applied biosystems

StepOne™ and StepOnePlus™ Real-Time PCR Systems

Installation, Networking, and Maintenance

for use with:
Applied Biosystems™ StepOne™ Real-Time PCR System Software

Catalog Number 4376357, 4376374, 4376373, 4379216, 4376600, 4376598, and 4376599
Publication Number 4376782
Revision H

ThermoFisher SCIENTIFIC

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Applied Biosystems StepOne™ and StepOnePlus™

Real-Time PCR Systems

Installation, Networking, and Maintenance Guide

The information in this guide is subject to change without notice.

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Revision history

Table 1 Revision history of Pub. no. 4376782

Revision	Date	Description
H	February 2016	Minor update in materials required for replacing external fuses. Update in logos and covers.
G	November 2011	Added information about excitation wavelength for custom dyes. Cleaned up website URLs (and some website-related instructions) in boilerplate areas so they now are LT, and changed SDS to MSDS. Replaced logos and back cover with current versions.

Limited Use Label License No. 474: Real-Time PCR System for Research Use Only

Notice to Purchaser: The purchase of this instrument conveys to the purchaser the limited, non-transferable right to use the purchased instrument only, under intellectual property rights that are owned and/or controlled by Life Technologies and relate specifically to the instrument. Purchase of the instrument includes the right to use the instrument for internal research and to perform services (including the right to report the results of services for a fee) by the purchaser only, but does not convey rights to use any other products, reagents, assays or methods such as the 5' nuclease assay process. The sale of this instrument is expressly conditioned on the purchaser not reselling, repackaging, or distributing this instrument, or any of its components, and no such rights are conveyed expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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TRADEMARKS

Contents

Preface

Purpose of This Guide

About the System Documentation

The guides listed below are shipped with the Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems (StepOne™ and StepOnePlus™ systems).

Guide	Purpose and Audience	PN
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Genotyping Experiments	Explains how to perform experiments on the StepOne™ and StepOnePlus™ systems. Each Getting Started Guide functions as both: • A tutorial, using example experiment data provided with the Applied Biosystems StepOne™ Real-Time PCR System Software (StepOne™ software). • A guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the StepOne™ or StepOnePlus™ system.	4376786
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Presence/Absence Experiments		4376787
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments		4376785
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Standard Curve Experiments		4376784
Applied Biosystems 7500/7500Fast, StepOne™, and StepOnePlus™ Real-Time PCR Systems Quick Reference Card for Comparative C_T Experiments and Studies		4411937
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Installation, Networking, and Maintenance Guide	Explains how to install and maintain the StepOne™ and StepOnePlus™ systems. Intended for laboratory staff responsible for the installation and maintenance of the StepOne™ or StepOnePlus™ system.	4376782
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Installation Quick Reference Card		4376783
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Reagent Guide	Provides information about the reagents you can use on the StepOne™ and StepOnePlus™ systems, including: • An introduction to TaqMan® and SYBR® Green reagents • Descriptions and design guidelines for the following experiment types: – Quantitation experiments – Genotyping experiments – Presence/absence experiments Intended for laboratory staff and principal investigators who perform experiments using the StepOne™ or StepOnePlus™ system.	4379704

Guide	Purpose and Audience	PN
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Site Preparation Guide	Explains how to prepare your site to receive and install the StepOne™ or StepOnePlus™ systems. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the StepOne™ or StepOnePlus™ system.	4376768
Applied Biosystems StepOne™ Real-Time PCR System Software Help	Explains how to use the StepOne™ software to: • Set up, run, and analyze experiments using the StepOne™ and StepOnePlus™ systems. • Monitor networked StepOne™ and StepOnePlus™ instruments. • Calibrate StepOne™ and StepOnePlus™ instruments. • Verify the performance of StepOne™ and StepOnePlus™ instruments with an RNase P run. Intended for: • Laboratory staff and principal investigators who perform experiments using the StepOne™ or StepOnePlus™ system. • Laboratory staff responsible for the installation and maintenance of the StepOne™ or StepOnePlus™ system.	NA

Text Conventions

This guide uses the following conventions:

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

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

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open**.

User Attention Words

Two user attention words appear in the 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: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection, you need a valid user ID.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see “Safety Alert Words” on page 15.

How to Obtain More Information

Related Documentation

StepOne™ and StepOnePlus™ System Documents Available for Sale

Document	PN
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Installation Performance Verification Protocol	4376791
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Installation Qualification-Operation Qualification Protocol	4376790
Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Planned Maintenance Protocol	4376788

Note: For more documentation, see “How to Obtain Support” on page 14.

Obtaining Information from the Software Help

The StepOne™ Software Help describes how to use each feature of the user interface. Access the Help from within the StepOne™ software by doing one of the following:

- Press **F1**.
- Click in the toolbar.
- Select **Help ▸ StepOne Help**.

To find topics of interest in the Help:

- Review the table of contents.
- Search for a specific topic.
- Search an alphabetized index.

How to Obtain Support

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

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order user documents, SDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Technical Support and Sales facilities.

Customer Care Center

Contact the Customer Care Center when directed by this guide, or to schedule maintenance for your instrument (such as, annual planned maintenance or temperature verification/calibration). To contact the Customer Care Center call 1-800-762-4001, Option 1 (U.S.A. only). For all other countries, please contact your local support service representative.

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in the 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 limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in the document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instruments (see “Safety Symbols” on page 16).

Examples

IMPORTANT! You must create a separate spreadsheet for each 96-well plate.

CHEMICAL HAZARD. TaqMan Universal PCR Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

PHYSICAL INJURY HAZARD. During instrument operation, the heated cover and sample block can reach temperatures in excess of 100°C.

ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation. Never operate the system with the grounding conductor disconnected.

Symbols on the Instruments

Electrical Symbols

The following electrical symbols may be displayed on the instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	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 the **On/Off** position of a push-push main power switch.

Symbol	Description
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following safety symbols may be displayed on the instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see “Safety Labels on Instruments” on page 17). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in this and other documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

Symbol	Description
	Indicates the presence of moving parts 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 laser inside the instrument and to proceed with appropriate caution.

Environmental Symbols

The following symbol applies to all all 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 Customer Service office for equipment pick-up and recycling. See **www.lifetechnologies.com** for a list of customer service offices in the European Union.

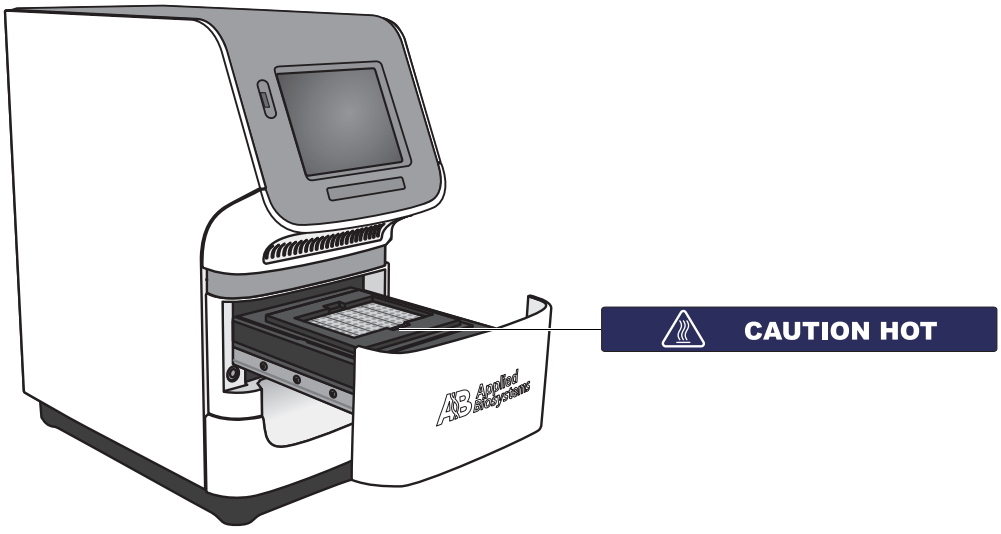
Safety Labels on Instruments

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

English	Francais
CAUTION Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Refer to SDS(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.
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 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 Thermo Fisher Scientific.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.
DANGER Class 3B (III) visible and/or invisible LED radiation present when open and interlocks defeated. Avoid exposure to beam.	**DANGER** Rayonnement visible ou invisible d'un faisceau LED de Classe 3B, (III) en cas d'ouverture et de neutralisation des dispositifs de sécurité. Evitez toute exposition au faisceau.

Locations of Warnings

The system contains a warning at the location shown below:



General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Using the instrument in a manner not specified by us 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 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 Safety Data Sheets (SDSs). See “About SDSs” on page 19.

Cleaning or Decontaminating the Instrument

CAUTION Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Chemical Safety

Chemical Hazard Warning

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

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 Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About SDSs” on page 19.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About SDSs

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

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

Obtaining SDSs

The SDS for any chemical supplied by us is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.lifetchnologies.com/support**
2. Click the **MSDS, COA & other Support Documents** link.
3. In the Redifine your search field:
   a. Type the chemical name, part number, or other information that you expect to appear in the SDS of interest.
   b. Select **Material Safety Data Sheets** in the drop-down list.
   c. Click **Search**.
4. To view, download, or print the document of interest:
   a. Click the link below MSDS Files.
   b. Click the link for the language of choice.
      The SDS opens in the web broswer. You can print or save the SDS.

