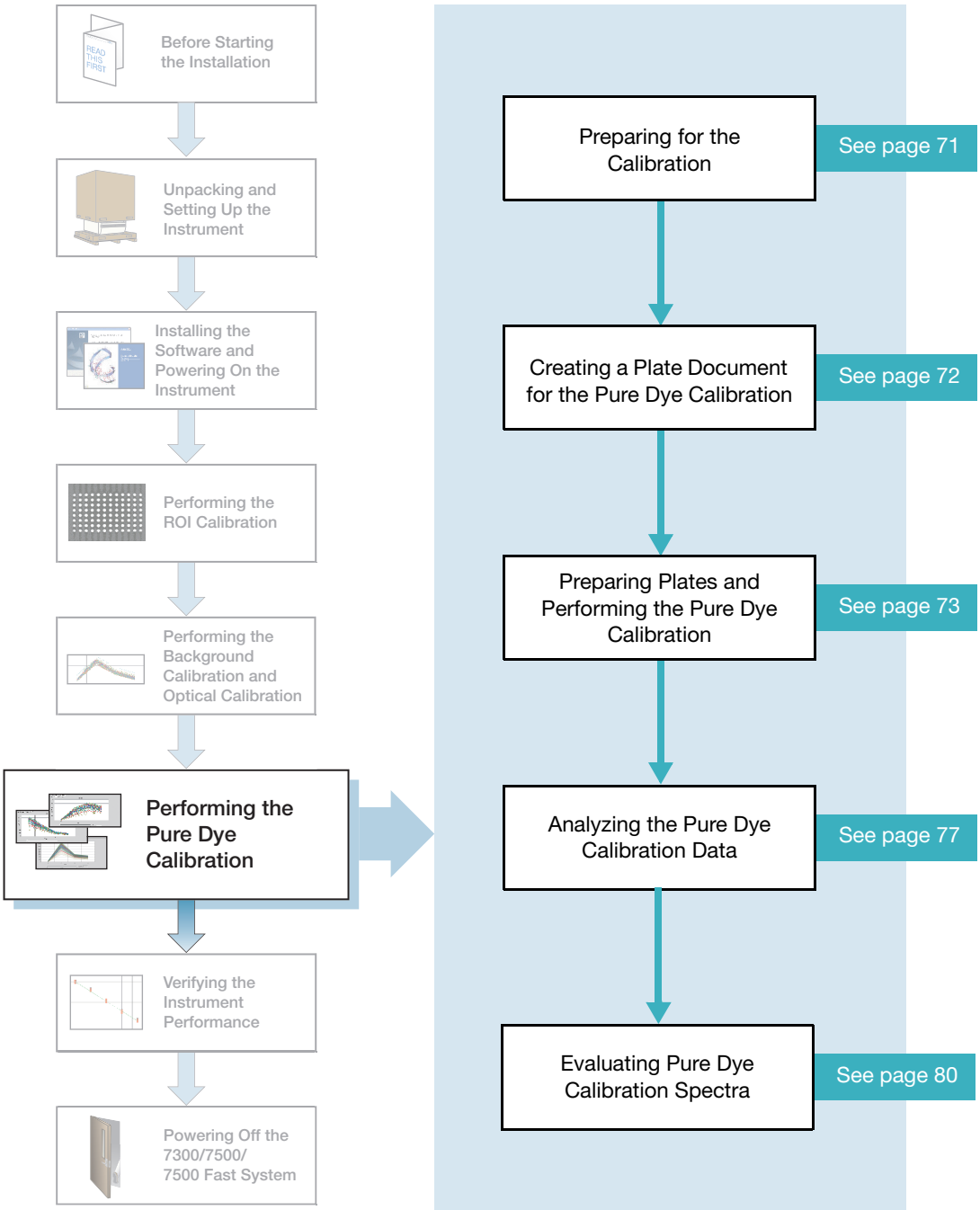




Notes



Performing the Pure Dye Calibration



Notes

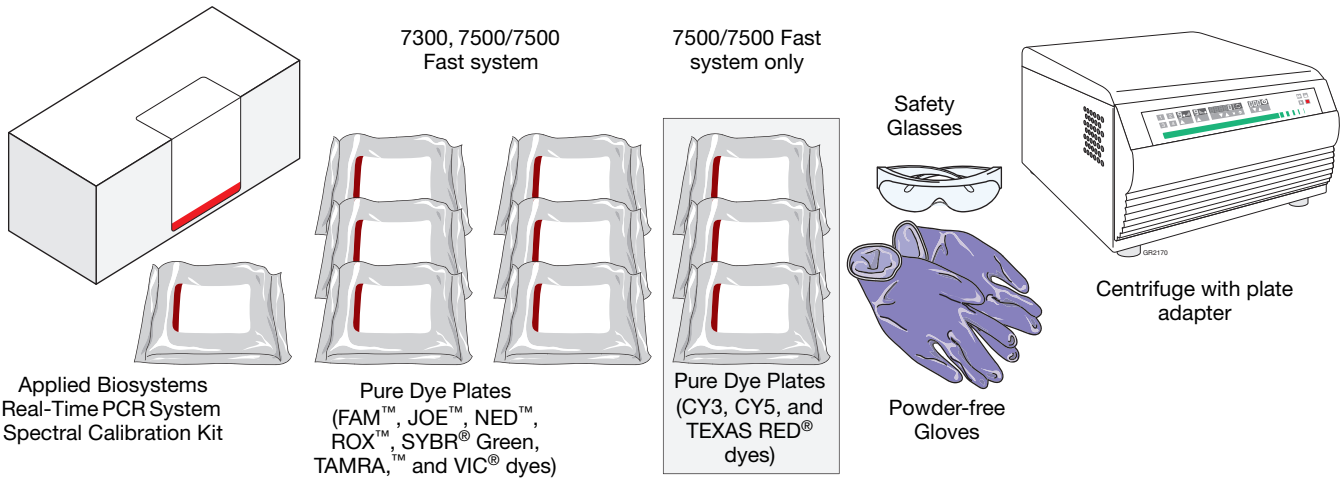
Overview

Note: You can also use the Real-Time System Setup wizard to perform the procedure in this chapter. To access the wizard, select start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.

Time Required

- **7300 systems:** 45 minutes
- **7500/7500 Fast systems:** 1 hour

Materials Required



When to Perform Pure Dye Calibrations

Perform a pure dye calibration:

- When installing the system (you must perform all calibrations in this order: ROI ▸ background ▸ optical (7500/7500 Fast systems only) ▸ pure dye ▸ instrument verification).
- Every 6 months, depending on instrument use.

IMPORTANT! You must perform a background run before every pure dye calibration. Because the age and use of instrument components can affect pure spectra readings, Applied Biosystems recommends performing a pure dye calibration at least every six months.

Notes

Purpose of Pure Dye Calibration

During a pure dye calibration run, the system:

- Collects spectral data from a series of pure dye standards.
- Stores the spectral information for the pure dye standards in the pure spectra run file, a calibration file in the SDS directory.

The software then uses the pure spectra data during subsequent runs to characterize pure dyes and distinguish the individual contribution of each dye in the collective fluorescence collected by the instrument during a run.

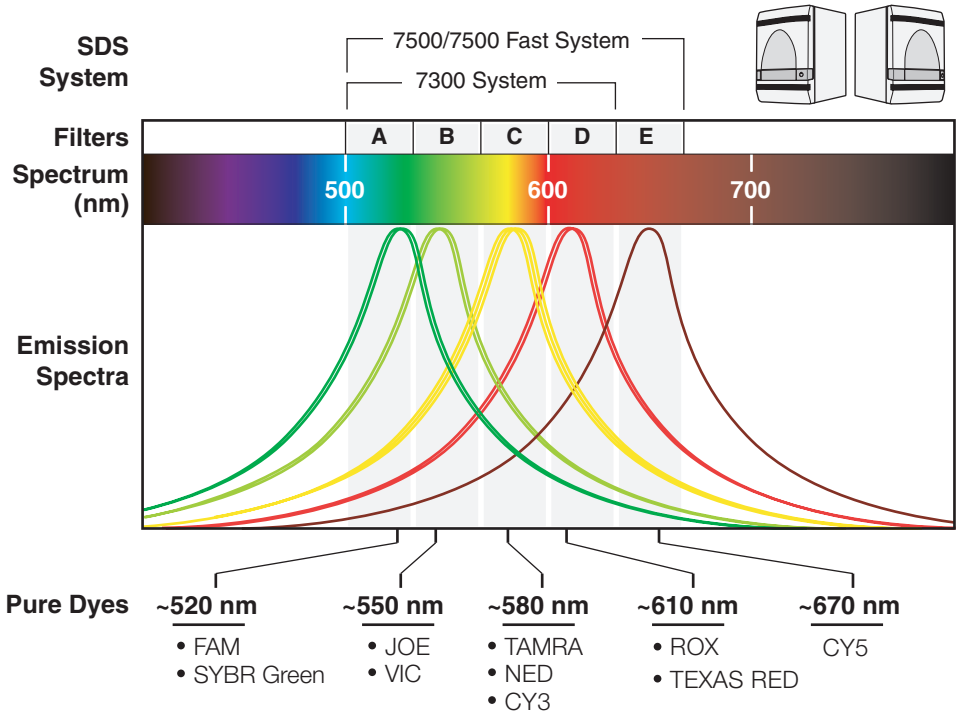
After each run, the SDS software receives run data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra file. When you save a plate document after analysis, the software stores the pure spectra file with the collected fluorescence data for that experiment.

Dye Sets

The Applied Biosystems Real-Time PCR Systems use the following dye sets for calibration:

- **7300 system** – FAM™, JOE™, NED™, ROX™, TAMRA™, VIC®, and the SYBR® Green I dsDNA binding dye, inside preloaded 96-well pure dye plates.
- **7500 and 7500 Fast systems** – All of the dyes listed above, plus CY3, CY5, and TEXAS RED® dyes (because of the additional filter).

The following figure shows the emission spectrum for each dye, and the filters and wavelengths at which each dye is read.



Notes

Custom Dyes

The 7300/7500/7500 Fast instrument supports the detection of custom pure dyes (dyes other than those provided by Applied Biosystems).

Custom dyes must fluoresce within the spectral range measured by the instrument:

- 500 to 650 nm for 7300 systems
- 500 to 700 nm for the 7500/7500 Fast systems

To add custom pure dyes to the Pure Dye set for your instrument, see "Creating a Custom Pure Dye Plate" on page 127.

Notes

Preparing for the Calibration

IMPORTANT! Before performing a pure dye calibration, you must perform an ROI calibration (see page 35), a background calibration (see page 49), and an optical calibration (7500 systems only, see page 59).

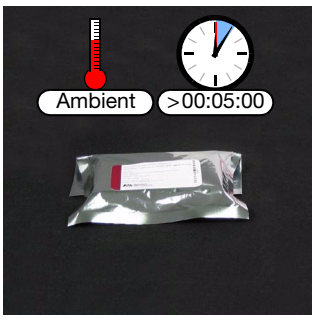
IMPORTANT! Wear powder-free gloves when you handle the pure dye plates.

1. Retrieve the spectral calibration kit from the freezer, then remove all of the pure dye plates.

2. Return the spectral calibration kit to the freezer.

3. Allow the pure dye plates to warm to room temperature (approximately 5 minutes).

   IMPORTANT! Do not remove a pure dye plate from its packaging until you are ready to run it. The fluorescent dye in the wells of each pure dye plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.



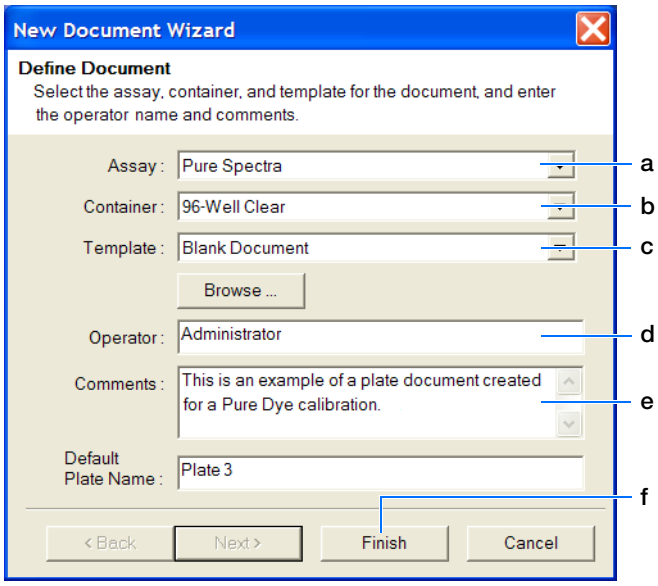
Continue with "Creating a Plate Document for the Pure Dye Calibration" on page 72.

Notes

Creating a Plate Document for the Pure Dye Calibration

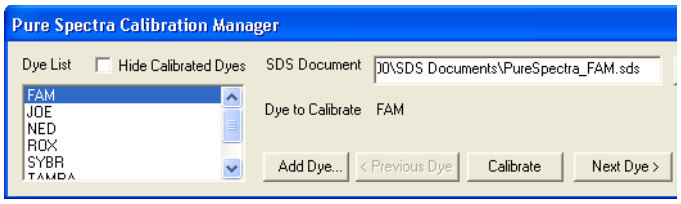
1. In the SDS software, click (or select **File ▸ New**).

2. Configure the New Document dialog box:
   a. Select **Assay ▸ Pure Spectra**.
   b. Select **Container ▸ 96-Well Clear**.
   c. Select **Template ▸ Blank Document**.
   d. In the Operator field, enter your name.
   e. In the Comments field, enter any information that you want to attach to the file (such as the plate bar code).
   f. Click Finish.



Note: It is not necessary to name or save the pure dye plate document. The SDS software automatically saves the pure dye data to a calibration file on the computer hard drive.

The Pure Spectra Calibration Manager is displayed.



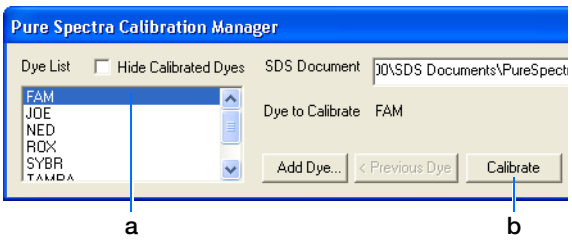
Continue with "Preparing Plates and Performing the Pure Dye Calibration" on page 73.

Notes

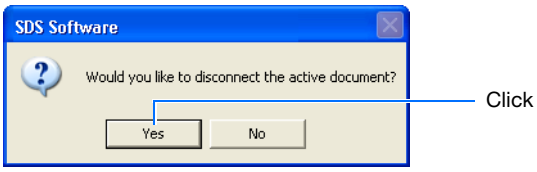
Preparing Plates and Performing the Pure Dye Calibration

Selecting the Dye

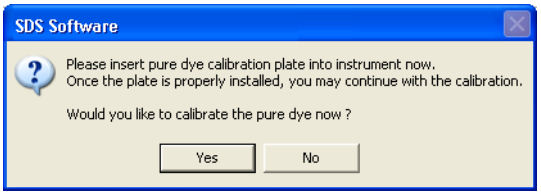
1. In the Pure Spectra Calibration Manager:
   a. In the Dye List field, select a pure dye to calibrate.
   b. Click Calibrate.



   c. If you are prompted to disconnect the plate document, click Yes.



   d. A message prompts you to load the plate. Do not click Yes or No at this time.



Preparing and Loading a Pure Dye Plate

1. Remove the appropriate pure dye plate from its packaging.

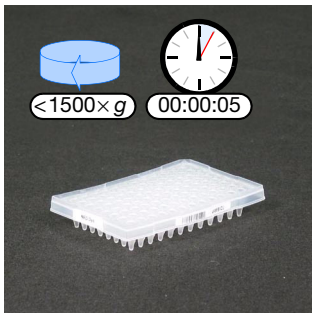
IMPORTANT! Do not discard the packaging for the pure dye plate. The pure dye plate can be used up to three times if it is stored in its original packaging sleeve.

2. **Standard plates only**: Vortex the plate for 5 seconds. Do not vortex Fast plates.

   (Remaining steps apply to both standard and Fast plates.)

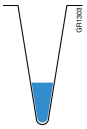
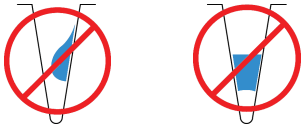
Notes

3. Briefly centrifuge the pure dye plate in a centrifuge with a plate adapter (<1500x*g*).

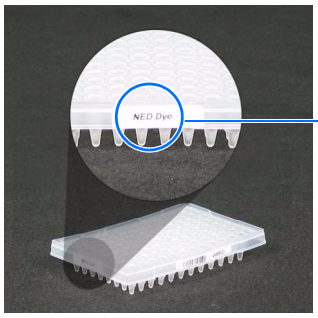


4. Verify that the dye standard in each well of the pure dye plate is at the bottom of the well.

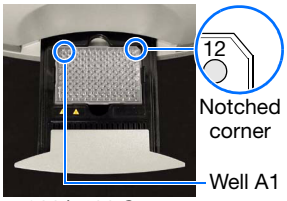
   If not, centrifuge the plate again at a higher speed and for a longer period.

Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

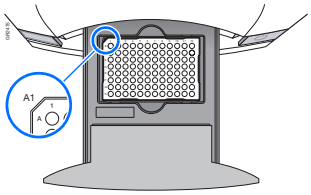
5. Verify that the pure dye plate that you are about to load matches the dye selected in the Pure Spectra Calibration Manager.



6. Load the plate in the instrument as described in "Loading the Plate" on page 40.

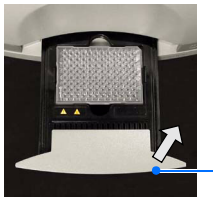


7300/7500 System
Standard plate



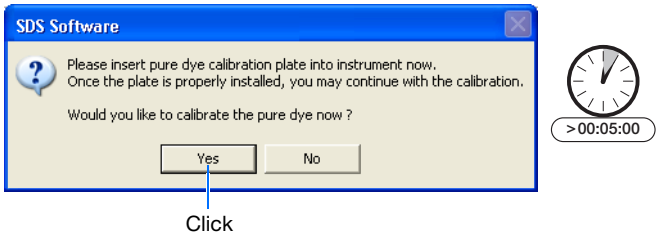
7500 Fast System
Fast plate

Note: When closing the instrument tray, apply pressure to the right side of the tray and at an angle.

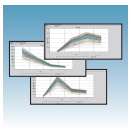


Performing the Pure Dye Calibration

In the dialog box that prompts you to load the plate (see step 1d on page 73, click Yes, then wait for the run to complete (~5 minutes).



Notes



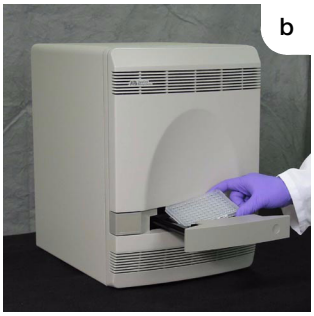
While the SDS Software performs the pure dye calibration, it locks the controls of the Pure Spectra Calibration Manager.

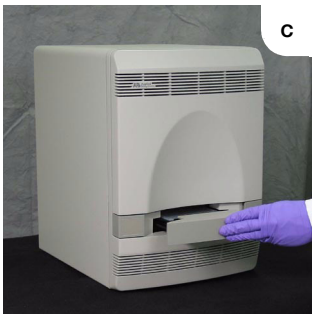
Unloading the Plate

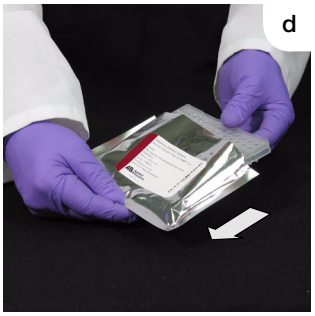
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. When the SDS software completes the run:
   a. Press the tray to open it.
   b. Remove the pure dye plate from the tray.
   c. Press the tray to move it into the instrument.
   d. Place the pure dye plate inside its packaging sleeve, and return it to the spectral calibration kit in the freezer.

   Note: Do not discard the pure dye plates. If you store the plates in their packaging sleeves at –20 to –25 °C, you can use the pure dye plates up to three times after you open them.



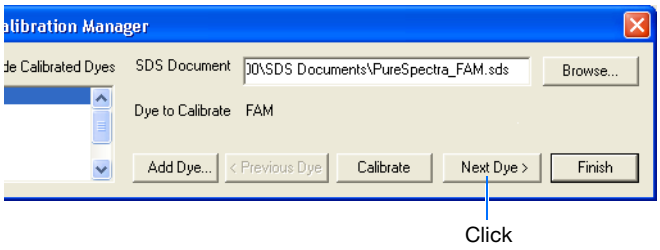




Calibrating Remaining Dyes

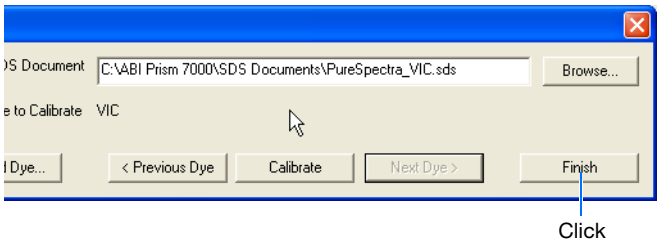
1. Click Next Dye >.
2. Repeat the procedures in "Preparing and Loading a Pure Dye Plate" on page 73 through "Calibrating Remaining Dyes" on page 75 to run the remaining pure dye plates (JOE, NED, ROX, SYBR Green, TAMRA, VIC).

   Note: If you are using a 7500 or 7500 Fast instrument, also perform calibrations for the CY3, CY5, and TEXAS RED pure dyes.



Notes

3. After you calibrate the instrument with all pure dyes provided in your spectral calibration kit, click Finish.

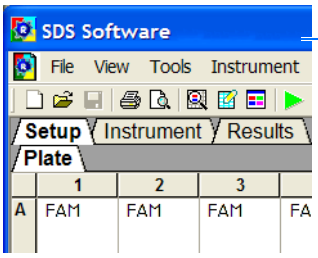


Continue with "Analyzing the Pure Dye Calibration Data" on page 77.

Notes

Analyzing the Pure Dye Calibration Data

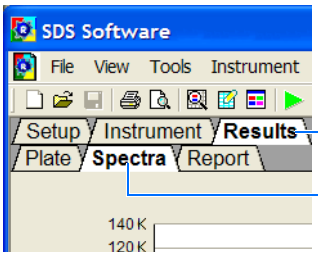
When you run each pure dye plate, the SDS Software automatically creates and saves a plate document for each dye. After you calibrate all of the pure dye plates, the plate documents remain open behind the plate document displayed by the software. To complete the calibration, analyze all open pure dye documents as explained below.



The SDS Software creates a plate document for each calibrated dye (behind the visible plate document)

1. From the Windows menu, select the plate document to analyze.

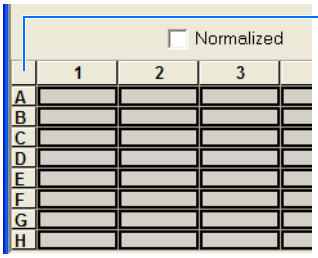
2. Select the **Results** tab, then select the **Spectra** tab.



Select

Select

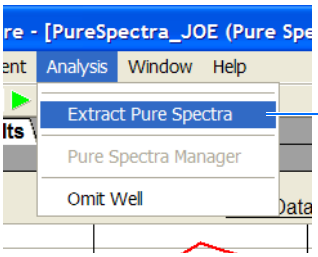
3. Select all wells of the plate document by clicking the upper-left corner of the plate grid.



Click

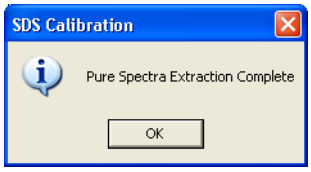
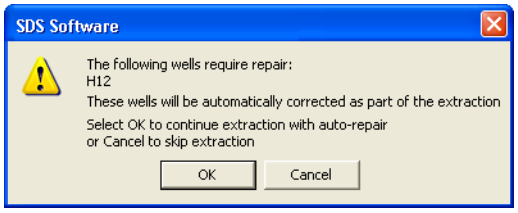
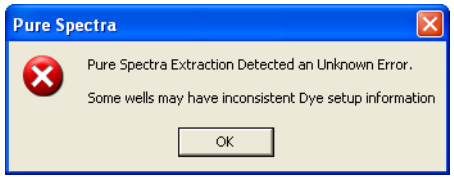
4. Click ▶ (or select **Analysis** ▸ **Extract Pure Spectra**).

   The SDS software completes the extraction, then displays a message (see next page).

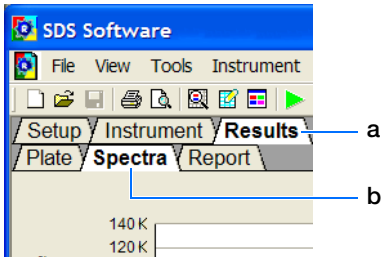


Click

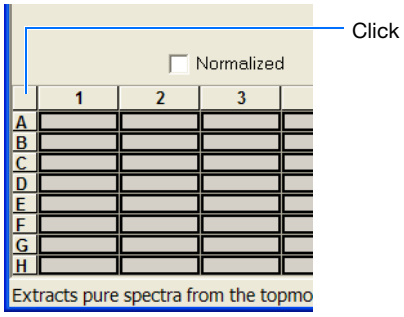
Notes

If the software displays:	Do this:
Pure Spectra Extraction Complete – The analysis is successful. 	Click OK, then go to step 5. **IMPORTANT!** The pure dye calibration is not complete at this point. Before closing the plate document you must inspect the Spectra plot as explained in steps 5 to 8.
Repair Message 	Click OK, then go to step 5. (For information on how the software auto-repairs calibration spectra, see "About Pure Dye Spectra" on page 79).
Error Message 	Click OK, load the plate, then run the pure dye plate again. If the calibration continues to fail, perform the calibration with a new pure dye plate.

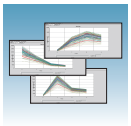
5. In the pure dye plate document:
   a. Select the **Results** tab.
   b. Select the **Spectra** tab.



6. Select all wells of the plate document by clicking the upper-left corner of the plate grid.

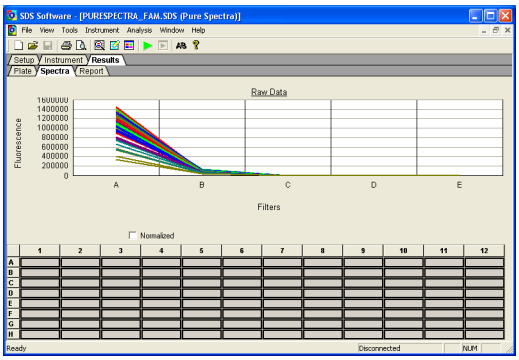


Notes



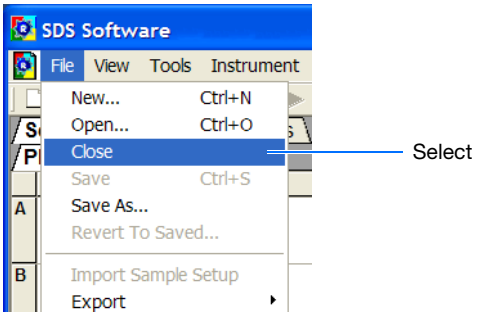
7. Using the tables in "Evaluating Pure Dye Calibration Spectra" on page 80 as a reference, verify that the peak for the spectrum of the pure dye occurs at the correct filter:
   - **7300 system** – see page 80
   - **7500 system** – see page 82
   - **7500 Fast system** – see page 84

   If the peak for the spectra of a dye occurs in the wrong filter, you may have run the wrong dye plate during the calibration. Repeat the procedure using the correct dye.



8. Select **File** ▸ **Close**.

   The SDS Software displays the plate document for the next pure dye plate.



   IMPORTANT! Do not close a plate document until you have extracted it. During the calibration, the software creates plate documents for each pure dye plate as it was run. You must extract each one individually before closing it.

9. Repeat steps 1 through 8 to extract the calibration data for the remaining pure dyes.

When you complete the pure dye spectra calibrations for the remaining dyes, close the remaining plate document, then continue with "Verifying the Instrument Performance" on page 87.

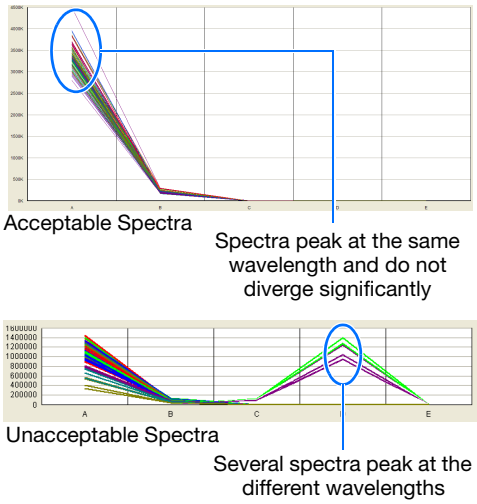
About Pure Dye Spectra

The product of a pure dye calibration is a collection of spectral profiles that represent the fluorescence signature of each pure dye standard. Each profile consists of a set of 96 lines that correspond to the fluorescence gathered from the 96 wells of the pure dye plate. The SDS software plots the resulting data for each spectral profile in a graph of fluorescence versus filter.

When the software extracts the calibration data from a pure dye run, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire plate. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths (divergence is not shown in the figure at the right).

The SDS software can compensate for some differences in a spectral profile by replacing (auto-repairing) the spectra of unacceptable wells with the spectra of neighboring wells. However, the software allows only a few replacements and may reject the calibration if the spectra between neighboring wells vary significantly.

Note: Because the wells in a pure dye plate contain the pure dye at the identical concentration, the resulting signals for all wells should be similar. The variations in spectral position and peak position are caused by minor differences in the optical and excitation energy between individual wells.



Notes

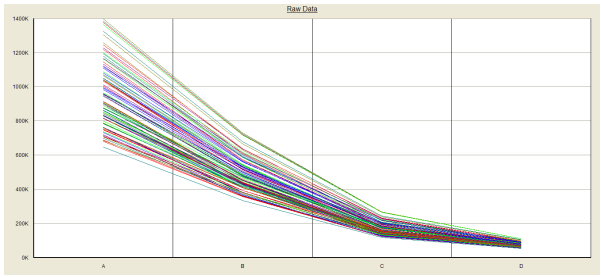
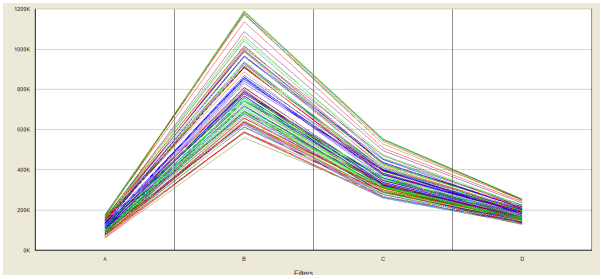
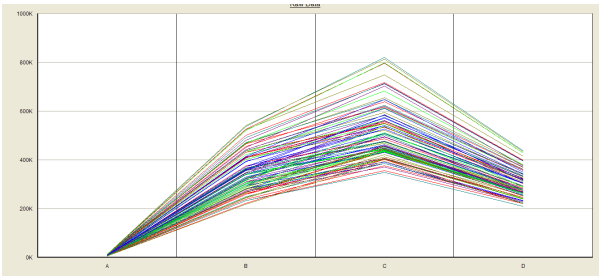
Evaluating Pure Dye Calibration Spectra

Use the tables in this section to verify that the peak for the spectra of the pure dye occurs at the correct filter for your system:

- **7300 system** – see page 80
- **7500 system** – see page 82
- **7500 Fast system** – see page 84

If the peak for the spectra of the dye occurs in the wrong filter, you may have run the wrong dye plate during the calibration. Repeat the procedures for only that dye.