Note: For the SDSs of chemicals not distributed by us, contact the chemical manufacturer.

Chemical Waste Safety

Chemical Waste Hazard

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

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by 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 Safety Data Sheets (SDSs) 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 SDS.
- 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 SDS.
- Handle chemical wastes in a fume hood.
- After emptying a 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

DANGER **ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the 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

WARNING **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.

WARNING **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 StepOne™ and StepOnePlus™ systems have an installation (overvoltage) category of II, and are classified as portable equipment.

LED Safety

To ensure safe LED operation:

- The system must be maintained by a Technical Representative from the company.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the LED is operating (during service with safety interlocks disabled), you may be exposed to LED emissions in excess of the Class **3B** rating.
- Do not remove safety labels or disable safety interlocks.

Biological Hazard Safety

General Biohazard

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, 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 equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

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.

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

U.S. and Canadian Safety Standards

The system has been tested to and complies with standard:

UL 61010-1 / CAN/CSA C22.2 No. 61010-1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”

Canadian EMC Standard

The instrument has been tested to and complies with ICES-001, Issue 4: “Industrial, Scientific, and Medical Radio Frequency Generators.”

European Safety, RoHS Directive, and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2014/35/EU). This instrument has been tested to and complies with standards EN 61010-1, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements.”

EN 61010-2-010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”

EN 61010-2-081, “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 2014/30/EU). 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.”

This instrument meets European RoHS Directive 2011/65/EU.

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS CISPR 11, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”

1 Get Started

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

About the StepOne™ and StepOnePlus™ Systems

There are two models available for this Real-Time PCR System:

System	Features
Applied Biosystems StepOne™ Real-Time PCR System (StepOne™ system)	• 48-well platform • Three-color system
Applied Biosystems StepOnePlus™ Real-Time PCR System (StepOnePlus™ system)	• 96-well platform • Four-color system • VeriFlex™ sample blocks

The Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems use fluorescent-based polymerase chain reaction (PCR) reagents to provide:

- Quantitative detection of target nucleic acid sequences (targets) using real-time analysis.
- Qualitative detection of targets using post-PCR (endpoint) analysis.
- Qualitative analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR).

Notes

About Data Collection

The StepOne™ and StepOnePlus™ systems collect raw fluorescence data at different points during a PCR, depending on the type of run that the instruments perform:

Run Type		Data Collection Point
Real-time runs	Standard curve	The instrument collects data following each extension step of the PCR.
	Relative standard curve	
	Comparative C_T ($\Delta\Delta C_T$)	
Post-PCR (endpoint) runs	Genotyping	The instrument collects data: • Before the PCR (For presence/absence experiments, data collection before the PCR is optional, but recommended.) • (Optional) During the PCR. The instrument can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting. • After the PCR
	Presence/absence	

Regardless of the run type, a data collection point or *read* on the StepOne™ or StepOnePlus™ instrument consists of three phases:

1. **Excitation** – The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.
2. **Emission** – The instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.
3. **Collection** – The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The StepOne™ software stores the raw fluorescent image for analysis.

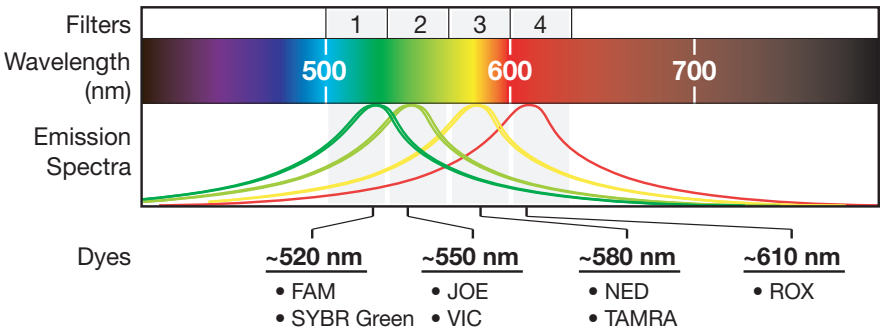
After a run, the StepOne™ software uses calibration data (spatial, dye, and background) to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal.

Notes

Filters and Supported Dyes

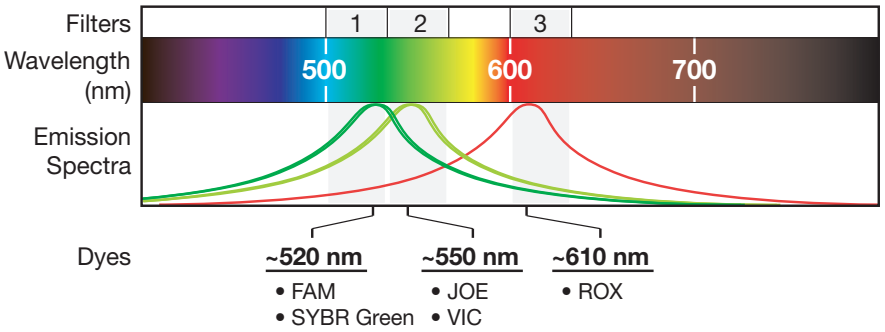
StepOnePlus™ System Filters and Dyes

The StepOnePlus™ instrument features a four-color filter set that supports the following dyes: FAM™ dye, JOE™ dye, NED® dye, ROX™ dye, TAMRA® dye, VIC® dye, and SYBR® Green dye. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



StepOne™ System Filters and Dyes

The StepOne™ instrument features a three-color filter set that supports the following dyes: FAM™ dye, JOE™ dye, ROX™ dye, VIC® dye, and SYBR® Green dye. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



About the VeriFlex™ Technology

The StepOnePlus instrument contains six independently thermally regulated VeriFlex™ blocks to help you optimize your thermal cycling conditions. You can set a different temperature for one or more of the VeriFlex blocks, creating up to six different zones for samples, or you can set the same temperature for each of the VeriFlex blocks.

Notes

Before You Begin

Before you install the Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System, familiarize yourself with this guide, read the supporting site preparation documentation, and obtain the materials required for the installation.

Review This Guide

You can use this guide to:

- Install the system.

  Chapters 2 through 5 of this guide contain all the information necessary for you to perform a complete installation of the system. You can install the system within 4 hours; however, the installation may require more time if you plan to connect the system to a network.

- Maintain the system.

  Chapter 6 contains all the information necessary to perform routine maintenance of the system.

  Go to Chapter 6 to learn how to:

  – Archive and back up data
  – Calibrate the instrument
  – Decontaminate the instrument
  – Move the instrument
  – Replace the instrument fuses
  – Ship the instrument for service

Prepare for the Installation

- Read the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide* and complete the preinstallation checklist contained in the guide.
- Obtain the materials for installation (see “Obtain the Required Materials” on page 30).
- Read “Select Protective Hardware and Software” on page 31 and obtain any protective hardware and software that you want to install to the system.

IMPORTANT! You can use this guide to reinstall the StepOne™ or StepOnePlus™ system after transportation, but you must have the necessary installation reagents. See “Check the Shipped Materials” on page 41 for a list of materials that are required for the installation and to order more materials as needed.

Notes

Obtain the Required Materials

- Tools and equipment
  - Centrifuge with a reaction plate adapter of the appropriate size
  - Powder-free gloves
  - Safety glasses
  - Scissors or box cutters
  - Screwdriver, flathead
- Computer (see below)
- (Optional) Protective hardware and software to install to the instrument and the computer (see "Select Protective Hardware and Software" on page 31).

Minimum Computer Requirements

If you did not order a computer with your system, supply one that meets the following requirements:

Component	Minimum Requirements‡	
	For operating the StepOne or StepOnePlus instrument	**For analyzing experiments in comparative C_T studies**
Computer	• Intel processor, 1.0 GHz • 512 MB of RAM • One 60-GB hard drive • 20/48X IDE CD-ROM • Ethernet network interface adapter (10BASE-T)§ • UL-listed • CE-marked • FCC-labeled	• Intel Core Duo T5500E processor, 1.66 GHz • 1 GB of RAM# • One 60-GB hard drive • 20/48X IDE CD-ROM • Ethernet network interface adapter (10BASE-T)§ • UL-listed • CE-marked • FCC-labeled
Monitor	• 1280 × 1024 pixel resolution for full-screen display • 32-bit color • UL-listed	• 1280 × 1024 pixel resolution for full-screen display • 32-bit color • UL-listed
Operating System‡‡	Microsoft Windows® XP Professional Operating System, Service Pack 2 or later	Microsoft Windows® XP Professional Operating System, Service Pack 2 or later

‡ A computer that meets the minimum requirement provides optimal software performance and is supported by Thermo Fisher Scientific.

§ Necessary only if you plan to connect the computer to the instrument or to a local area network.

The number of reaction plates (experiments) that can be analyzed in a comparative C_T study depends on the processor speed and the amount of RAM.

‡‡We support the Windows® Vista Business Operating System, Service Pack 1, for performing data analysis (that is, analyzing your experiments with the computer). We do not support the Vista operating system for instrument control purposes.

Notes

Select Protective Hardware and Software

Before installing the system, obtain any of the following optional hardware and/or software.

Electrical Protective Devices

We recommend several protective devices to prevent loss of data and to protect the system from damage resulting from electrical hazards.

Power Line Regulator

We recommend 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 system.

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

Uninterruptible Power Supply (UPS)

We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the system can corrupt data and possibly damage the system.

IMPORTANT! UPSs have finite battery lives and they provide power for a limited time. 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, power off the system unless you expect to regain power within the battery life of the UPS.

Surge Protector

We recommend 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 system.

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

Data Backup/ Storage Devices

We recommend the use of one or more backup storage devices to prevent potential loss of data caused by unforeseen failures of the instrument or the computer. If your system includes a computer, then the CD/DVD drive of the computer can serve as the backup storage device for your system. By saving your experiment files (EDSs) to one or more writable CD or DVDs on a weekly basis, you can back up the data generated by your system. Before installing the system, decide on a method for backing up your data ("Archive and Back Up Data" on page 142).

Notes

Third-Party Software

We recommend the use of several types of commercial software to ensure optimal performance of the system and software, including:

- File compression software for archiving data generated by the system
- System optimization software for defragmenting the computer hard drive(s)
- Antivirus and firewall software for protecting the system if you plan to connect it to a network

Before you install third-party software to the computer running the StepOne™ software, confirm that the software will not:

- Restrict Ethernet communication
- Interfere with StepOne™ software operation (see below)

To confirm that third-party software does not interfere with the StepOne™ software:

1. Install the software to the computer that contains the StepOne™ software.
2. Perform several test experiments using "dummy" plates (plates that do not contain reagents).

   Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.

3. Confirm that the system performs each test experiment without producing errors.

   If the system performs the tests successfully, perform experiments normally. If the system encounters errors during the test runs, the software may not be compatible with the StepOne™ software.

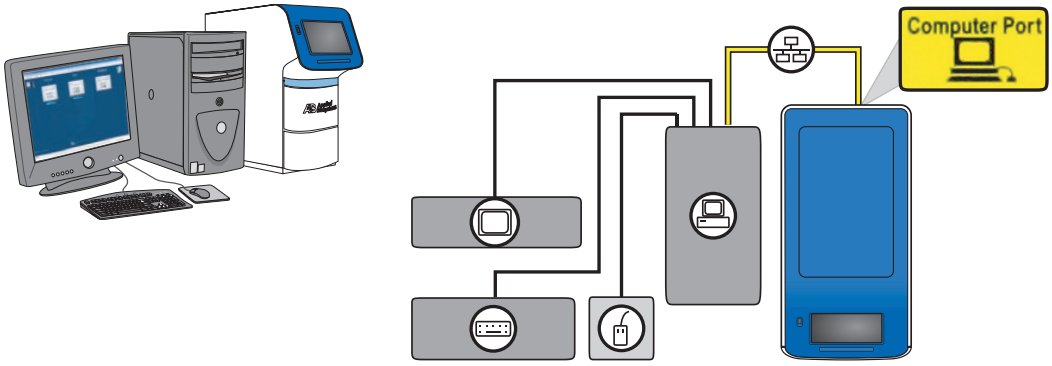
Notes

Select an Installation Layout

Before you install your StepOne™ or StepOnePlus™ system, select the installation layout that best suits your laboratory environment. The layout that you select affects the flow of the installation, so note your choice for later reference. The StepOne™ and StepOnePlus™ instruments can be installed in the two layouts described below (standalone or colocated).

Colocated Layout

In the colocated layout, the instrument is directly connected to the computer by the yellow StepOne system cable. In this configuration, you can set up, run, and analyze experiments from the colocated computer. The following figure shows an instrument in a colocated layout.



Note: You can control a colocated instrument from the instrument touchscreen, but only when the StepOne™ software is not performing a run.

Note: Experiment data can be transferred between the standalone instrument and computer over an Ethernet network (🖧) connection. See “Networked Colocated and Standalone Layouts” on page 34 for more information.

The following steps summarize the experiment workflow when using a colocated system:

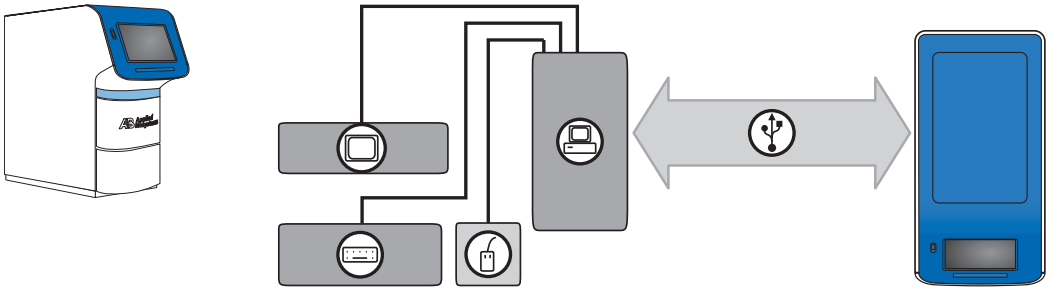
1. Create the experiment on the colocated computer using the StepOne™ software.
2. Start the run from the colocated computer.

   During the run, use the colocated computer to:
   - Monitor the status of a run in progress.
   - View temperature and amplification data as they are collected.
3. Analyze the experiment on the colocated computer using the StepOne™ software.

Notes

Standalone Layout

In the standalone layout, the instrument is *not* connected to the computer. Instead, a USB drive ( ) is used to transfer data between the instrument and the computer. In this configuration, you set up and analyze experiments from the computer but run them from the instrument touchscreen. The following figure shows an instrument in a standalone layout.



Note: Experiment data can be transferred between the standalone instrument and computer over an Ethernet network ( ) connection. See "Networked Colocated and Standalone Layouts" below for more information.

The workflow for the standalone layout is more complicated than for the colocated layout because the system components do not share a direct connection. The following steps summarize the experiment workflow for a standalone system:

1. Create the experiment on the standalone computer using the StepOne™ software.
2. Transfer the experiment to the standalone instrument on a USB drive.
3. Start the experiment from the instrument touchscreen.

   During the run, monitor the run from the instrument touchscreen.
4. Transfer the experiment to the standalone computer on a USB drive.
5. Analyze the experiment on the standalone computer using the StepOne™ software.

Networked Colocated and Standalone Layouts

You can expand the functionality of a colocated or standalone instrument by connecting it to an Ethernet network. When an instrument is part of a network, other computers on the network that are running the StepOne™ software can:

- Monitor the status of the instrument
- Send and download experiments to and from the instrument

IMPORTANT! Computers on the network cannot control the instrument, only monitor it.

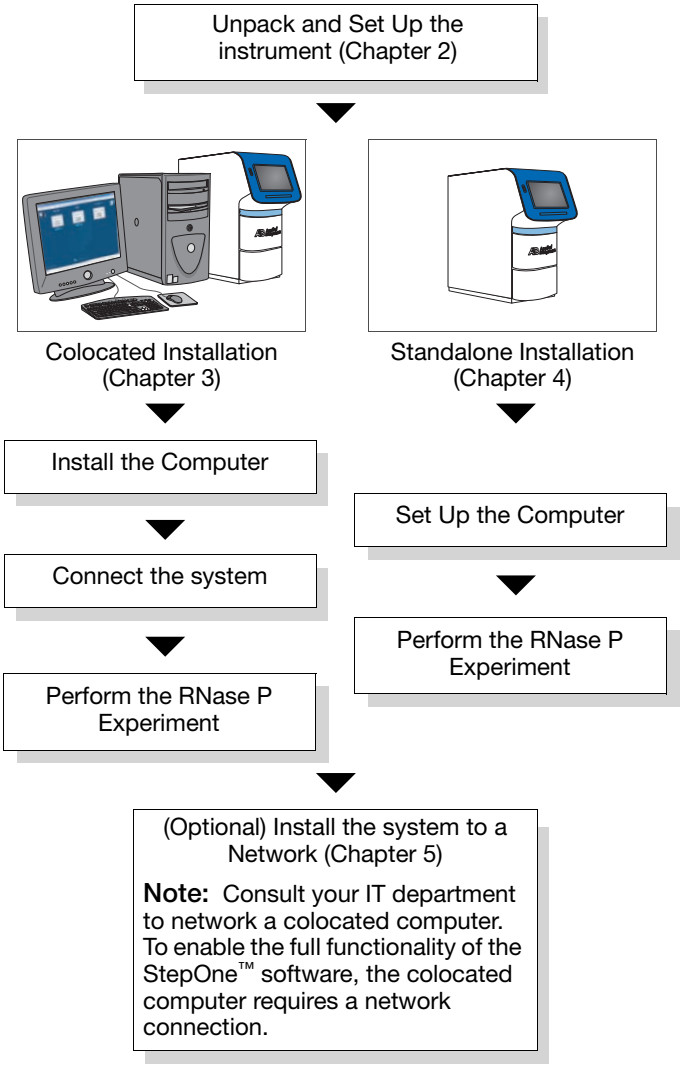
Note: Chapter 5, "Connect the System to a Network," on page 101 contains a detailed explanation of how to install the instrument to a network.

Notes

Plan the Installation

You can install the system within a 4-hour period. The installation does not require your constant attention; however, plan to spend most of your time working with the system.