Table 1 Spectra for pure dyes supported by the 7300 system

Instrument	Pure Dye	Filter	Peak (nm)	
7300 System	FAM	A	~520 nm	
	JOE	B	~550 nm	
	NED	C	~550 nm	

Notes

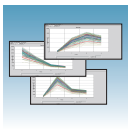
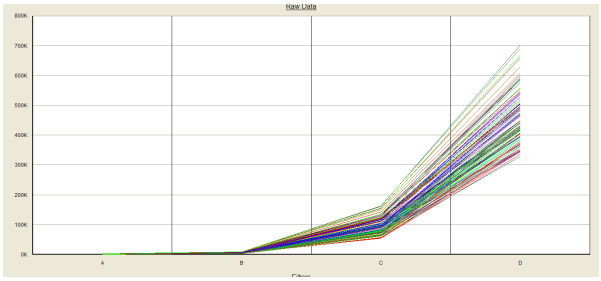
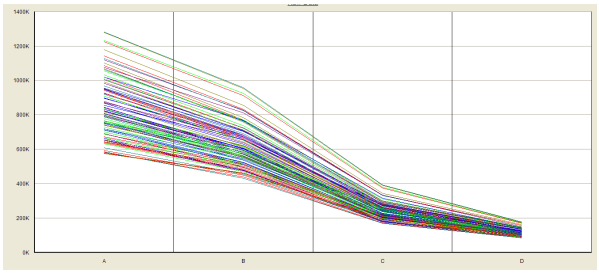
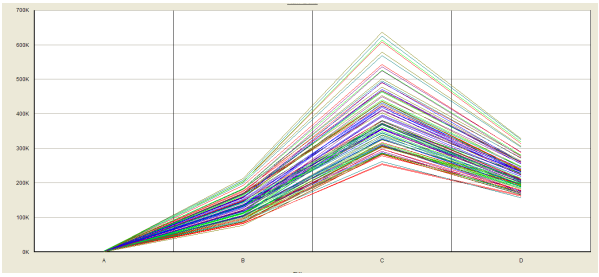
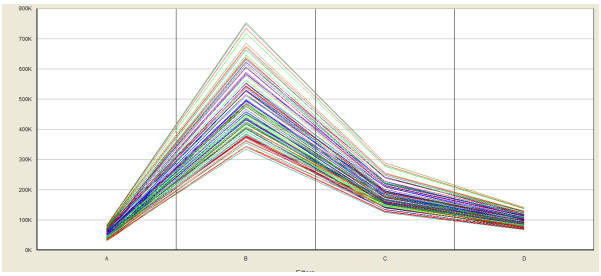
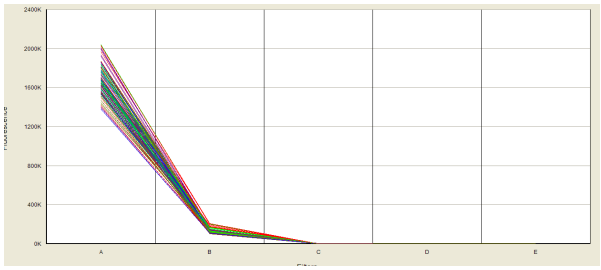
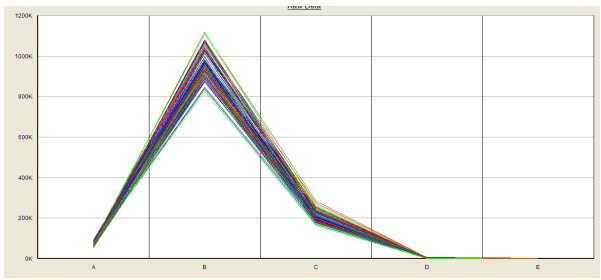
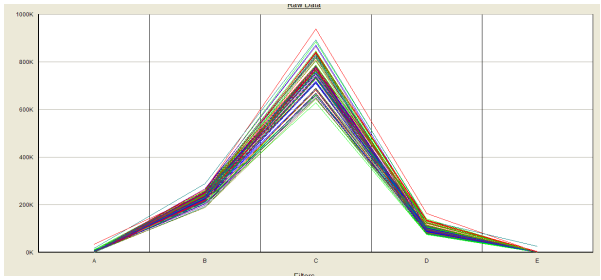
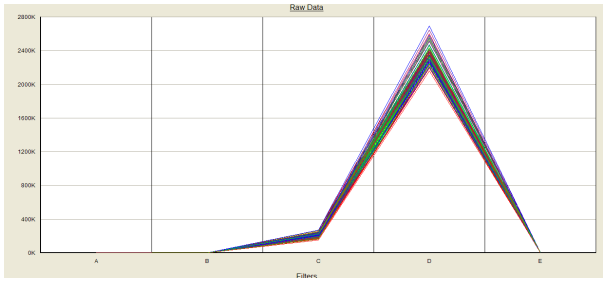
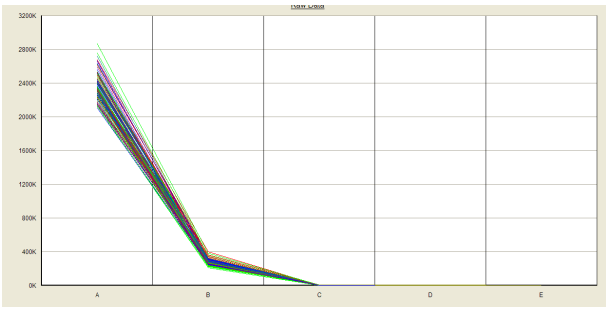


Table 1 Spectra for pure dyes supported by the 7300 system *(continued)*

Instrument	Pure Dye	Filter	Peak (nm)	
7300 System	ROX	D	~610 nm	
	SYBR Green	A	~520 nm	
	TAMRA	C	~580 nm	
	VIC	B	~550 nm	

Notes

Table 2 Spectra for pure dyes supported by the 7500 system

Instrument	Pure Dye	Filter	Peak (nm)	
7500 System	FAM	A	~520 nm	
	JOE	B	~550 nm	
	NED	C	~550 nm	
	ROX	D	~610 nm	
	SYBR Green	A	~520 nm	

Notes

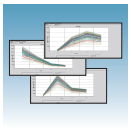
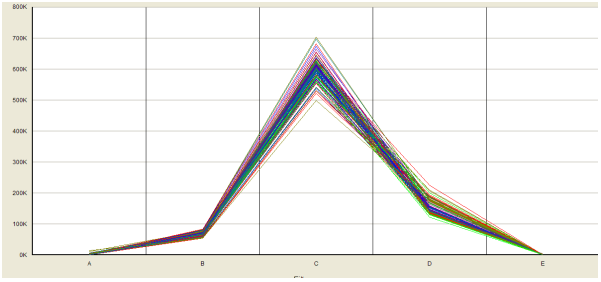
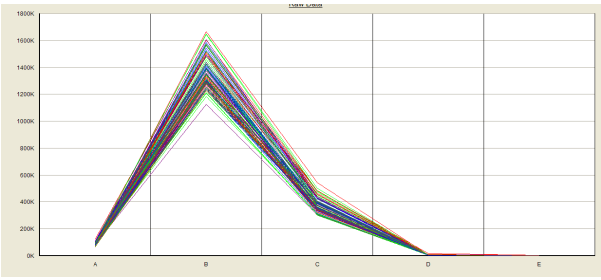
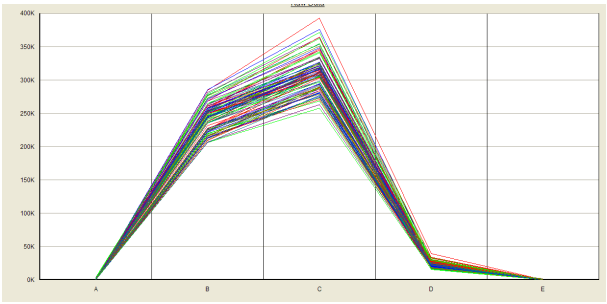
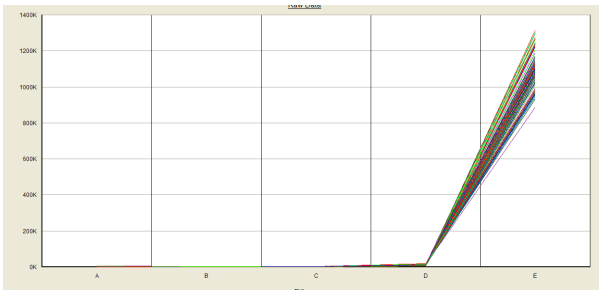
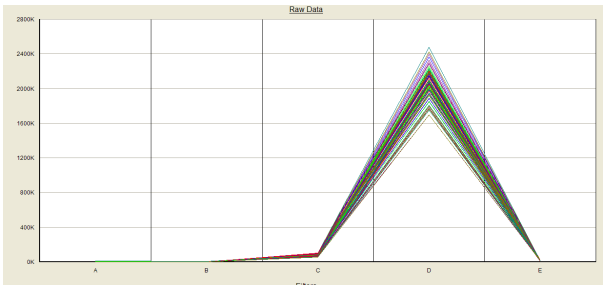


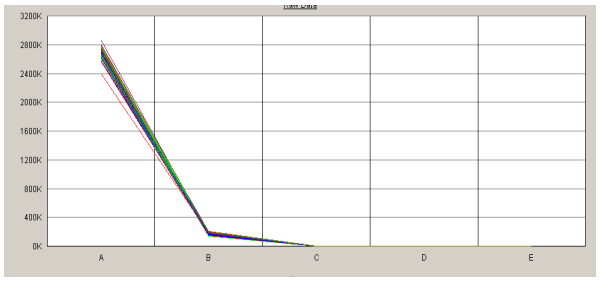
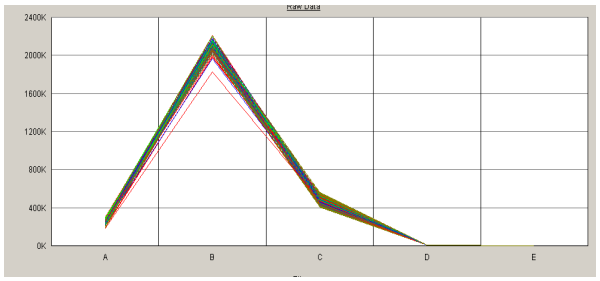
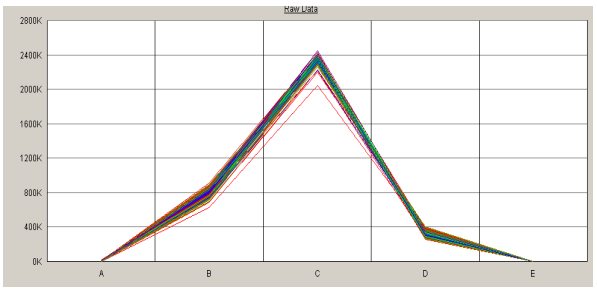
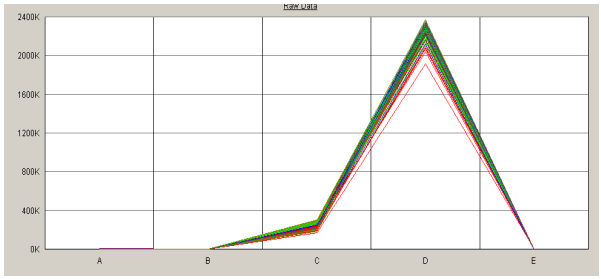
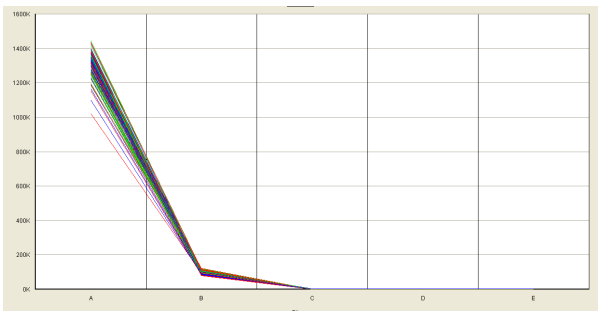
Table 2 Spectra for pure dyes supported by the 7500 system *(continued)*

Instrument	Pure Dye	Filter	Peak (nm)	
7500 System	TAMRA	C	~580 nm	
	VIC	B	~550 nm	
	CY3	C	~580 nm	
	CY5	E	~670 nm	
	TEXAS RED	D	~610 nm	

6

Notes

Table 3 Spectra for pure dyes supported by the 7500 Fast system

Instrument	Pure Dye	Filter	Peak (nm)	
7500 Fast System	FAM	A	~520 nm	
	JOE	B	~550 nm	
	NED	C	~550 nm	
	ROX	D	~610 nm	
	SYBR Green	A	~520 nm	

Notes

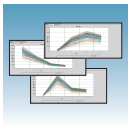
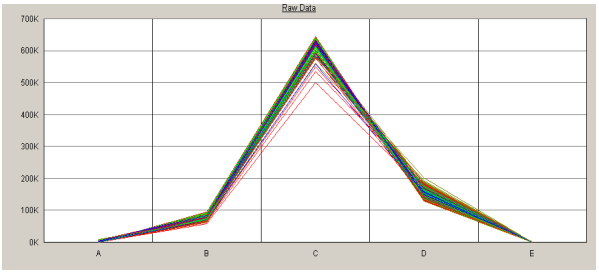
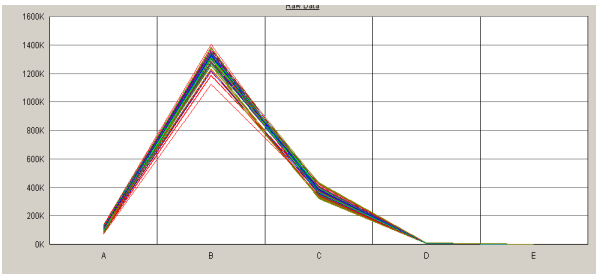
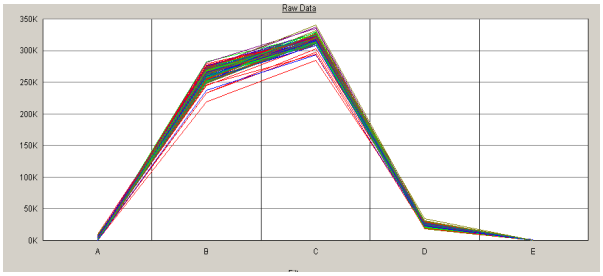
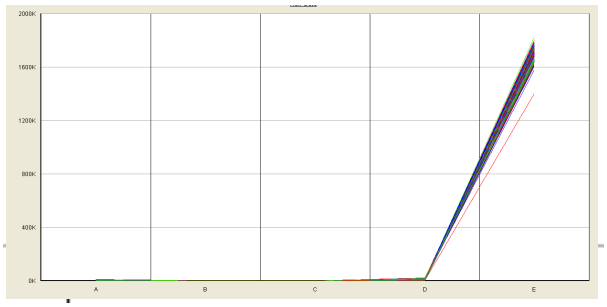
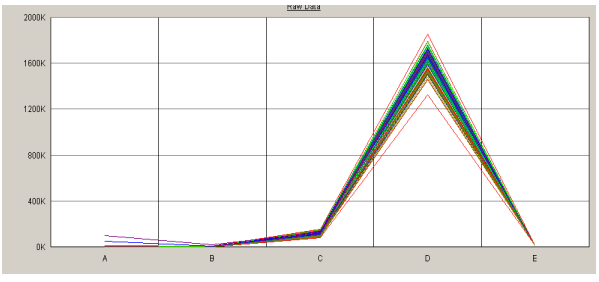


Table 3 Spectra for pure dyes supported by the 7500 Fast system ***(continued)***

Instrument	Pure Dye	Filter	Peak (nm)	
7500 Fast System	TAMRA	C	~580 nm	
	VIC	B	~550 nm	
	CY3	C	~580 nm	
	CY5	E	~670 nm	
	TEXAS RED	D	~610 nm	

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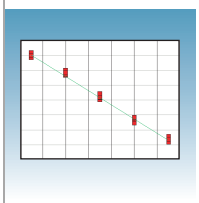
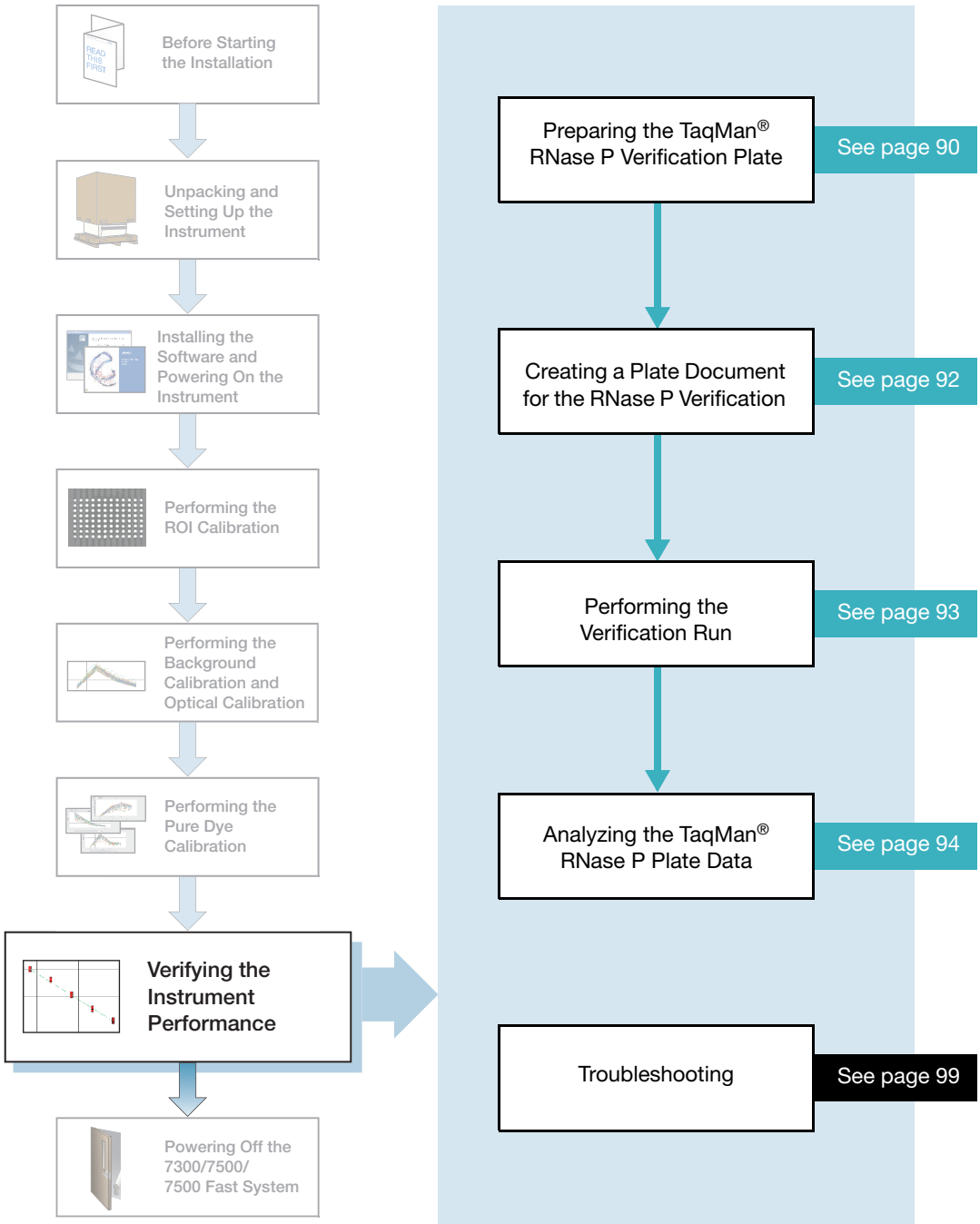


Verifying the Instrument Performance



Notes

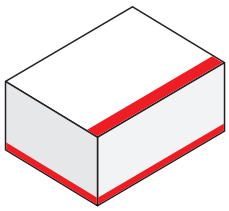
Overview

Note: You can also use the Real-Time System Setup wizard to perform this procedure. To access the wizard, select start ▸ **Program Files** ▸ **7300/7500/7500 Fast System** ▸ **Real-Time System Setup Wizard**.