Recommended Workflow



Notes

Notes

2 Set Up the Instrument

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

Notes

About the Installation

This chapter explains how to install the Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR Instrument for both colocated and standalone layouts.

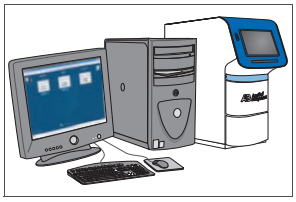
Installation Workflow

Chapter 1, Get Started

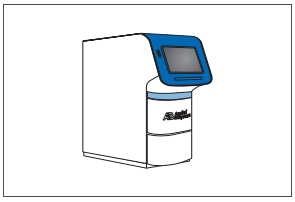
Unpack and Set Up the Instrument

1. Unpack and place the instrument.
2. Check the shipped materials.
3. Set up the instrument.
   a. Prepare the instrument.
   b. Set the instrument settings.

Chapter 3, Install the Colocated Layout



Chapter 4, Install the Standalone Layout



Notes

Unpack the Instrument

Prepare the installation site and unpack the instrument.

Before Unpacking

- Review and complete the preinstallation checklists in the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide*. The guide is shipped to you before the instrument arrives, and it contains important environmental and electrical requirements for the system.
- Prepare the installation site as described in the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide*.

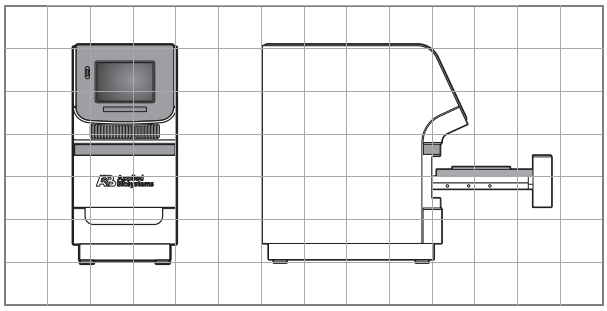
Confirm the Site Requirements

IMPORTANT! Avoid placing the system on surfaces that are subject to constant or intermittent vibration. Tabletop centrifuges, vortex mixers, and other laboratory equipment can vibrate the instrument during a run and produce data collection errors.

Confirm that the installation site meets the physical and environmental requirements for the instrument listed in the table below. Refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide* for a complete summary.

Requirement	Instrument	Additional	Total
Length (drawer closed)	48.5 cm (19.1 in)	• Front‡ – 18.8 cm (7.4 in) • Rear§ – 15.2 cm (6.0 in)	82.5 cm (32.5 in)
Width	24.6 cm (9.7 in)	15.2 cm (6.0 in)#	39.8 cm (15.7 in)
Height	51.8 cm (20.4 in)	30.5 cm (12.0 in)	81.7 cm (32.4 in)
Weight	• StepOne™ Instrument – 23.59 ± 0.45 kg (52 ± 1 lbs)‡‡ • StepOnePlus™ Instrument – 24.04 ± 0.45 kg (53 ± 1 lbs)		
Temperature	15 to 30°C (59 to 86°F) / Maximum change < 15°C every 24 hrs		
Humidity	15 to 80% relative humidity, noncondensing		
Power	100 to 240 VAC (50 to 60 Hz)		

‡ Clearance required to provide adequate space for the instrument drawer to open.
§ Clearance required to ensure adequate airflow and cooling.
# Clearance at the left and right sides to ensure adequate airflow and cooling.
‡‡ Or at least 38.32 kg (84.5 lbs) if you plan to place a computer with the instrument. Actual weights depend on the computer model you use.



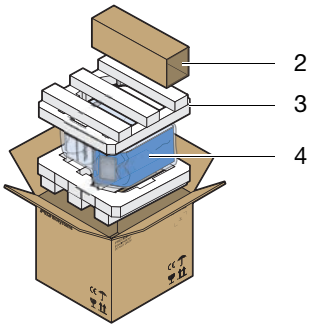
1 square. = $(10\ cm)^2$ $(3.94\ in)^2$

Notes

Place the Instrument

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 two people are required to lift the instrument.

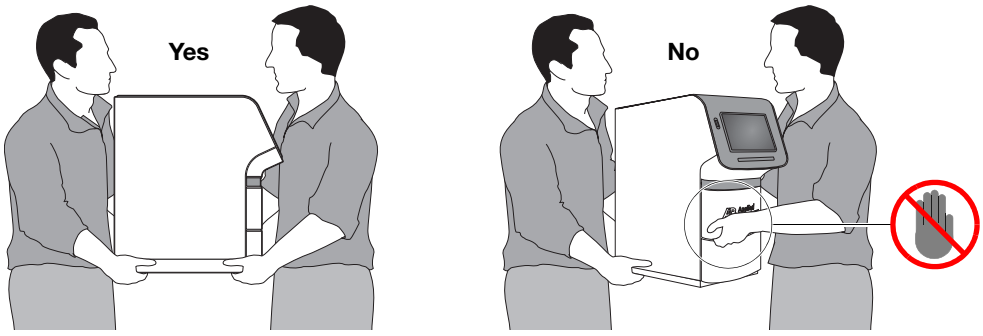
1. Cut the tape securing the top flaps of the crate and open them.
2. Remove the system packing kit from the instrument and set it aside.
3. Remove the packing material from the crate.
4. Remove the protective cover from the instrument.



5. Position yourselves on either side of the instrument and grasp it firmly at the corners.
6. Lift and move the instrument to the installation location.

   Guidelines for lifting and moving the instrument:
   - Keep your spine in a 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.

CAUTION Do not carry the instrument by the instrument drawer. Carrying the instrument by the drawer can damage the instrument optics.



Notes

Check the Shipped Materials

After unpacking the instrument, confirm that you have received all of the components shipped with the Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems.

Instrument and Computer

✓	Component
	StepOne™ and StepOnePlus™ instrument
	(Optional) Dell® computer: • Dell® tower computer with a Dell® flat screen monitor, *or* • Dell® laptop computer with a PCMCIA Network Card **IMPORTANT!** If you did not order a computer from us, provide one that meets the requirements listed in "Minimum Computer Requirements" on page 30.

Packing Kit

StepOnePlus™ System Packing Kit

✓	Component	PN
	Business card holder	4333831
	Cable, Ethernet (blue)	4376698
	Cable, StepOne™ system (yellow)	4376700
	Cables, universal voltage kit‡	4377117
	MicroAmp® 96-Well Support Base	4379590
	MicroAmp® 96-Well Tray for VeriFlex Blocks	4379983
	MicroAmp® Adhesive Film Applicator	4333183
	MicroAmp® Cap Installing Tool (Handle)	4330015
	MicroAmp® *Fast* 8-Tube Strip (0.1 mL)	4358293
	MicroAmp® *Fast* Optical 96-Well Reaction Plate with Barcode (0.1-mL)	4346906
	MicroAmp® *Fast* Reaction Tube with Cap (0.1-mL)	4358297
	MicroAmp® Optical 8-Cap Strip	4323032
	MicroAmp® Optical Adhesive Film	4360954
	StepOnePlus™ System USB Drive	4333831
	Warranty card, release notes	—

‡ Contains Australian, British, European, North American, and Japanese power cords.

Notes

StepOne™ System Packing Kit

✓	Component	PN
	Business card holder	4333831
	Cable, Ethernet (blue)	4376698
	Cable, StepOne™ system (yellow)	4376700
	Cables, universal voltage kit‡	4377117
	MicroAmp® *Fast* Reaction Tube with Cap (0.1-mL)	4358297
	MicroAmp® 48-Well Base Adapter	4375284
	MicroAmp® 48-Well Optical Adhesive Film	4375928
	MicroAmp® 96-Well Support Base	4379590
	MicroAmp® *Fast* 48-Well Tray	4375282
	MicroAmp® *Fast* 8-Tube Strip (0.1-mL)	4358293
	MicroAmp® *Fast* Optical 48-Well Reaction Plate	4375816
	MicroAmp® Optical 8-Cap Strip	4323032
	StepOne™ System USB Drive	4333831
	Warranty card, release notes	—

‡ Contains Australian, British, European, North American, and Japanese power cords.

Chemistry Installation Kit

StepOnePlus™ System Chemistry Installation Kit

✓	Component	PN
	TaqMan® RNase P Fast 96-Well Instrument Verification Plate	4351979

StepOne™ System Chemistry Installation Kit

✓	Component	PN
	TaqMan® RNase P Fast 48-Well Instrument Verification Plate	4371439

Notes

Software and Documentation Kit

✓	Component	PN
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide	4376768
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Quick Reference Card	4376783
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking, and Maintenance Guide (this document)	4376782
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Genotyping Experiments	4376786
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Presence/Absence Experiments	4376787
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments	4376784
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments	4376785
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide	4379740
	Applied Biosystems StepOne™ Real-Time PCR System Software	4379099
	Primer Express® Software v3.0	4363991
	Miscellaneous items (mouse pad, registration card, and others)	—

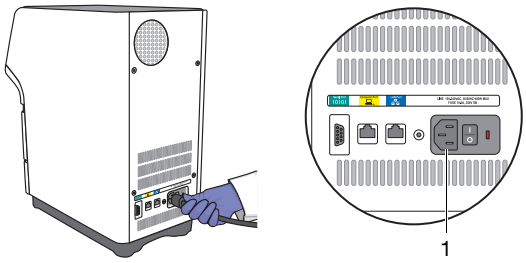
Notes

Set Up the Instrument

After confirming that you have received all system components, perform an initial system test of the instrument, then define the instrument settings.