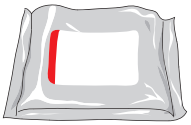
Time Required

- **7300/7500 systems** – ~ 2 hours
- **7500 Fast systems** – ~ 40 minutes

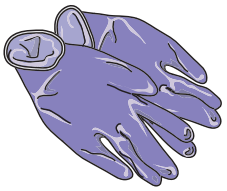
Materials Required



TaqMan® RNase P Instrument Verification Plate Kit

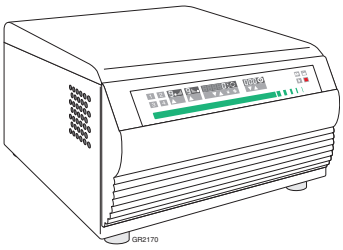


TaqMan® RNase P Instrument Verification Plate



Powder-free Gloves

Safety Goggles



Centrifuge with plate adapter

When to Verify Instrument Performance

Applied Biosystems recommends running a TaqMan® RNase P Instrument Verification plate:

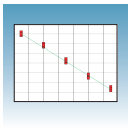
- When installing the system (you must perform all calibrations in this order: ROI ▸ background ▸ optical (7500/7500 Fast systems only) ▸ pure dye ▸ instrument verification).
- After moving the instrument to another location.
- As needed to verify the function of the 7300/7500/7500 Fast instrument.

Purpose of RNase P Runs

The TaqMan RNase P Instrument Verification Plate run verifies the performance of an Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System. The RNase P plate is pre-loaded with the reagents necessary for the detection and quantification of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme). Each well contains:

- Reaction mix:
  - **7300/7500 systems:** 1× TaqMan® Universal PCR Master Mix
  - **7500 Fast systems:** 1× TaqMan® Fast Universal PCR Master Mix
- RNase P primers
- FAM™ dye-labeled probe)
- Known concentration of human genomic DNA template

Notes



The figures below illustrate the arrangement of the standard and unknown populations on the RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10,000, and 20,000 copies), two unknown populations (5000 and 10,000 copies), and four no template control (NTC) wells.

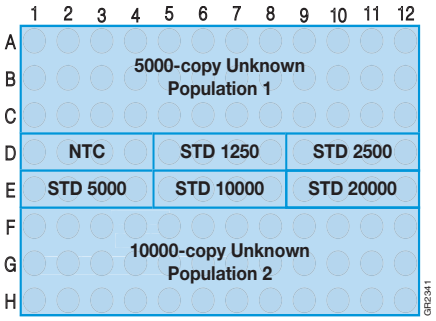


Plate for 7300/7500 system

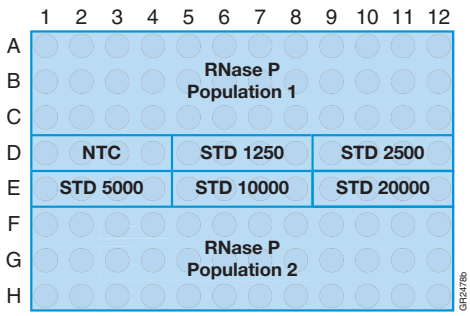


Plate for 7500 Fast system

After the run, the SDS software:

- Generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards.
- Calculates the concentration of the two unknown populations using the standard curve.
- Calculates the following using the mean quantity and standard deviation for the 5,000- and 10,000-copy unknown populations to assess the instrument performance:

$$[(\text{CopyUnk}_2) - 3(\sigma_{\text{CopyUnk2}})] > [(\text{CopyUnk}_1) + 3(\sigma_{\text{CopyUnk1}})]$$

where:

- CopyUnk_1 = Average copy number of unknown #1 (5,000-copy population)
- σ_{CopyUnk1} = Standard deviation of unknown #1 (5,000-copy population)
- CopyUnk_2 = Average copy number of unknown #2 (10,000-copy population)
- σ_{CopyUnk2} = Standard deviation of unknown #2 (10,000-copy population)

The instrument passes the verification if the analyzed data demonstrates that the instrument distinguishes between 5,000 and 10,000 genome equivalents with a 99.7% confidence level.

IMPORTANT! Up to six outlier wells from each unknown replicate group in a 96-well TaqMan RNase P Instrument Verification Plate can be omitted from the analysis to meet specifications.

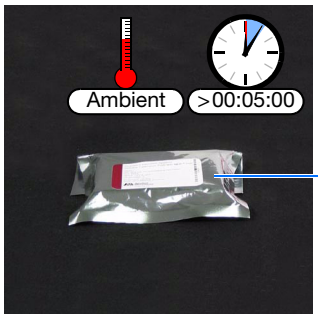
Notes

Preparing the TaqMan® RNase P Verification Plate

IMPORTANT! You must perform an ROI calibration (see page 35), a background calibration (see page 49) and a pure dye calibration (see page 67) before running an RNase P plate.

IMPORTANT! Wear powder-free gloves when you handle the RNase P Verification plate.

1. Retrieve the TaqMan RNase P Verification Plate Kit from the freezer, remove the RNase P plate, then allow the plate to warm to room temperature (approximately 5 minutes).



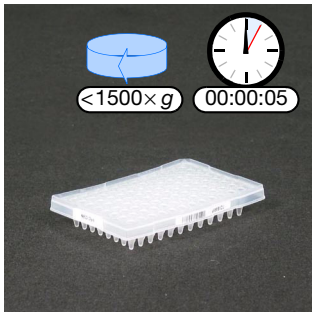
2. Remove the RNase P plate from its packaging.

Note: If the RNase P plate contains a compression pad, remove it from the plate. Applied Biosystems does not recommend the use of compression pads with the 7300/7500/7500 Fast system.

3. **Standard plates only**: Vortex the plate for 5 seconds. Do not vortex Fast plates.

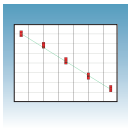
   (Remaining steps apply to both standard and Fast plates.)

4. Centrifuge the plate for 2 minutes in a centrifuge with a plate adapter (<1500 x *g*).



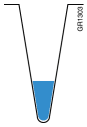
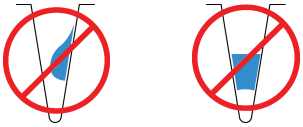
IMPORTANT! The plate must be well mixed and centrifuged.

Notes



5. Verify that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher speed and for a longer period of time.

Continue with "Creating a Plate Document for the RNase P Verification Run" on page 92.

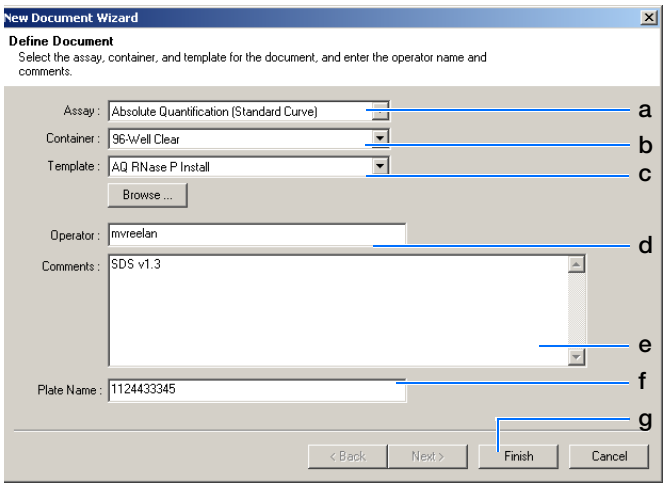
Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

Notes

Creating a Plate Document for the RNase P Verification Run

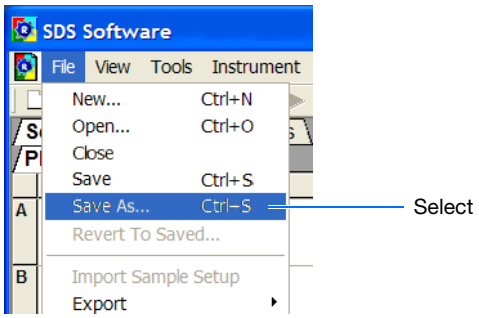
1. In the SDS software, click (or select **File ▸ New**).

2. In the New Document dialog box:
   a. Select **Assay ▸ Absolute Quantification (Standard Curve)**.
   b. Select **Container ▸ 96-Well Clear**.
   c. Select **Template ▸ AQ RNase P Install**.
   d. In the Operator field, enter your name.
   e. In the Comments field, enter any information that you want to attach to the file (such as the plate bar code).
   f. In the Default Plate Name field, enter the bar code of the RNase P plate.
   g. Click Finish.



3. In the SDS software, select **File ▸ Save As**.

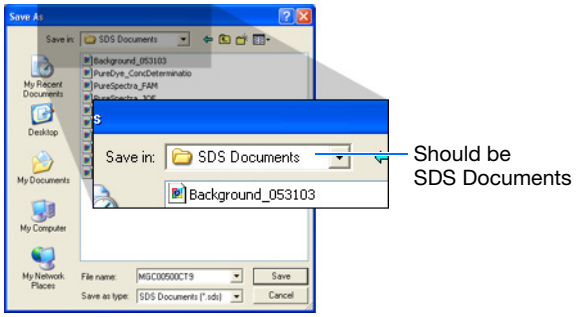
IMPORTANT! Do not modify the plate document. The sample, detector, and method for the run are coded into the software.



4. Save the document. In the Save As dialog box, select the folder:

   If the Save in field does not display SDS Documents, navigate to **D: drive ▸ Applied Biosystems ▸ SDS Documents**.

   After selecting the folder, Click Save. Prepare and run the RNase P plate as explained on page 93.

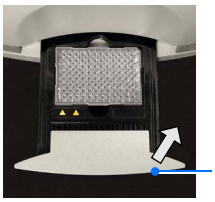


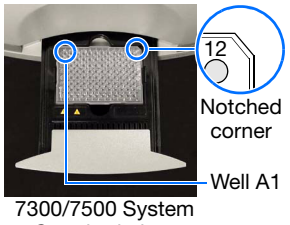
Notes

Performing the Verification Run

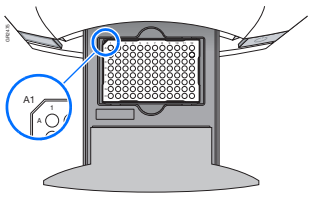
1. Load the plate in the instrument as described in "Loading the Plate" on page 40.

   Note: When closing the instrument tray, apply pressure to the right side of the tray and at an angle.





7300/7500 System
Standard plate



7500 Fast System
Fast plate

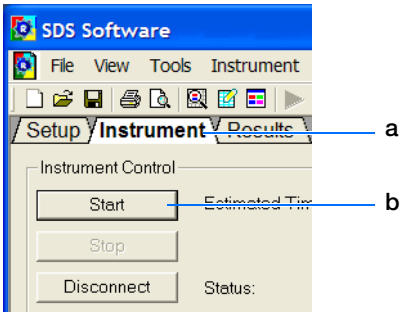
2. In the plate document:

   a. Select the **Instrument** tab.

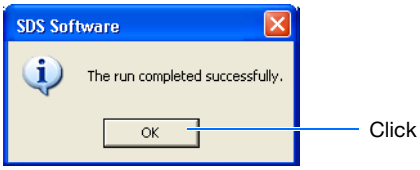
   b. Click Start.

   The instrument begins the run, which lasts 1.5 to 2 hours using a 7300/7500 Real-Time PCR system or <40 minutes using a 7500 Fast Real-Time PCR system.

   Note: Before starting the run, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the correct temperature.



3. When the run is complete, click OK.

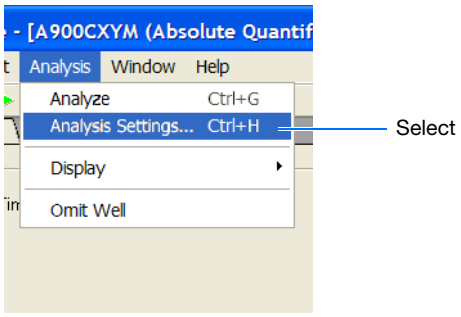


Continue with "Analyzing the TaqMan® RNase P Plate Data" on page 94.

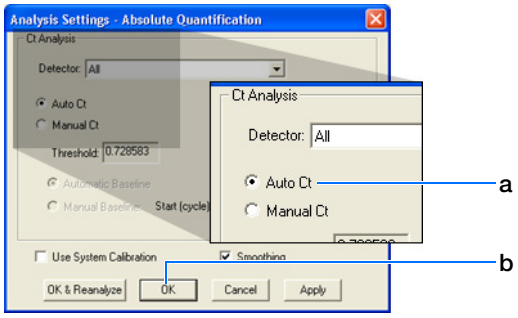
Notes

Analyzing the TaqMan® RNase P Plate Data

1. In the SDS software, select **Analysis** ▸ **Analysis Settings**.

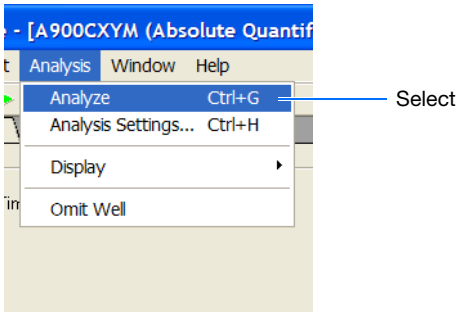


2. In the Analysis Settings dialog box:
   a. Select **Auto Ct.**
   b. Click OK.

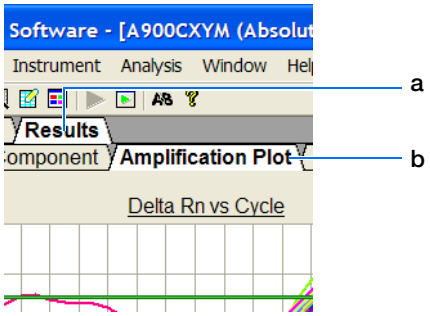


3. In the SDS software, click ▶ (or select **Analysis** ▸ **Analyze**).

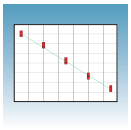
   The SDS software analyzes the run data and displays the results in the Results tab.



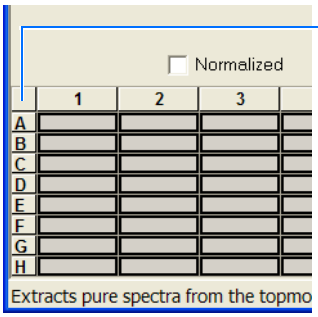
4. Access the results. In the plate document:
   a. Select the **Results** tab.
   b. Select the **Amplification Plot** tab.



Notes



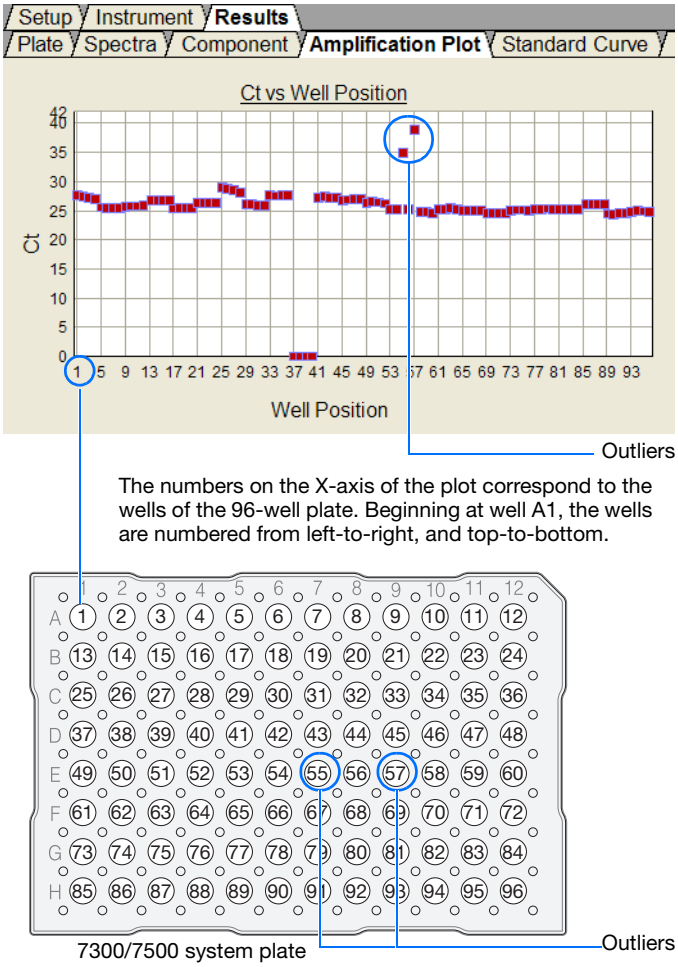
5. Display the data. In the Amplification Plot tab:
   a. Click the upper-left corner of the plate grid to select all wells.
   b. Select **Data ▸ Ct vs. Well Position** to display the Ct vs. Well Position plot.