Prepare the Instrument

1. Connect the power cord to the system.



2. (Optional) Install electrical protective devices to the power cord.

   We recommend that you install at least one of the electrical protective devices (power line regulator, uninterruptible power supply, and/or surge protector) to prevent loss of data and to protect the instrument from damage resulting from electrical hazards.

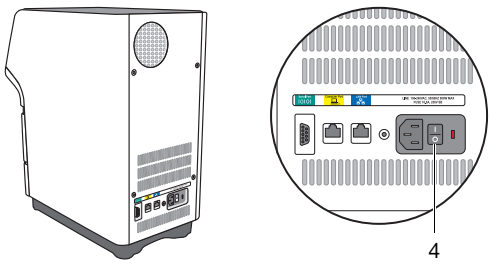
   Note: For more information on selecting electrical protective devices for the instrument, see "Select Protective Hardware and Software" on page 31.

3. Connect the power cord to the power receptacle.

4. Power on the instrument, then wait for it to perform a diagnostic of the system components.

   When you power on the instrument for the first time, it may require more than 5 min to boot. Proceed with the installation when the touchscreen displays the Main Menu, indicating that the instrument has completed the boot.

   Note: If you are updating the instrument firmware, the instrument may pause for up to 20 min before completing the boot.



   If the instrument displays an error, power off the instrument, wait 30 sec, then power on the instrument. If the instrument displays the error again, contact Support as explained in "How to Obtain Support" on page 14.

Notes

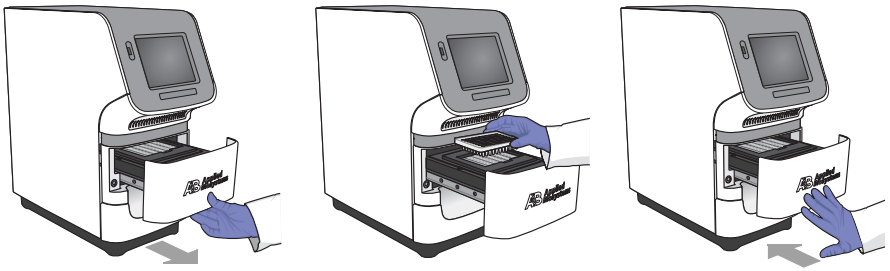
5. Remove the packing material from the sample block(s):

   a. Open the instrument drawer.

   b. Remove the packing material from the sample block(s).

   c. Close the instrument drawer.

   When removing the packing material from the sample block(s), you must power on the instrument to lower the instrument drawer. The drawer is in the "raised" position during transportation and it will not open until the instrument is powered on.



Define the Settings

1. Set the instrument name:

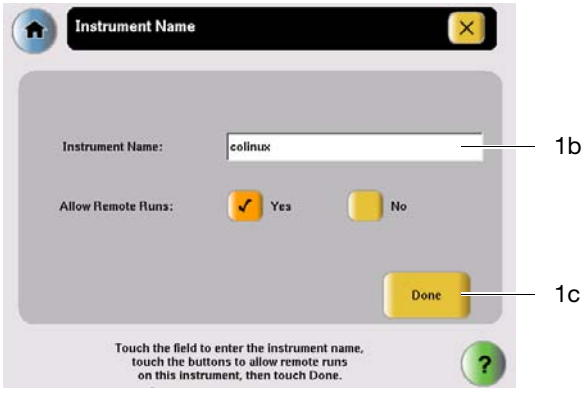
   a. In the Main Menu, touch **Settings Menu**, then touch **Admin Menu**, then touch **Set Instrument Name**.

   b. Touch the **Instrument Name** field, enter a name for the instrument, then touch **Done**.

   The instrument name identifies your computer on the network. If you want to connect the instrument to a network, the name must be unique.

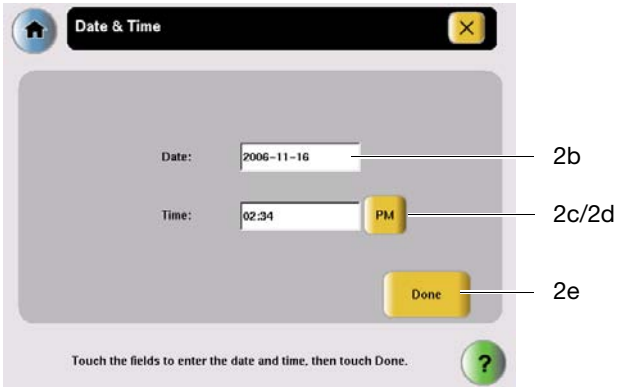
   Note: The instrument does not restrict the length or content of the instrument name. However, if you plan to connect the instrument to a network, limit the instrument name to fifteen characters and do not include spaces or special characters (such as ; : " < > * + = \ | ? ,).

   c. Touch **Done** to save the settings, then touch **OK** when prompted.



Notes

2. Define the date and time settings:
   a. In the Admin Menu, touch **Set Date & Time**.
   b. Touch the Date field, then enter the current date (in *year*/*month*/*day* format), then touch **Done**.
   c. Touch the Time field, then enter the time in *hour*:*minute* format, then touch **Done**.
   d. Touch **AM**/**PM** to display the correct time period.
   e. Touch **Done** to save the settings, then touch **OK** when prompted.



3. Touch , then touch **Yes** in the Confirmation Screen to enter standby mode.

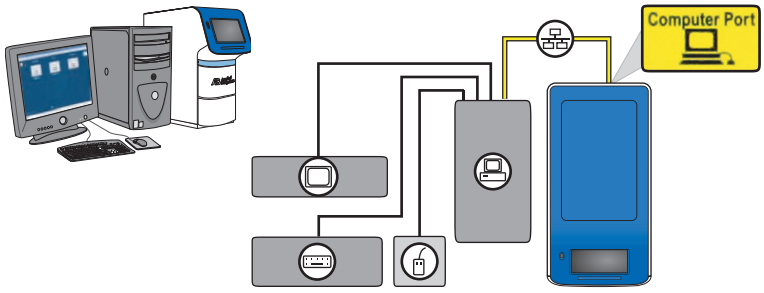
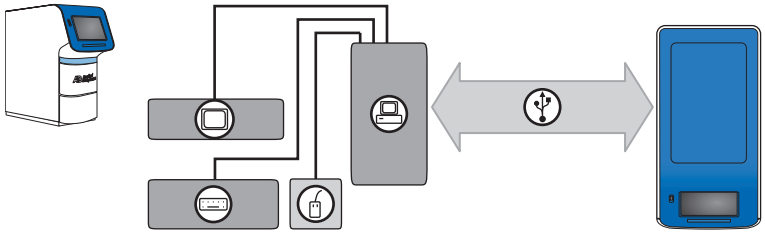
Notes

Complete the Installation

After setting up the instrument, install the remaining system components according to the layout you selected (standalone or colocated), then perform the RNase P verification experiment.

Perform the Appropriate Installation

As explained in "Select an Installation Layout" on page 33, the system supports two basic installation layouts: colocated and standalone. Perform the installation according to the layout you selected.

If you want to install the…	Go to…
instrument and the computer to the same location, connected by the yellow StepOne system cable. 	Chapter 3, "Install the Colocated Layout," on page 49.
instrument and the computer to different locations. 	Chapter 4, "Install the Standalone Layout," on page 75.

IMPORTANT! Do not install the instrument to an Ethernet network until you complete the installation by performing an RNase P experiment that passes. See Chapter 5, "Connect the System to a Network," on page 101 to connect the instrument to a network after the installation.

Notes

Notes

3 Install the Colocated Layout

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

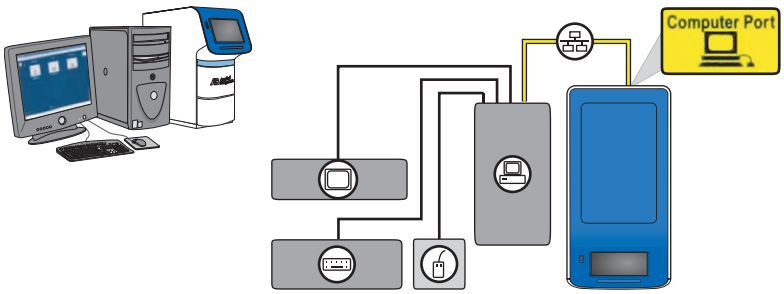
Notes

About the Colocated Installation

This chapter explains how to install the Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR System for colocated operation.

When to Perform a Colocated Installation

In this layout, the yellow StepOne system cable connects the instrument to the colocated computer. Install the system in the colocated layout when both the computer and the instrument will be placed together in the same location. See “Colocated Layout” on page 33 for a complete description of the layout.



Note: If you *do not* want to connect the computer to the instrument, perform the standalone installation as described in Chapter 4, “Install the Standalone Layout.”