6. Verify the uniformity of each replicate population by comparing the groupings of C_T values.

Note: Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_T values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

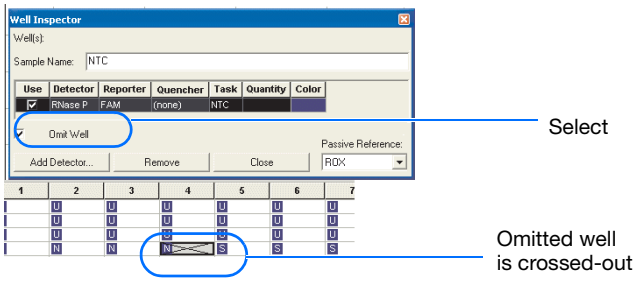
7. If outliers are present, reanalyze the run:
   a. In the plate grid, select the wells that produced the outlying data.
   b. Select **View ▸ Well Inspector**.



Notes

c. In the Well Inspector, select **Omit Well**.

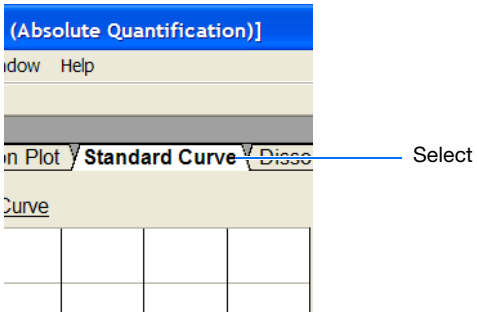
d. Click ▶ (or select **Analysis ▸ Analyze**) to reanalyze the run without the outlying data.



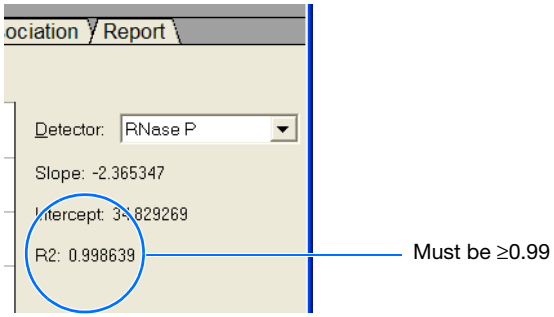
8. Repeat step 7 on the previous page for other wells with outlying data.

IMPORTANT! Up to six wells from each replicate group in a RNase P plate can be omitted to meet specifications. If the analysis contains more than six outliers, troubleshoot the RNase P verification run. See "Troubleshooting" on page 99.

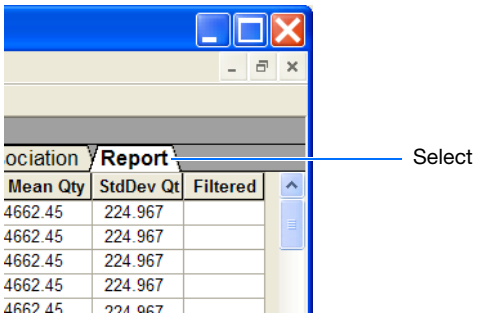
9. In the Results tab, select the **Standard Curve** tab.



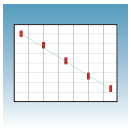
10. In the Standard Curve tab, verify that the R2 value is ≥0.990.



11. In the Results tab, select the **Report** tab.



Notes

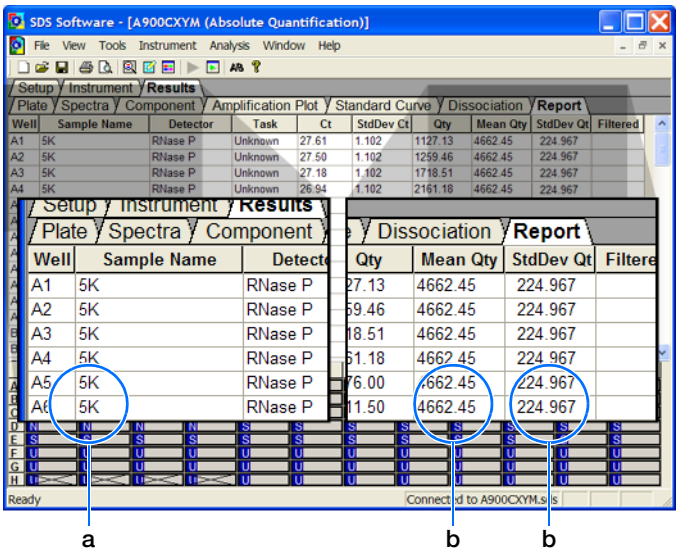


12. Calculate the verification value for the 5000-copy population:

a. In the Report tab, scroll to a sample in the 5K population.

b. Apply the values in the Mean Qty and StdDev Qty columns to the following equation:

5K value = Mean Qty + 3(StdDev Qty)



13. Calculate the verification value for the 10000-copy population:

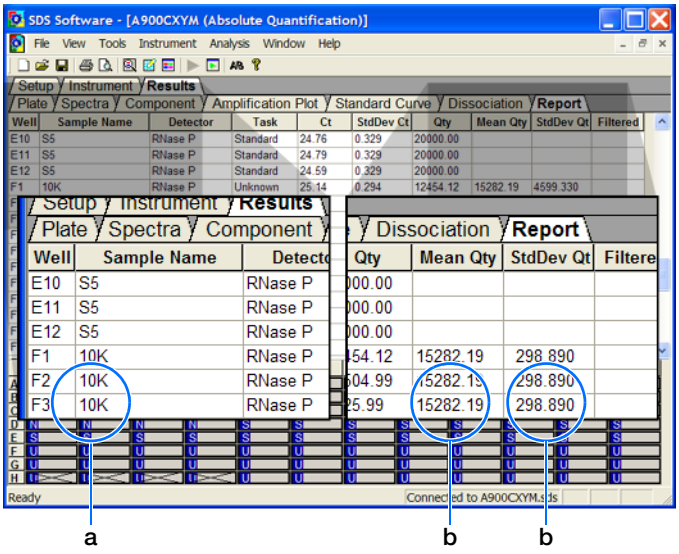
a. In the Report tab, scroll to a sample in the 10K population.

b. Apply the values in the Mean Qty and StdDev Qty columns to the following equation:

10K value = Mean Qty – 3(StdDev Qty)

14. Compare the verification values for the 10000- and 5000-copy populations:

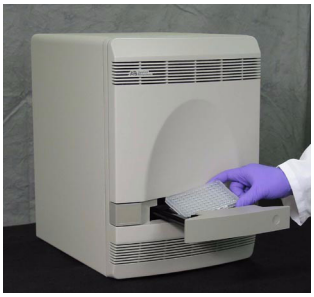
- If the 10K value (step 13) is greater than the 5K value (step 12), the 7300/7500/7500 Fast system has passed the installation verification.
- If the 10K value is not greater than the 5K, the 7300/7500/7500 Fast system has failed the installation verification. Troubleshoot the RNase P verification run. See "Troubleshooting" on page 99.



Notes

Unloading the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.



1. Remove the RNase P plate:
   a. Press the tray to open it.
   b. Remove the RNase P plate.
   c. Press the tray to move it into the instrument.
2. Discard the plate.

Notes

Troubleshooting

Troubleshooting – RNase P Plate Run

Condition: More than six outliers present in RNase P plate data

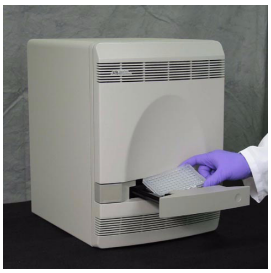
Contact your Applied Biosystems service and sales representative to order a replacement TaqMan RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Applied Biosystems technical support or your service representative for further assistance.

Condition: RNase P plate verification run failed

1. Remove the RNase P plate:
   a. Press the tray to open it.
   b. Remove the RNase P plate from the tray.
   c. Push the tray back into the instrument.
2. Hold the plate up to a light source and verify that all wells contain the same volume of fluid.

   If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.

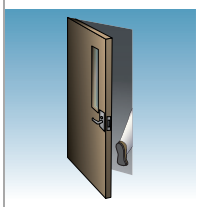
   Also, compare the position of the wells that have lower volumes with the outliers that you have removed from the plate. If the well positions coincide, the heat seal on the plate may be defective and resulted in the evaporation of the associated samples.
3. Contact your Applied Biosystems service and sales representative to order a replacement TaqMan RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Applied Biosystems technical support or your service representative for further assistance.



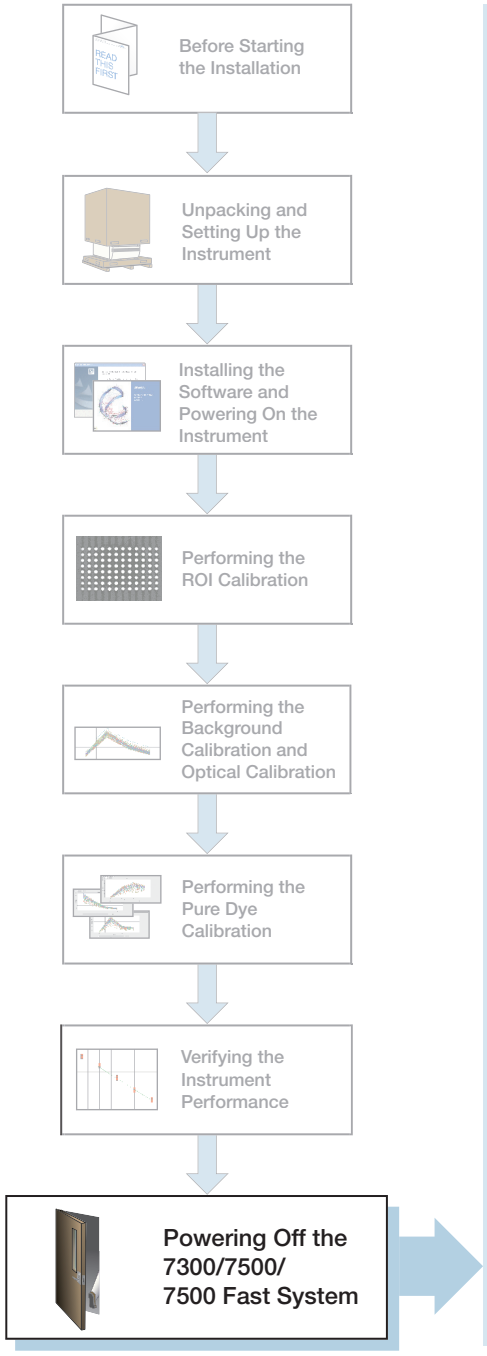
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

Notes

Notes



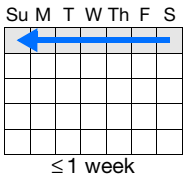
Powering Off the 7300/7500/7500 Fast System



Notes

Performing a Short-Term Shutdown

Perform the short-term shutdown procedure if you will use the instrument within 7 days.



Time Required

5 minutes

Performing a Short-term Shutdown

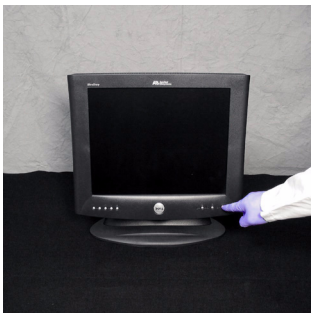
1. Press the tray to open it.

2. If the tray contains a plate, remove it, then press the tray to move it into the instrument.

3. Press the instrument power button.

Notes

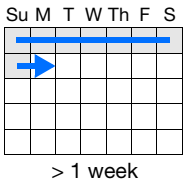
4. Power off the computer and monitor:
   a. Select start ▸ **Shut Down**.
   b. In the Shut Down Windows dialog box (not shown), select **Shut Down**.
   c. Power off the monitor.



Notes

Performing a Long-Term Shutdown

Perform the long-term shutdown procedure if the instrument will be inactive for more than 7 days.



Time Required

5 minutes

Materials Required

Plate, packaging (from step 8 on page 21)

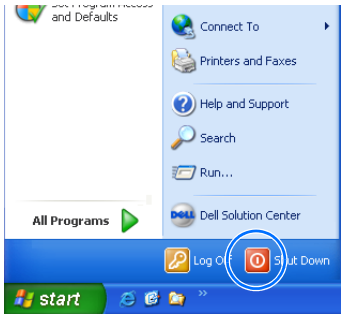
Performing a Long-term Shutdown

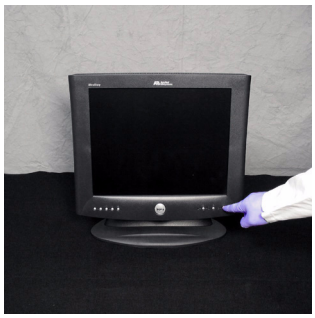
1. Press the tray to open it.
2. If the tray contains a plate, remove it.
3. Load the packaging plate into the tray.

> **Note:** If the shipping plate is not available, substitute an unused reaction plate. During storage, the instrument optics block rests on the plate to protect the optics block.

4. Press the tray to move it into the instrument.
5. Press the instrument power button.

6. Power off the computer and monitor:
   a. Select start ▸ **Shut Down**.
   b. In the Shut Down Windows dialog box (not shown), select **Shut Down**.
   c. Power off the monitor.





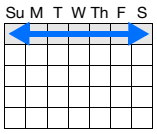
Notes

A

Maintaining the Instrument

Recommended Maintenance Schedule

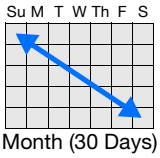
Weekly Maintenance Tasks



- Check disk space
- Archive or back up SDS plate document files (see page 107)
- Cycle the computer and instrument power (power off, then power on the computer and instrument)
- Wipe instrument surfaces with a lint-free cloth

IMPORTANT! Never use organic solvents to clean the 7300/7500/7500 Fast system.

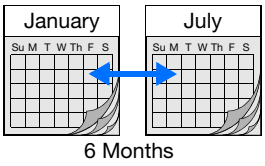
Monthly Maintenance Tasks



- Perform a background calibration (see page 49)
- Perform an optical calibration on 7500/7500 Fast systems (see page 59)
- Clean up and defragment the computer hard drive (see page 114)

Notes

Semi-Annual Maintenance Tasks



- Perform a background calibration (see page 49)
- Perform an optical calibration on 7500/7500 Fast systems (see page 59)
- Perform a region of interest (ROI) calibration (see page 35)
- Perform a pure dye calibration (see page 67)

Note: You can run a background calibration to check for contamination.If any parts of the optics are replaced or moved, you must run a background calibration, an ROI calibration, an optical calibration (7500/7500 Fast only), and a pure dye calibration.

Miscellaneous Maintenance Tasks

Perform the following tasks as needed to resolve problems as they arise:

- Decontaminate the sample block (see page 108)
- Move the 7300/7500/7500 Fast system (see page 115)
- Replace the halogen bulb (see page 117)
- Replace the instrument fuses (see page 122)
- Update the Microsoft Windows operating system (see page 124)

Notes

A

Archiving and Backing Up SDS Files

Archiving SDS Files

To conserve space on the computer hard drive, SDS plate document files can be archived using a data compression utility. Several commercially available compression utilities are available. PKZIP and *.arc are archive formats common to the Microsoft® Windows® operating system.

Backing Up SDS Files

Applied Biosystems strongly recommends that you back up the plate documents generated by your 7300/7500/7500 Fast system because backing up:

- Protects against potential data loss of data caused by an unforeseen failure of the computer or its hard drive(s).
- Conserves space on the hard drive and optimizes performance, if you remove old data after backing up.

See "Choosing a Backup Storage Device" on page 10 for more information about backup storage devices.

Developing a Data Management Strategy

Applied Biosystems recommends developing a strategy for dealing with the files produced by the SDS software. During a single day of real-time operation, the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System can generate over 0.5 MB of data. Data management is a concern only if you perform absolute or relative quantification experiments on your 7300/7500/7500 Fast system. These real-time runs generate significantly more data than allelic discrimination or plus/minus experiments.

Checking disk space

If you perform real-time experiments on your 7300/7500/7500 Fast system, check the amount of available space on your hard drive weekly. When the hard drive is within 20% of maximum capacity, transfer the older data to a backup storage device.