Notes

Colocated Installation Workflow

Chapter 2, Set Up the Instrument

Set Up the Computer

1. Install the computer.
2. Install the StepOne™ software.

IMPORTANT! Perform step 2 only if you did not order a computer from us.

Check for Software Updates

Connect the System

1. Connect the instrument to the computer.
2. Confirm the connection.

Perform the RNase P Experiment

1. Set up the experiment.
2. Run the experiment.
3. Analyze the experiment.

Chapter 5, Connect the System to a Network

Notes

Set Up the Computer

After you install the StepOne™ or StepOnePlus™ instrument, set up the colocated computer.

IMPORTANT! If you ordered a computer from us, you need only to unpack the computer as explained on page 53. Computers shipped by us are ready for use with the instrument and already contain the StepOne™ software.

Materials Required

- CD, Applied Biosystems StepOne™ Real-Time PCR System Software
- Computer

  IMPORTANT! If you did not order a computer from us, provide a computer that satisfies the requirements listed on "Minimum Computer Requirements" on page 30.

- Screwdrivers, flathead and Phillips
- StepOne system cable, yellow (from the system packing kit)
- (Optional) Protective hardware to install to the computer

  We recommend that you install one or more of the following electrical devices to the computer to prevent loss of data and to protect the computer from damage resulting from electrical hazards:

  - Power line regulator
  - Uninterruptible power supply (UPS)
  - Surge protector
  - Backup storage device

  For more information, see "Select Protective Hardware and Software" on page 31.

Notes

Install the Computer

After obtaining the required materials, install the computer.

Confirm the Site Requirements

Confirm that the installation site meets the physical and environmental requirements for the computer. See the documentation for your computer for the site requirements.

Place the Computer

CAUTION **PHYSICAL INJURY HAZARD**. Improper lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the computer. We recommend safety training for proper lifting techniques. 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 the monitor, this may require two or more people.

1. If you have not done so already, unpack the monitor, computer, keyboard, and mouse, and assemble the computer components as described in the computer installation guide.

Guidelines for lifting and moving the computer:

- Make sure that you have a secure grip on the computer when lifting.
- Make sure that the pathway from the beginning of the lift to the final location is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a neutral position while lifting with your legs.
- Coordinate lift and movement with all participants before lifting and carrying the computer.
- Instead of lifting the computer/monitor from the packing box, tilt the box on its side, then slide the contents out of the box.

2. Make the following connections:

Connect				Do Not Connect
Power	Mouse	Keyboard	Monitor	Ethernet
	or	or	or	

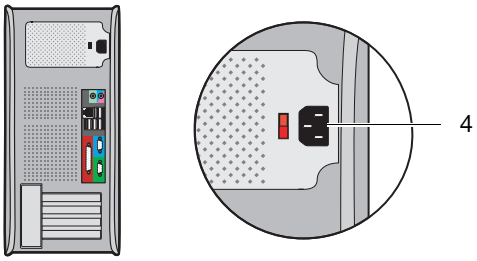
Notes

3. If you ordered a laptop computer from us, install the PCMCIA Network Card as described in the documentation accompanying the card.

   After installing the PCMCIA Network Card, wait several minutes to allow the computer to enable the device. The computer may require time to register the network card with the operating system before it is available for use.

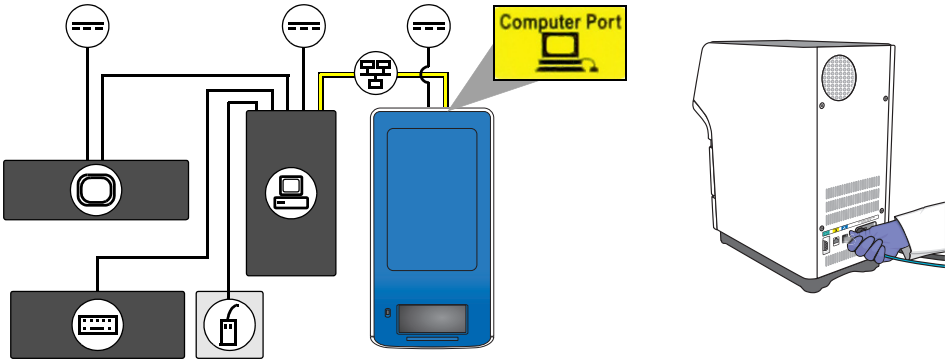
   Note: If you are installing your system in a colocated setup, do not connect the StepOne system cable to the Ethernet port of the PCMCIA card. If you have already installed the instrument connection through the card, unplug and connect the StepOne system cable to the appropriate port.

4. If you ordered a tower computer from us, set the voltage appropriately for your region as described in the computer installation guide.



5. Get the yellow StepOne system cable from the system packing kit (see "Check the Shipped Materials" on page 41), then connect the:
   - *Yellow* Ethernet port ( ) of the instrument, to the
   - Ethernet port ( ) of the computer

   IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port ( ); this port is reserved for a network connection.



6. (Optional) Install electrical protective devices to the computer.

   Note: For more information, see "Select Protective Hardware and Software" on page 31.

Notes

Power On the Computer

1. Power on the computer and monitor.
2. Log onto the operating system:
   - If you are using a computer that you provided, log on to the operating system as a member of the Administrators user group.
   - If you are using a computer from us, enter **Administrator** in the User field, then click **Login**. Leave the Password field blank.

   IMPORTANT! If you are installing a computer that you provided, you must log onto the Windows® operating system using a user account that belongs to the Administrators user group.

3. In the Getting Started with Windows® XP window, deselect **Show this screen at startup**, then click **Exit**.
4. Determine the next step:
   - If you are installing a computer that you provided, go to "Install the StepOne™ Software" on page 56.
   - If you are installing a computer from us, go to "Check for StepOne™ Software Updates" on page 60.

Notes

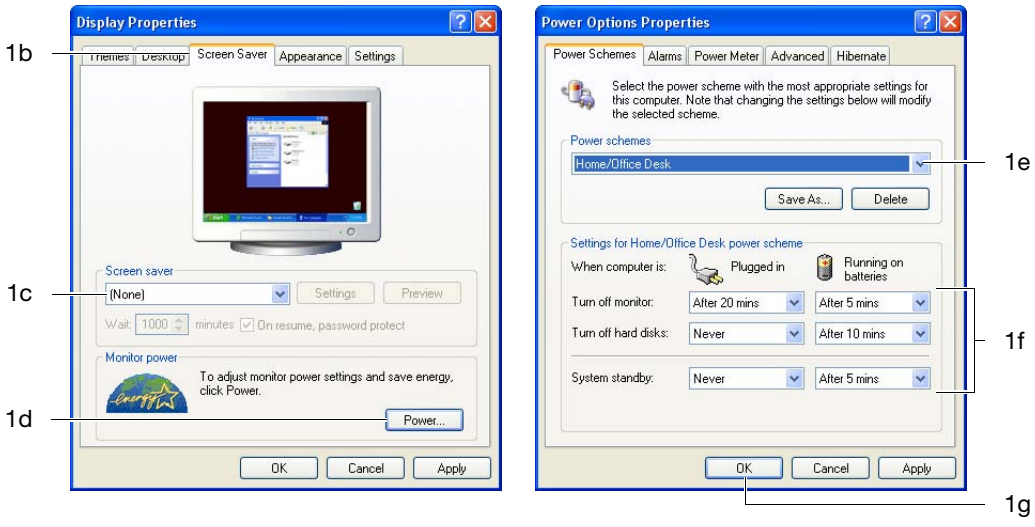
Install the StepOne™ Software

After setting up the computer, define the display properties, computer name, and the date and time settings, then install the StepOne™ software.

IMPORTANT! If you ordered a computer from us, skip "Install the StepOne™ Software" and go to "Check for StepOne™ Software Updates" on page 60. Computers that are supplied by us already contain the StepOne™ software, and are ready for use with the instrument.

Prepare the Operating System

1. Change the screen saver and power options settings:
   a. Right-click the desktop, then select **Properties**.
   b. In the Display Properties dialog box, select the **Screen Saver** tab.
   c. In the Screen saver group box, select **(None)** in the dropdown menu.
   d. Click **Power**.
   e. In the Power Options Properties dialog box, select **Home\Office Desk** from the Power schemes dropdown list.
   f. Set the Plugged in power scheme settings:
      - Monitor – Select **After 20 mins** from the Plugged in dropdown menu.
      - Hard Disks – Select **Never** from the Plugged in dropdown menu.
      - System standby – Select **Never** from the Plugged in dropdown menu.
   g. Click **OK** to save the settings.



Notes

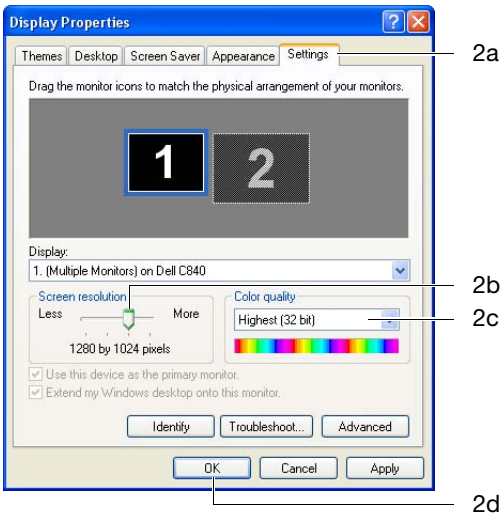
2. Change the screen resolution:

   a. In the Display Properties dialog box, select the **Settings** tab.

   b. In the Screen Area group box, use the slider to select **1280 by 1024 pixels**.

   c. In the Colors group box, select **Highest (32 bit)** in the dropdown menu.

   d. Click **OK** twice, then click **Yes** to accept the new resolution.