Notes

Decontaminating the Sample Block

WARNING **PHYSICAL INJURY HAZARD.** Do not remove the instrument cover. There are no components inside the 7300/7500/7500 Fast system that you can safely service yourself. If you suspect a problem, contact an Applied Biosystems Service Representative.

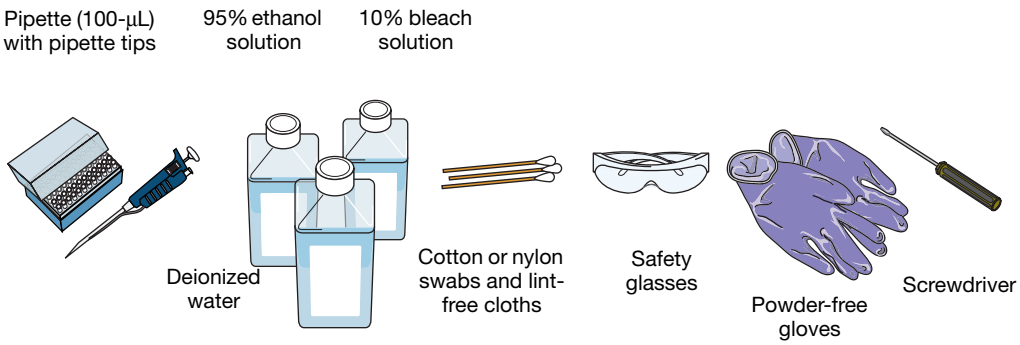
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

The following procedure explains how to eliminate fluorescent contaminants from the sample block of the 7300/7500/7500 Fast instrument. Perform the procedure to resolve problematic background runs where one or more wells consistently exhibit abnormally high signals, indicating the presence of a fluorescent contaminant.

Time Required

30 minutes

Materials Required



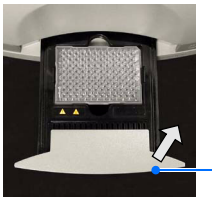
Notes

A

Cleaning the Sample Wells

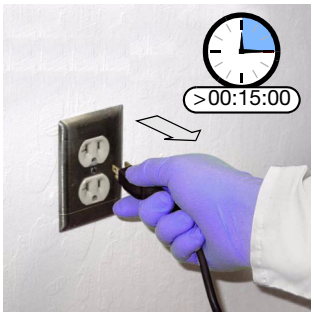
IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block (see "Troubleshooting" on page 64).
2. Remove the plate and the tray holder.
3. Close the tray. Apply pressure to the right side of the tray and at an angle.



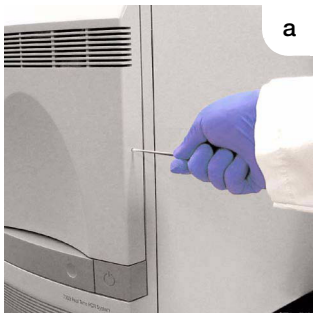
4. Manually raise the block from the ROI Inspector window:
   a. Click (or select **File** ▸ **New**).
   b. In the New Document wizard, click Finish.
   c. In the SDS software, select **Instrument** ▸ **Calibrate**.
   d. In the warning dialog box, click Yes to lower the sample block.

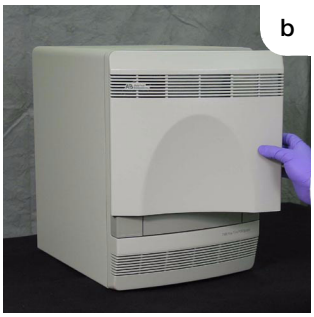
      The ROI Inspector dialog box opens.
   e. In the ROI Inspector dialog box, click Block Up.
5. Power off, then unplug the 7300/7500/7500 Fast system. Allow it to cool for 15 min.



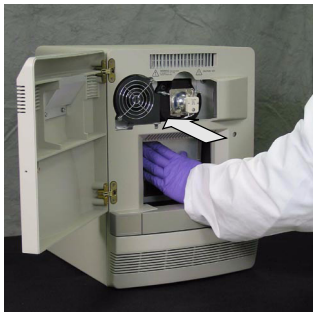
Notes

6. Open the access door to the 7300/7500/7500 Fast system.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.

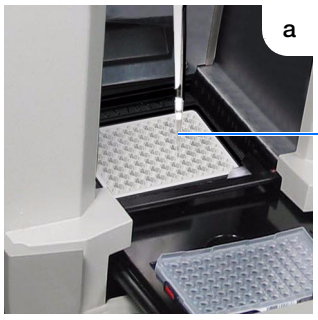




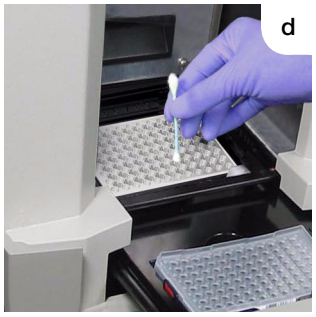
7. Move the heated cover door to the back of the instrument.

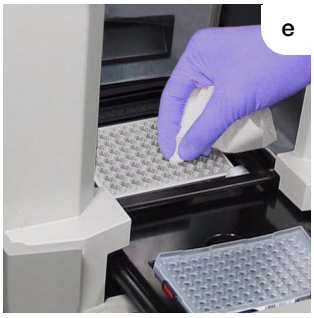


8. Clean the contaminated wells of the sample block using a small volume of deionized water:
   a. Pipette a small volume of deionized water into each contaminated well.
   b. Pipette the water up and down several times to rinse the well.
   c. Pipette the water to a waste beaker.



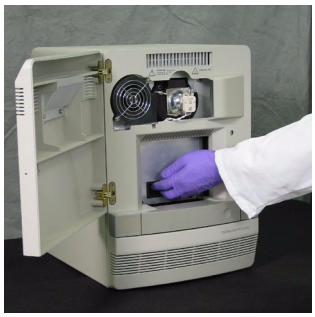
   d. Using a cotton swab, scrub inside of each contaminated well.
   e. Using a lint-free cloth, absorb the excess deionized water.



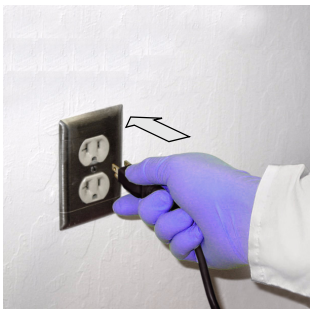


Notes

9. Pull the heated cover door to the front of the instrument, then close the access door.



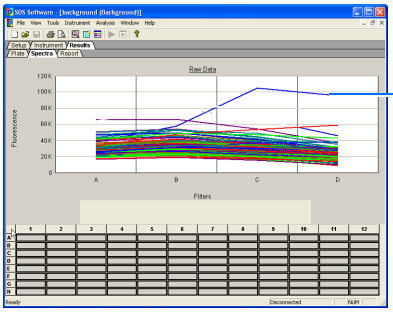
10. Plug in, then power on the 7300/7500/7500 Fast system.



11. Create a new background plate (see Appendix B, "Creating a Background Plate" on page 125.)

12. To confirm that you have eliminated the contamination, perform a background calibration run (see "Performing the Background Calibration and Optical Calibration" on page 49).

13. If the contamination is still present, repeat steps 1 through 7, then go to step 14.



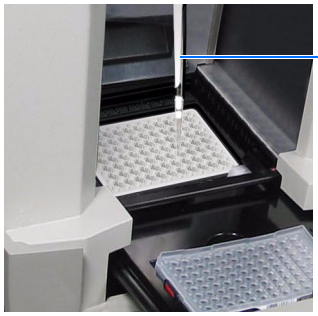
WARNING **CHEMICAL HAZARD.** **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Notes

14. Clean the contaminated wells of the sample block using a small volume of 95% ethanol solution:
    a. Pipette a small volume of 95% ethanol solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the ethanol solution to a waste beaker.

15. Repeat steps 8 through 11 to rinse the wells of the sample block and to verify that you have eliminated the contamination.

    If the contamination is still present, repeat steps 1 through 7, then go to step 16.



IMPORTANT! Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

WARNING **CHEMICAL HAZARD.** **Sodium hypochlorite (bleach)** is a liquid disinfectant that can be corrosive to the skin and can cause skin depigmentation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and powder-free gloves.

16. Clean the contaminated wells of the sample block using a small volume of 10% bleach solution:
    a. Pipette a small volume of 10% bleach solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the bleach solution to a waste beaker.

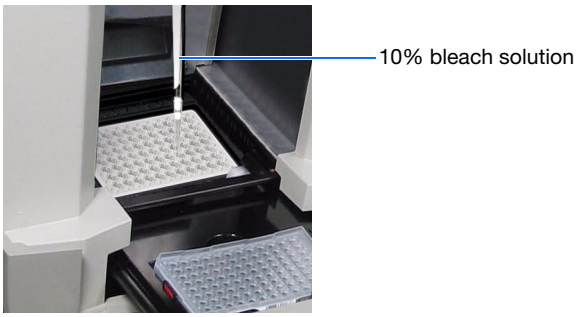
Notes

17. Repeat steps 8 through 11, to rinse the wells of the sample block, and to verify that you have eliminated the contamination.

    IMPORTANT! Always use deionized water to rinse wells after cleaning with bleach or EtOH solution.

    If contamination is present, contact Applied Biosystems technical support (see page xiii).

18. Ensure that the heated cover assembly door is completely closed. If it is not, an error message is displayed.



Notes

Cleaning Up and Defragmenting the Hard Drive

When to Clean Up and Defragment the Hard Drive

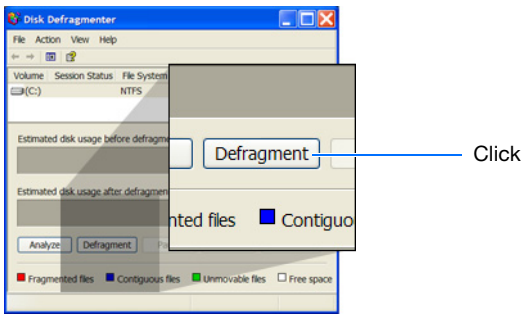
- At least once every month
- When a message is displayed by the Windows operating system instructing you to defragment

Cleaning Up the Disk

1. In the Windows desktop, select start ▸ **All Programs**.
2. Select **Accessories** ▸ **System Tools** ▸ **Disk Cleanup**.
3. Select the drive to clean up, then click OK. Click OK at any additional prompts.
4. Repeat for remaining drives.

Defragmenting

1. In the Windows desktop, select start ▸ **All Programs**.
2. Select **Accessories** ▸ **System Tools** ▸ **Disk Defragmenter**.
3. At the top of the dialog box, select the Volume (hard drive) to defragment.
4. Click Defragment.
5. When the Defragmentation Complete dialog box displays, click **Close**.
6. Repeat steps 3 through 5 for the remaining drives on the computer.



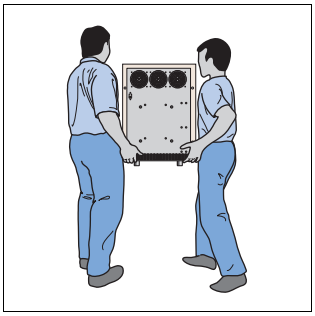
Notes

A

Moving the 7300/7500/7500 Fast System

WARNING **PHYSICAL INJURY HAZARD.** Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the 7300/7500/7500 Fast instrument.

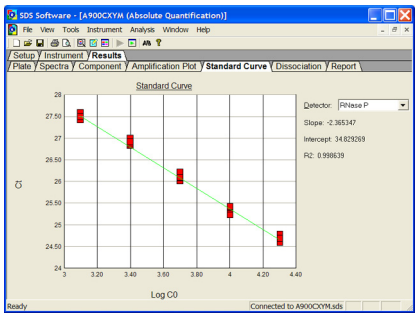
IMPORTANT! Moving your Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System can create subtle changes in the alignment of the instrument optics.



1. Load the packing plate or empty 96-well plate in the instrument.
2. Manually raise the block: from the ROI Inspector window:
   a. Click (or select **File** ▸ **New**).
   b. In the New Document wizard, click Finish.
   c. In the SDS software, select **Instrument** ▸ **Calibrate**.
   d. In the warning dialog box, click Yes to lower the sample block.
      The ROI Inspector dialog box opens.
   e. In the ROI Inspector dialog box, click Block Up.
3. Move your 7300/7500/7500 Fast system according to the guidelines on page 12.
4. Connect the components of the system (see "Setting Up the 7300/7500/7500 Fast Instrument" on page 20).

Notes

5. Run a TaqMan® RNase P Instrument Verification plate (see page 87).
   - If the run passes, recalibrations are not necessary.
   - If the run fails, perform steps steps 6 through 9 to recalibrate the instrument.



6. Perform an ROI calibration (see page 35).

7. Perform a Background calibration (see page 49). Perform an optical calibration if you are using a 7500 or 7500 Fast system (see page 59).

8. Perform a pure dye calibration (see page 67).

9. Perform an instrument verification run (see page 87).

Notes

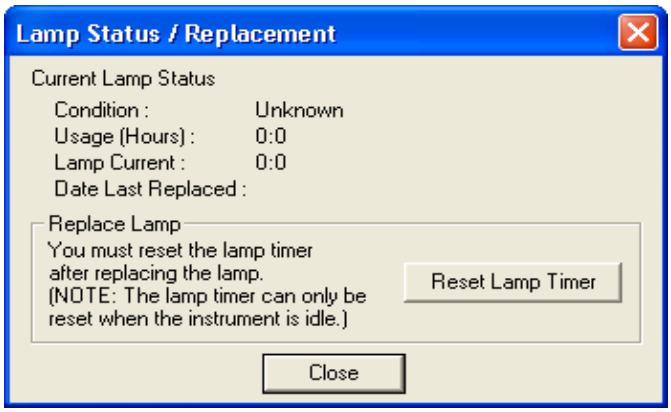
A

Monitoring Lamp Status

Checking Status

To determine whether the halogen lamp has enough electrical current, select **Instrument ▸ Lamp Status/Replacement**.

In the Lamp Status/Replacement dialog box, the Lamp Current: field indicates a percentage figure for the electrical current.The Condition: field indicates one of the following:



- **Good** – The lamp is functioning well. There is no need to replace the lamp bulb at this time. Click Close.
- **Failed** – The lamp bulb must be replaced. Click Close, then replace the lamp as explained below.
- **Change Soon** – The lamp bulb usage is above 2000 hours. It is recommended to replace the lamp soon. Click Close, then decide whether to replace the lamp.

If the bulb needs replacement, see "Replacing the Halogen Lamp" on page 118.

Warning Messages

Three warning messages can be displayed before or during a run that indicate low lamp current:

Message	Description
Warning – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced.	Displayed at the start of a run if the lamp current has fallen below the acceptable percentage. You cannot proceed with the run. You must replace the halogen bulb.
Warning – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced.	Displayed if the lamp current falls below the acceptable percentage during a run. The run is terminated. Click **OK** in the message box, inspect the Instrument Log, then replace the lamp bulb. You cannot proceed with the run. You must replace the halogen bulb.
Warning - The lamp usage has exceeded 2000 hours. We recommend replacing the lamp soon to ensure optimal assay performance.	Displayed at the start of a run if the lamp usage exceeds 2000 hours. Click **Cancel Run**, then replace the lamp, or click **Continue Run**.

Notes

Replacing the Halogen Lamp

WARNING **PHYSICAL INJURY HAZARD.** The 7300/7500/7500 Fast system and lamp are hot! The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective, powder-free gloves before handling it.

CAUTION **PHYSICAL INJURY HAZARD.** Wear disposable, powder-free gloves when handling the lamp to prevent burns and to prevent shortening the life of the replacement lamp.

CAUTION **WARNING.** This instrument is designed for 12V, 75W halogen lamps only.

Replace the halogen lamp after approximately 2000 hours of life.

Time Required

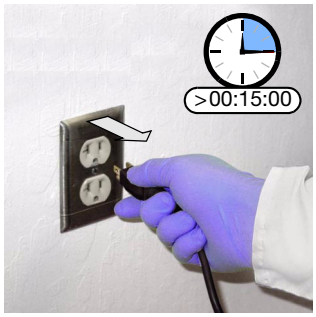
30 minutes

Materials Required

Replacing the Lamp

IMPORTANT! Wear powder-free gloves when you handle the lamp.

1. Power off, then unplug the 7300/7500/7500 Fast system. Allow the instrument to cool for 15 minutes.



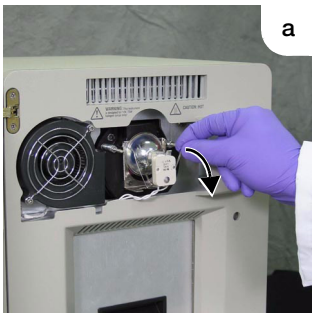
Notes

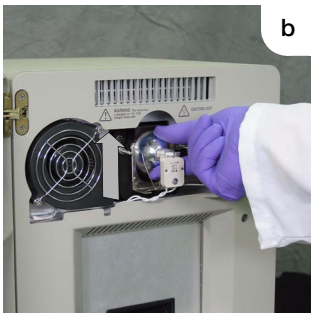
A

2. Open the access door to the 7300/7500/7500 Fast system.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.

WARNING **PHYSICAL INJURY HAZARD**. The 7300/7500/7500 Fast system and lamp are hot! The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective, powder-free gloves before handling it.