3. Define the date and time settings:

   a. Right-click the time readout in the toolbar, then select **Adjust Date/Time**.

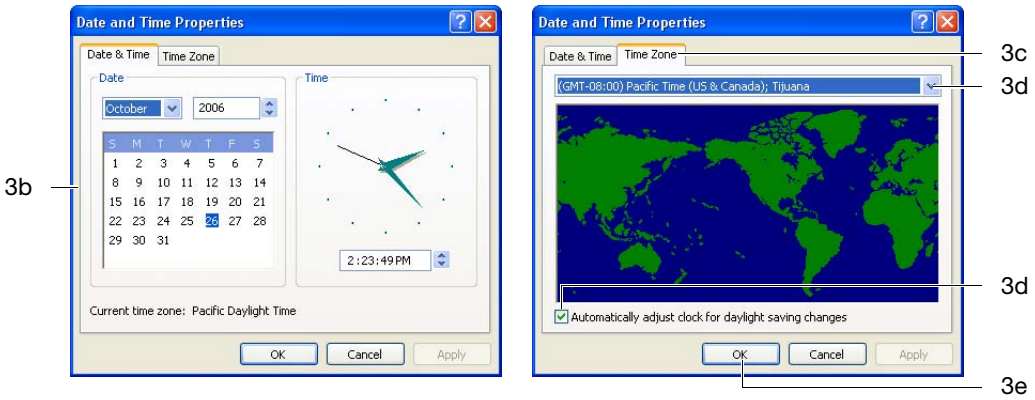
   b. In the Date & Time tab, define the date and time settings.

   c. Select the **Time Zone** tab.

   d. Select a time zone from the dropdown menu, then select **Automatically adjust clock for daylight saving changes** if necessary.

   e. Click **OK**.

Note: Computers on a network synchronize their date/time settings with the server.



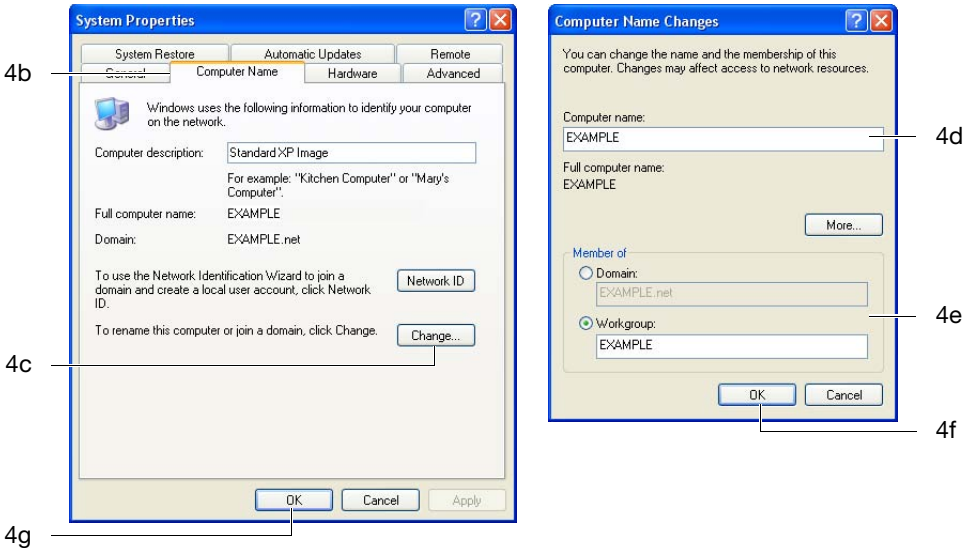
Notes

4. Change the computer name:
   a. In the desktop, right-click **My Computer,** then select **Properties**.
   b. In the System Properties dialog box, select the **Computer Name** tab.
   c. Click **Change**.
   d. In the Computer Name field, enter the computer name.

   CAUTION Do not give the computer the same name as the instrument. A shared name could lead to network conflicts if you install both devices to an Ethernet network.

   e. If necessary, define the Domain or Workgroup settings if you are going to connect the computer to a network.
   f. Click **OK** twice.
   g. In the Network Identification dialog box, click **OK**.

Note: The computer name identifies the computer on the network and must be different than that of the instrument. After you install the system, you can change the computer name without disrupting the function of the StepOne™ software.



5. In the Systems Settings Change dialog box, click **Yes** to restart the computer.
6. Log onto the operating system as a member of the Administrators user group.

Notes

Install the Software

IMPORTANT! You must be logged into the Windows® operating system as an administrator to install the StepOne™ software.

Note: If you encounter errors during the installation of the StepOne™ software, reinstall the software as described in "Uninstall the StepOne™ Software" on page 160.

1. Insert the Applied Biosystems StepOne™ Real-Time PCR System Software CD into the CD drive of the computer and wait for the installer to start.

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

2. In the Welcome page of the InstallShield Wizard, click **Next**.

3. In the License Agreement page, click **Yes** to accept the agreement.

4. In the Choose Destination Location page, click **Next** to accept the default location.

   Note: We recommend installing the StepOne™ software to the D drive; however, you can install the software to another location.

5. In the Default Instrument Type page, select the type of instrument that you are installing (**StepOnePlus™ Instrument** or **StepOne™ Instrument**), then click **Next**.

6. In the Start Copying Files page, confirm that the installation location displayed in the Current Settings field is correct, then click **Next** to begin installing the StepOne™ software.

7. When the StepOne™ software completes the installation, click **Finish**.

Notes

Check for StepOne™ Software Updates

Updates and/or patches may be available for the StepOne software. Applied Biosystems recommends that you check for software updates before you proceed with the installation.

Note: To periodically check for software updates after the StepOne or StepOnePlus system is installed (as part of routine maintenance), see "Update the StepOne™ Software or the Operating System" on page 154.

To check for software updates:

1. Go to: **http://www.lifetechnologies.com/support** and click the **Instrument Software, Patches & Updates** link.
2. In the Instrument Software, Patches & Updates page, click the link for the appropriate software.
3. Under Software Download, view the available downloads.
4. If there are updates and/or patches for StepOne software v2.1 or later, select the appropriate option, then follow the prompts to begin the download.

   You may be asked for your instrument serial number. The serial number is on the back panel of your instrument, or can be obtained from the instrument touchscreen.

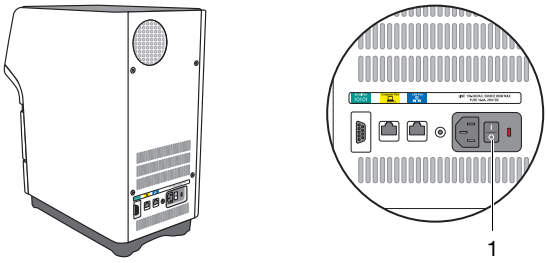
Notes

Connect the System Components

After preparing the computer, connect the instrument to the computer, then confirm that the StepOne™ software can access the instrument by retrieving the maintenance data.

Establish the Connection

1. If necessary, power on the instrument.



2. Power on the computer and monitor.
3. Log in as Administrator, or as a member of the Administrators user group.

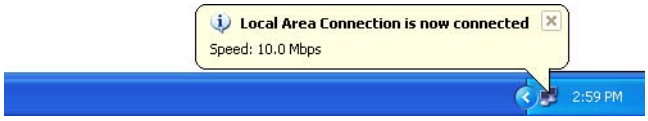
IMPORTANT! If you install the system using a computer that you provide, you must log onto the Windows® operating system using a user account that belongs to the Administrators user group.

4. In the desktop, double-click (StepOne software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **StepOne Software** ▸ ***<software name>***

   where *<software name>* is the current version of the StepOne software.

   While the StepOne™ software loads:

   - The software establishes communication with the instrument.
   - The Windows XP operating system automatically detects the connection and displays a Local Area Connection alert in the taskbar.



Note: You can disable the network connection messages displayed when the StepOne™ software and instrument communicate. See "(Optional) Disable the Local Area Connection Messages" on page 63 for more information.

Notes

5. In the Login dialog box, create a user name:

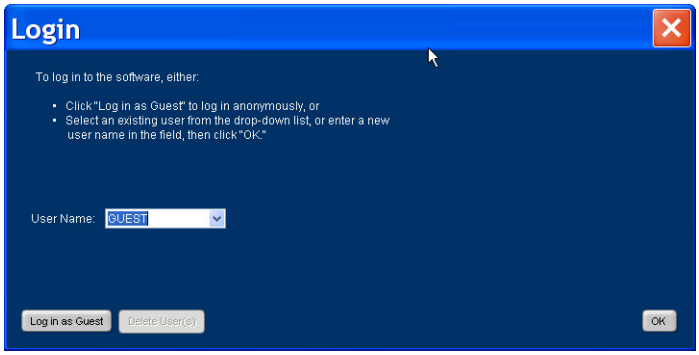
   a. In the User Name field, enter a user name.