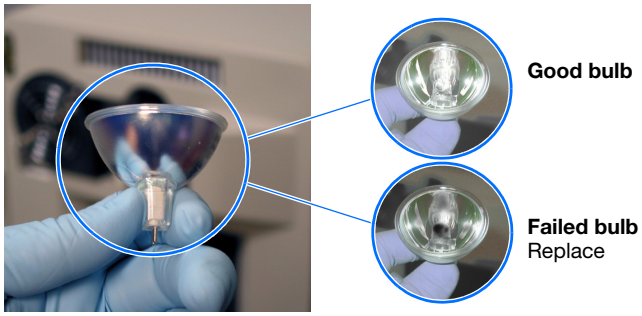
3. Remove the lamp from the instrument:
   a. Slide the lamp release lever downward.
   b. Firmly grasp the lamp and lift it up and out of the slotted mount.



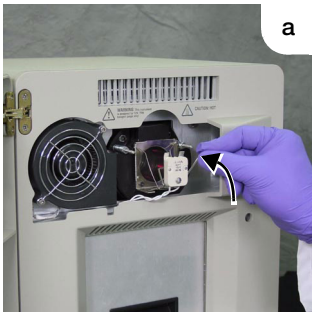


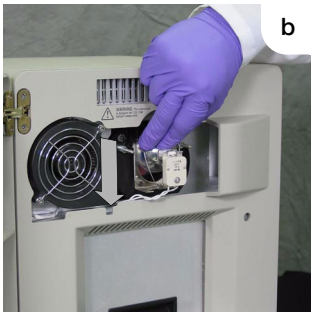
CAUTION Do not touch the lamp without powder-free gloves. Finger prints shorten the lamp life.

4. Inspect the lamp for signs of failure (carbon typically coats the inside of failed lamps).



5. Place the new lamp into the instrument:
   a. Slide the lamp release lever upward.
   b. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.



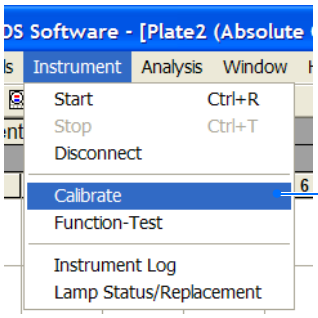


Notes

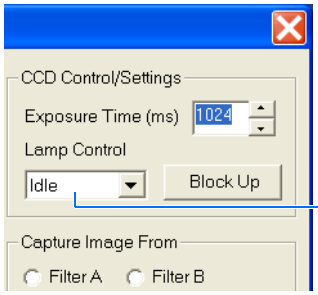
6. Close the access door.

7. Plug in and power on the 7300/7500/7500 Fast system.

8. In the SDS software, select **Instrument ▸ Calibrate**.



9. In the ROI Inspector dialog box, select **Lamp Control ▸ Idle**.



Notes

10. While the instrument is running, look through grating of the access door and verify that the lamp is illuminated, then click Done.

If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the instrument fuses for failure (see page 122).

11. Perform the calibrations listed below after replacing the lamp. See:

- Chapter 4, Performing the Regions of Interest (ROI) Calibration
- Chapter 5, Performing the Background Calibration and Optical Calibration
- Chapter 6, Performing the Pure Dye Calibration
- Chapter 7, Verifying the Instrument Performance

Notes

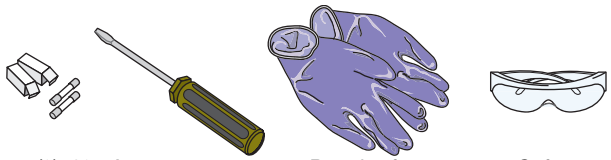
Replacing the Instrument Fuses

CAUTION **FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the instrument.

Time Required

30 minutes

Materials Required



Fuses (2), 12.5A, 250V, 5 x 20 mm | Flathead screwdriver | Powder-free gloves | Safety glasses

Replacing the Fuses

1. Turn off the instrument, then unplug it.

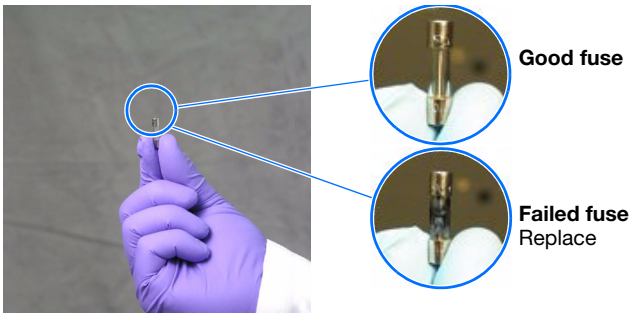
2. Using a flat-head screwdriver, unscrew and remove the fuse holders from the instrument.

Notes

3. Remove each fuse from its fuse holder and inspect it for damage. Carbon typically coats the inside of failed fuses.

4. Replace failed fuses with a 12.5A, 250V, 5 x 20-mm fuse.

   Note: The voltage and amperage ratings are on the fuse holder.



5. Replace the fuse holder into the instrument.

6. Plug in, then power on the instrument.

   The installation is successful if the instrument powers on.

   Note: Fuse failure can result from fluctuations in the supplied power to the instrument. To prevent further failures, consider installing an electrical protective device (see page 9).

Notes

Updating the Operating System Software and Service Packs

Do not upgrade the operating system of the computer connected to the 7300/7500/7500 Fast system unless instructed to do so by an Applied Biosystems representative. New versions of the Microsoft Windows operating system can conflict with the SDS software and make the instrument inoperable.

Service Pack Updates

To install a service pack to update the operating system, review the release notes provided with the SDS software installation for compatibility issues.

Note: Applied Biosystems service engineers maintain the operating system software as part of planned maintenance visits. During a visit, an engineer updates the computer operating system as upgrades become available and are validated by Applied Biosystems.

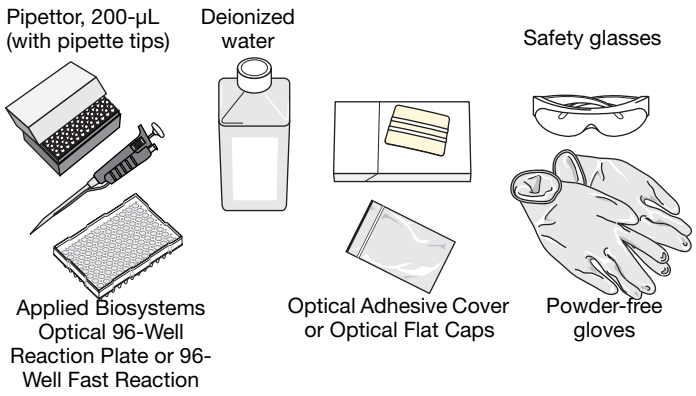
Notes

Creating a Background Plate

Overview

Whenever possible, use a background plate included with the spectral calibration kit. The plates supplied in the kit contain a buffer that accurately models the reagents used for PCR, and, therefore, produces high-quality calibration data. However, if a background plate from a spectral calibration kit is not available, you can create one by following the procedure below.

Materials Required



Creating a Background Plate

IMPORTANT! Wear powder-free gloves while creating the background plate.

1. Remove an Applied Biosystems 96-Well Optical Reaction Plate or 96-Well Fast Reaction Plate from its box and place it on a clean, dry surface.
2. Aliquot 50 µL (7300/7500 system) or 20 µL (7500 Fast system) of deionized water to each well of the reaction plate.
3. Seal the plate using an optical adhesive cover or optical flat caps.

Use the plate for background calibration in the same way you use a background plate from the spectral calibration kit. See Chapter 5, "Performing the Background Calibration and Optical Calibration."

Notes

Notes

Creating a Custom Pure Dye Plate

Overview

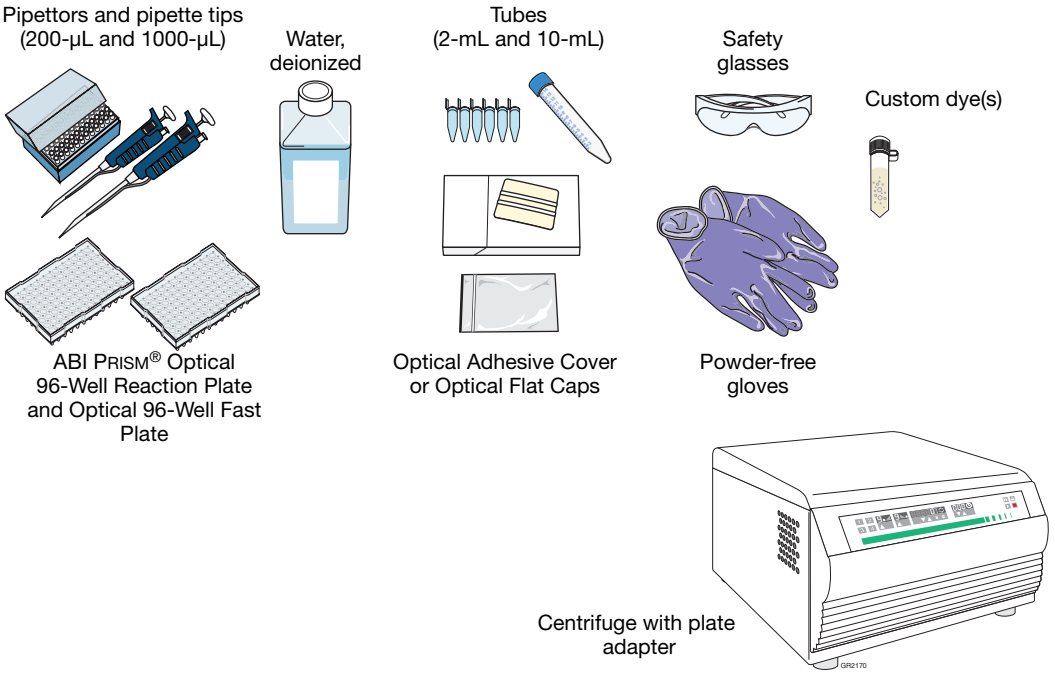
The Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System can be used to run assays designed with custom dyes (dyes not manufactured by Applied Biosystems). Custom dyes must fluoresce within the spectral range measured by the instrument:

- 500 to 650 nm for 7300 systems
- 500 to 700 nm for the 7500/7500 Fast systems

Before using custom dyes with the 7300/7500/7500 Fast instrument, you must:

- Determine optimum dye concentration
- Create a custom pure dye plate
- Add the custom dye to the software
- Perform a pure dye calibration (see Chapter 6, "Performing the Pure Dye Calibration."

Materials Required

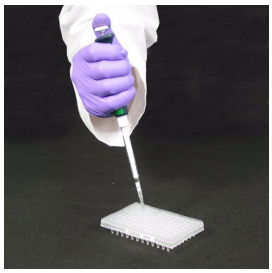


Notes

Determining Optimum Dye Concentration

IMPORTANT! Wear powder-free gloves while creating a custom pure dye plate.

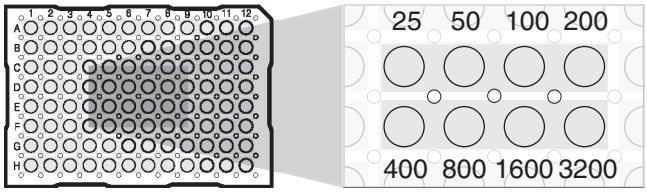
1. In the center wells of a 96-well plate, prepare a dilution series of the custom dye (for example, 25, 50, 100, 200, 400, 800, 1600, and 3200 nM concentrations) using 50-µL volumes for the 7300/7500 system or 20-µL volumes for the 7500 Fast system.



2. Seal the wells of the reaction plate using an optical adhesive cover.

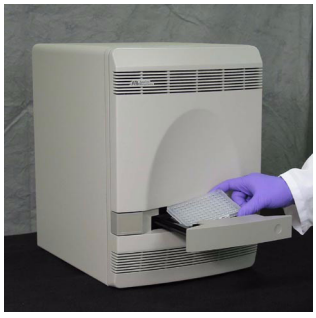
3. In the SDS software, click (or select **File ▸ New**).

4. In the New Document dialog box, click Finish.



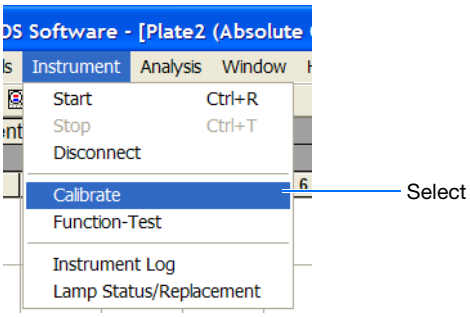
Note: It is not necessary to configure detector, sample, and method information for the plate document. The purpose of the run is to establish the correct working concentration for the dye by viewing the intensity of the raw spectra produced by the wells in the dilution series.

5. Load the prepared plate:
   a. Press the tray door to open it.
   b. Load the pure dye plate into the plate holder.
   c. Press the tray door to move the drawer into the instrument.

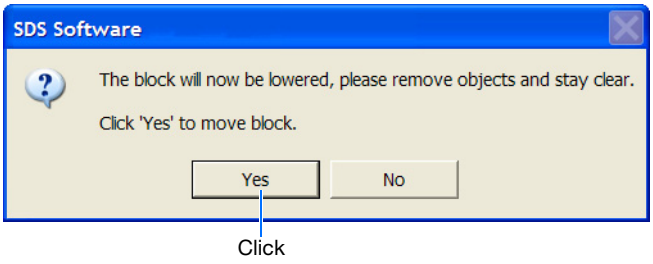


Notes

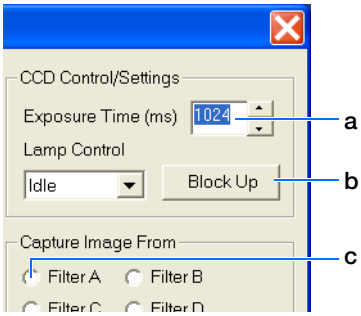
6. In the SDS software, select **Instrument ▸ Calibrate**.



7. In the warning dialog box, click Yes to move the block.

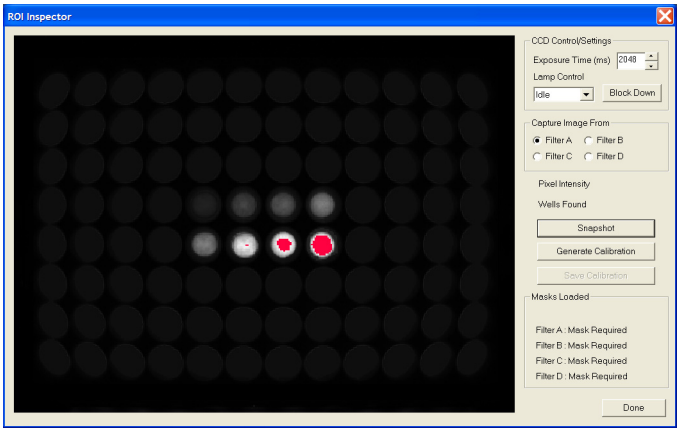


8. In the ROI Inspector, create the ROI image for each filter, beginning with Filter A:
   a. In the Exposure Time field, enter **1024**.
   b. Click Block Up.
   c. Select **Filter A**.
   d. Click Snapshot.



   e. Check the image for saturation.

No Saturation	Normal Saturation	Over Saturated
		
Good	Bad	



   f. Record the coordinate of the well that displays the brightest possible signal without saturation. This well contains the best concentration of the custom pure dye for Filter A.

Notes

9. Repeat step 8 (steps c through f) for the remaining filters.

10. After you determine the optimum concentration for each filter, determine the optimum concentration for the custom dye:
    - Compare the results from all filters.
    - Select the concentration that yields the highest possible signal in all filters, but does not saturate.

Unloading the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. In the ROI Inspector. click Block Down.

2. Remove the plate from the instrument:
    a. Press the tray to open it.
    b. Remove the plate.
    c. Press the tray to move it into the instrument.

    Note: If you cannot open the tray, the sample block may be in its raised position, locking the tray position. To lower the block, select **Instrument ▸ Calibrate**, then exit the ROI Inspector.

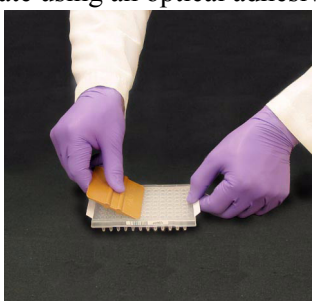
3. Click Done.

Creating a Custom Pure Dye Plate

1. Prepare 5 mL (7300/7500 system) or 2 mL (7500 Fast system) of the custom pure dye at the concentration determined in step 10 on page 130.

2. Pipette 50 µL (7300/7500 system) or 20 µL (7500 Fast system) of the diluted custom dye to all wells of an optical reaction plate.

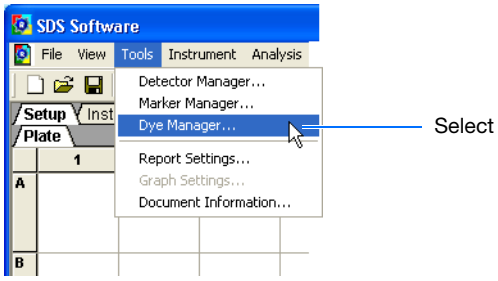
3. Seal the wells of the reaction plate using an optical adhesive cover.



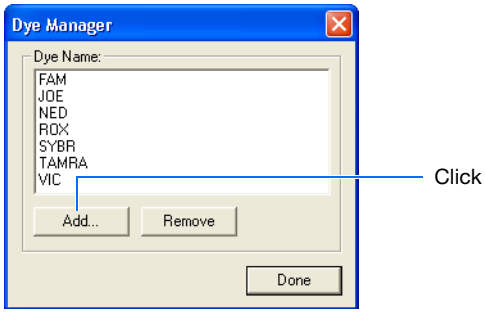
Notes

Adding the Custom Dye to the Software

1. In the SDS software, select **Tools ▸ Dye Manager**.

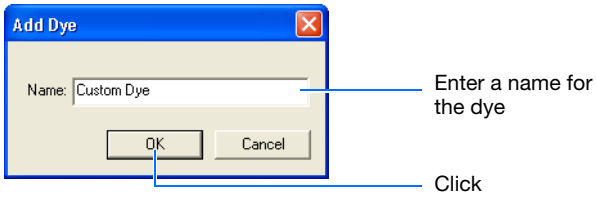


2. In the Dye Manager dialog box, click Add....



3. In the Add Dye dialog box, enter a name for the custom dye, then click OK.

4. Click Done.



Run the custom pure dye plate (see Chapter 6, "Performing the Pure Dye Calibration.")

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Notes

Setting Up the Computer

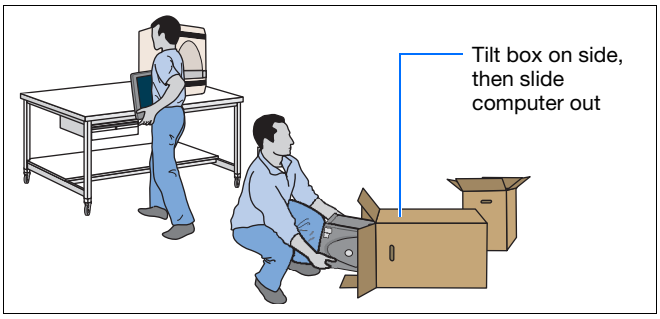
Note: This appendix duplicates the information in the *Applied Biosystems Real-Time System Computer Setup Guide*.

Notes

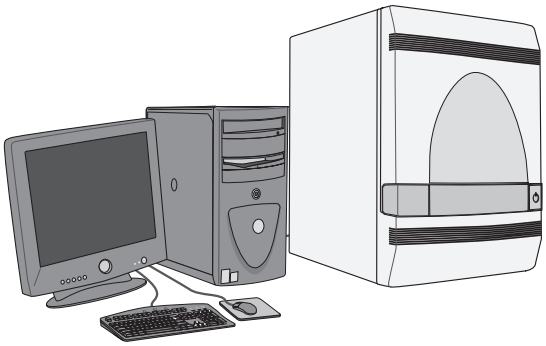
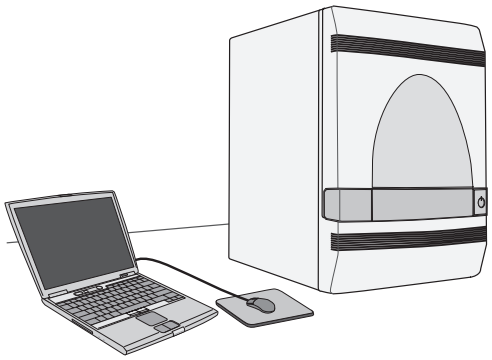
Unpacking the Computer and Setting Voltage

Note: The photos and graphic depictions in this appendix are general and are intended to guide you through the setup process. The photos and graphics might vary slightly from your system. The instrument shown below is to illustrate placement of instrument and computer.

1. Unpack the computer boxes.



2. Place the monitor, computer, keyboard, and mouse on the bench as shown. Do not connect components or the USB cable to the instrument or computer at this time. Doing so may result in installation of incorrect drivers.

Tower Computer	Laptop Computer
	

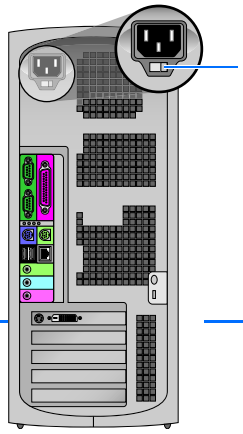
D

Notes

3. **Tower computers:** Set voltage and connect the power cord:

   a. Examine the back of the computer tower.

   b. If your computer has a voltage selection switch, set the voltage:



Country	Voltage
US/Japan	115
Europe/Australia	230

CAUTION Always check the voltage configuration at the rear of the computer to ensure that it matches the site voltage. Failure to properly set the voltage switch can damage the computer.

   If your computer does not contain a voltage selection switch, it contains an auto-sensing voltage selector and automatically detects the correct operating voltage.

   c. **Inside the US**: Use the power cord shipped with the computer.

   Outside the US: Locate the international voltage kit (PN603615) provided in the instrument packing kit. Select the appropriate power cord (you can discard remaining power cords).

IMPORTANT! Outside the US, use the power cord from the international voltage kit, not the power cord provided in the computer carton.

4. **Laptop computers:** Connect the power cord (laptop computer AC adaptors contain an auto-sensing voltage detector and automatically detect the correct operating voltage):

   a. **Inside the US**: Use the power cord shipped with the computer.

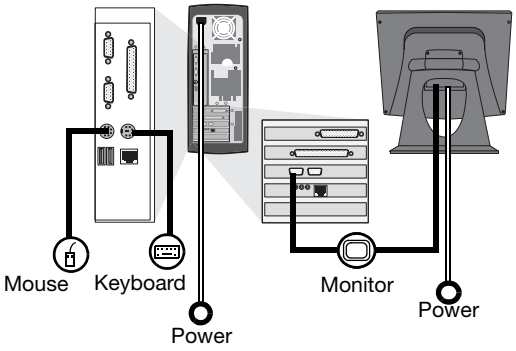
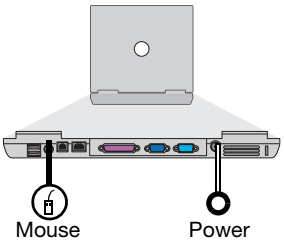
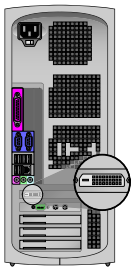
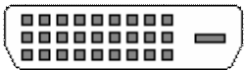
   Outside the US: Locate the international voltage kit (PN4346883) provided in the instrument packing kit. Select the appropriate power cord, then connect the power cord between the AC adaptor and the laptop computer (you can discard remaining power cords).

IMPORTANT! Outside the US, use the power cord from the international voltage kit, not the power cord provided in the computer carton.

Notes

Connecting Components and Powering Up

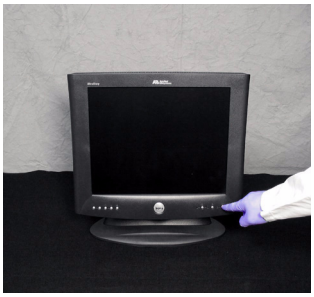
1. Connect the mouse, the keyboard, and the monitor as instructed by the quick start guide that accompanied the computer. Refer to the diagram below to verify connections.

Tower Computer	Laptop Computer
	
 If two computer-to-monitor cables are provided, make sure to use the cable with the DVI connector. (shown at right). 	

2. Power on the computer and monitor (tower computer shown at right).

   The power switch on a laptop is at the top-center of the keyboard.

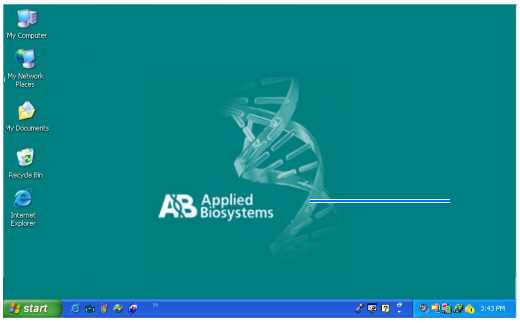
   Wait for the computer to boot.



3. Log on using an account with windows administrator privileges (see your system administrator for more information).

Notes

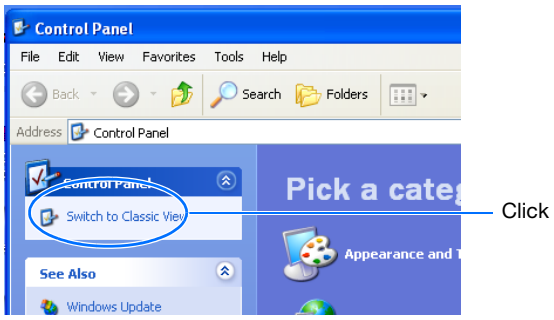
The Windows operating system desktop is displayed.



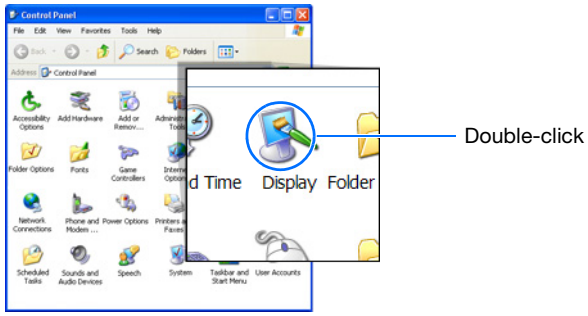
Setting the Display Settings and Power Options

IMPORTANT! You must have administrator rights to set the display and power options for the computer.

1. Select start ▸ **Control Panel**.
2. In the top left of the Control Panel, click **Switch to Classic View**.



3. In the Control Panel window, double-click **Display**.



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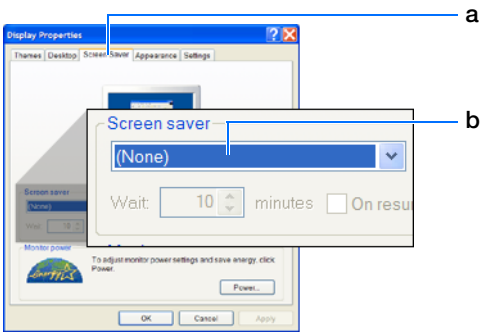
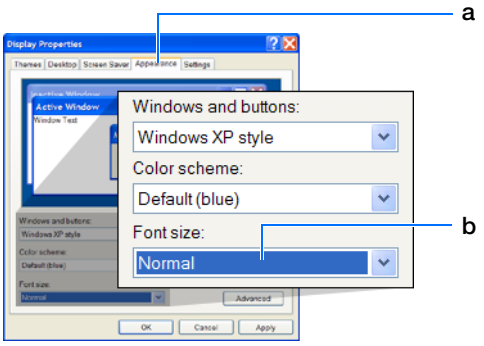
4. In the Display Properties dialog box, select the screen saver:
   a. Click the **Screen Saver** tab.
   b. Select **Screen Saver** ▸ **(None)**.



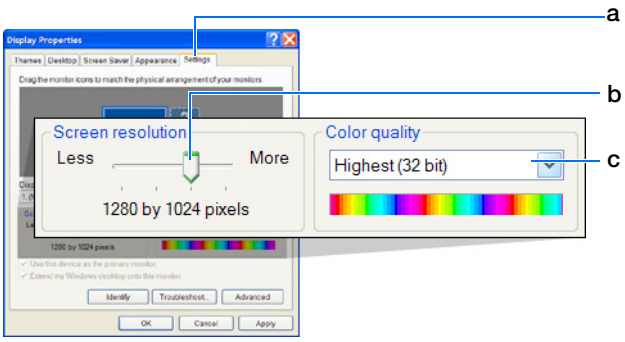
5. In the Display Properties dialog box, select the font:
   a. Click the **Appearance** tab.
   b. Select **Font size** ▸ **Normal**.



6. In the Display Properties dialog box, select color quality:
   a. Click the **Settings** tab.
   b. In the Screen resolution box, select **1280 by 1024 pixels** using the slider.

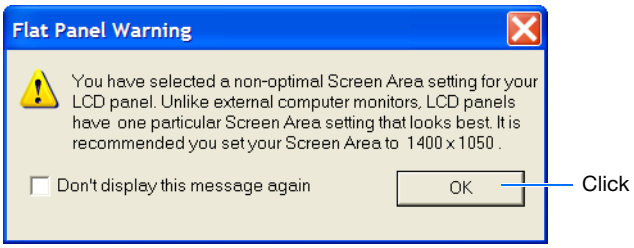
   Note: Some laptop models have a maximum resolution setting of 1024 by 768 pixels.

   c. Select **Color quality** ▸ **Highest (32 bit)**.



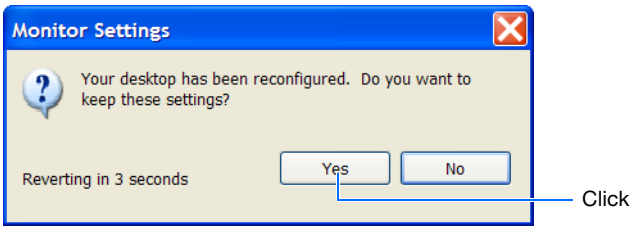
7. Click OK.

8. If you are using a laptop computer and the computer displays a Flat Panel Warning dialog box, click OK.

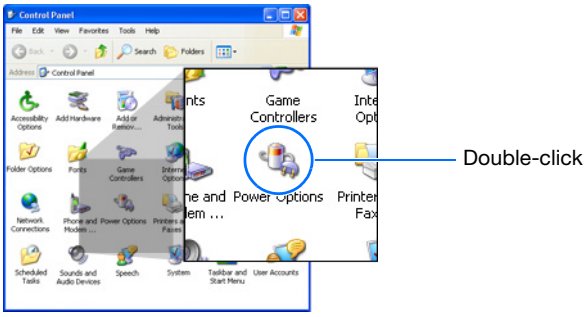


Notes

9. In the Monitor Settings dialog box, click Yes.



10. In the Control Panel window, double-click **Power Options**.



11. Set the Power Options. In the Power Options Properties dialog box:

a. Select the **Power Schemes** tab.

b. For Turn off monitor, select **Never**.

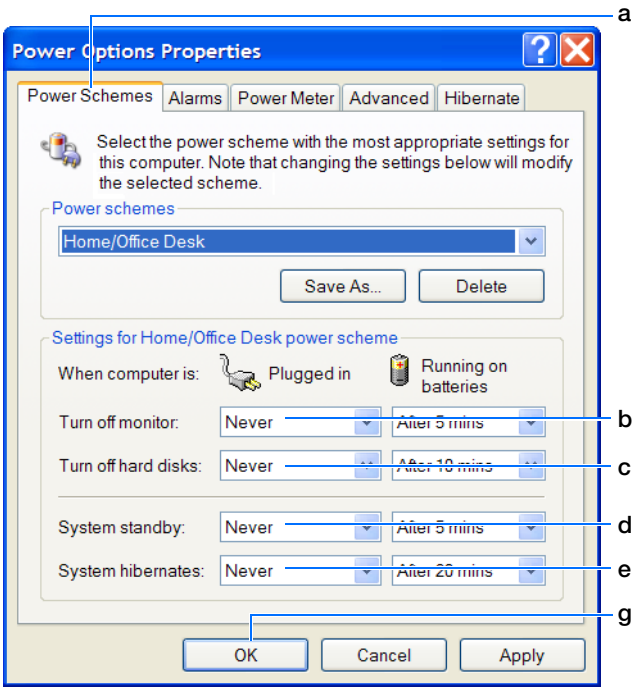
c. For Turn off hard disks, select **Never**.

d. For System standby, select **Never**.

e. For System Hibernates, select **Never** (this selection on the Power Schemes tab is not displayed on laptop computers).

f. Select the Hibernate tab (not shown), then ensure that the **Enable Hibernation checkbox is not checked**.

g. Click OK.



IMPORTANT! Make sure that the computer Hibernate power setting is disabled. If the Hibernate setting is enabled, data collection will stop when the computer goes into Hibernate mode.

12. Close the Control Panel window.

Notes

Connecting to the Network and Downloading Adobe Acrobat

1. Connect your computer to the network. For help in connecting your computer to the network, see your system administrator.

   IMPORTANT! Do not use the 7300/7500/7500 Fast System on a wireless network. Use of a wireless network can interfere with data collection and may result in data loss.

2. Download Adobe® Acrobat® Reader (free shareware) from www.adobe.com. Note that you must download Adobe® Acrobat® Reader to view document links referenced in the Real-Time System Setup wizard and the Sequence Detection Systems Online Help.

3. After the computer is set up, proceed with the installation by using either:
   - The Real-Time System Setup wizard provided on the Installation CD (which starts automatically after a slight delay when you insert the CD in to the CD drive of the computer, *or*
   - This installation and maintenance guide.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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