   Note: You cannot use the following characters in the User Name field: space, forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (|), colon (:), or semicolon (;).

   b. Click **OK**.

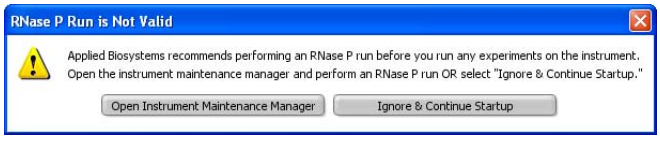
We recommend that you log in with a user name. If you log in with a user name, you can set preferences in the software. The next time you log in to the software with the same user name, the software uses the preferences you set as the defaults.

IMPORTANT! If you log in to the software as a Guest, you cannot set preferences.



6. If you are prompted to update the instrument firmware, click **Upgrade Firmware Now**.

7. When the StepOne™ software prompts you to perform the RNase P experiment, click **Open Instrument Maintenance Manager**.



Wait while the StepOne™ software downloads the maintenance data from the instrument. If successful, the StepOne™ software automatically opens the RNase P Run wizard in the Instrument Maintenance Manager.

If the StepOne™ software displays the “Instrument Connection Failed” message:

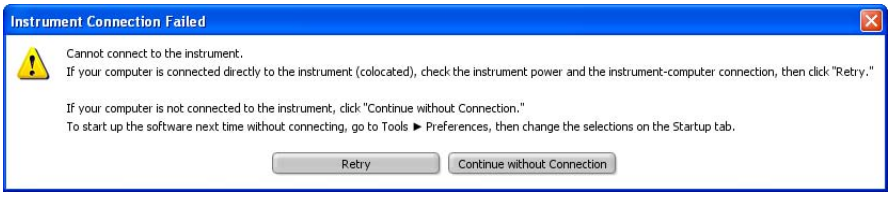
   a. Confirm that the computer and instrument are connected by the yellow StepOne system cable as explained in step 5 on page 54.

   b. Power off the instrument, then after 30 sec power on the instrument.

Notes

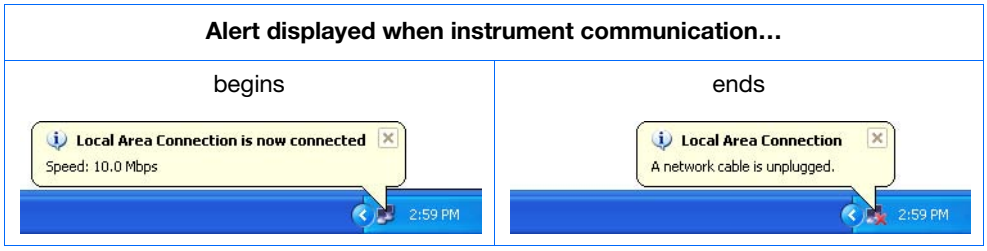
c. When the instrument displays the main screen, click **Retry**.

   If the problem persists, contact Support as explained in "How to Obtain Support" on page 14.



(Optional) Disable the Local Area Connection Messages

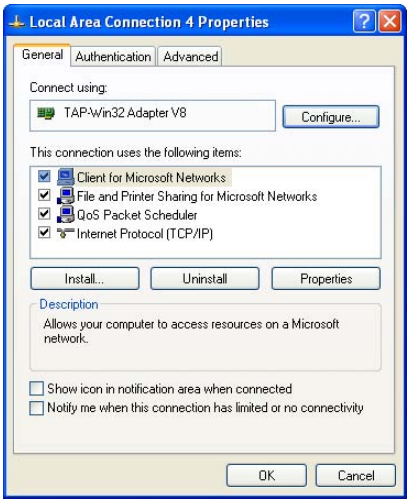
During normal operation, the Windows® operating system displays Local Area Connection messages when the StepOne™ software and instrument communicate. You can disable these messages by changing the network connection settings for the Ethernet network interface adapter as explained below.



To disable the network connection alerts for the instrument connection:

1. In the desktop, select **Start** ▸ **Control Panel**, then double-click **Network Connections**.
2. Right-click **Local Area Connection**, then select **Properties**.
3. Deselect **Show icon in notification area when connected**.
4. Deselect **Notify me when this connection has limited or no connectivity**.
5. Click **OK**.

Notes



6. Close the Network Connections window.

Notes

Perform the RNase P Experiment

After you establish system communication, confirm the function of the system by running a TaqMan® RNase P Fast Instrument Verification Plate.

Materials Required

- TaqMan® RNase P Fast Instrument Verification Plate
- Powder-free gloves
- Safety glasses
- Centrifuge with reaction plate adapter

When to Perform the RNase P Experiment

We recommend performing an RNase P experiment:

- After installing the system.
- After moving the instrument to another location.
- As needed to confirm the function of the instrument, depending on your laboratory and regulatory requirements.

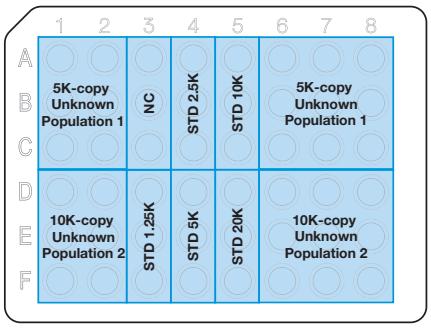
Purpose of the Experiment

The TaqMan® RNase P Fast Instrument Verification Plate experiment verifies the performance of the instrument. The RNase P plate is preloaded with the reagents necessary for the detection and quantitation of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme).

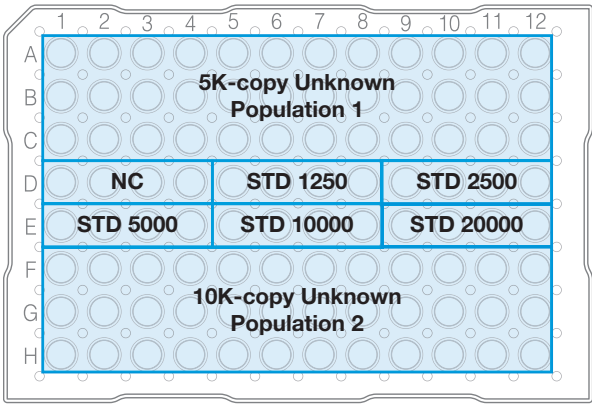
Each well contains:

- 1✕ TaqMan® Fast Universal PCR Master Mix, No AmpErase® UNG
- RNase P primers
- FAM™ dye-labeled probe
- Known concentration of human genomic DNA template

The figure below illustrates 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 negative control wells (NC).



TaqMan® 48-well RNase P Plate for the StepOne™ System



TaqMan® 96-well RNase P Plate for the StepOnePlus™ System

Notes

After the run, the StepOne™ software:

1. Generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards.
2. Calculates the concentration of the two unknown populations using the standard curve.
3. Calculates the following to assess the instrument performance:

   $$[(CopyUnk_2) - 3(\sigma_{CopyUnk2})] > [(CopyUnk_1) + 3(\sigma_{CopyUnk1})]$$

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

Installation Specification

The instrument passes the installation specification if the inequality holds and the instrument successfully distinguishes between 5,000 and 10,000 copies with greater than 99.7% confidence.

To meet the installation specification, you can omit a limited number of outlier wells from the 5,000- and 10,000-copy unknown populations. The number of wells that you can remove depends on the instrument that you are installing.

Instrument	Maximum number of outlier wells that can be removed from each…			Total
	Unknown Population	Standard (STD)	Negative Controls (NC)	
StepOnePlus™ System	6	0	0	12
StepOne™ System	2	0	0	4

Notes

Set Up the Experiment

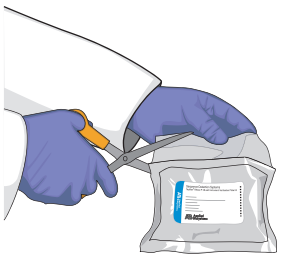
Prepare the TaqMan® RNase P Fast Instrument Verification Plate for the run.

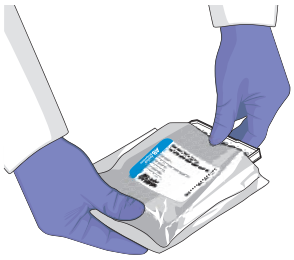
Prepare the RNase P Plate

IMPORTANT! Do not use an RNase P plate for another Thermo Fisher Scientific instrument to verify the performance of the StepOne™ system. RNase P plates for other instruments contain the TAMRA™ dye, which is not supported by the StepOne™ system.

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

1. Get the TaqMan® RNase P Fast Instrument Verification Plate from the freezer, then allow the reaction plate to warm to room temperature (for approximately 5 min).
2. Remove the RNase P plate from its packaging.



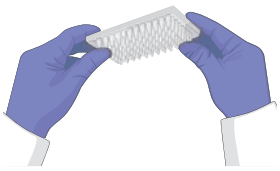


3. Vortex the reaction plate for 5 sec.
4. Centrifuge the reaction plate for 2 min at less than 1500 rpm.

IMPORTANT! The reaction plate must be well mixed and centrifuged.

5. Confirm that the liquid is at the bottom of each well of the reaction plate. If not, centrifuge the reaction plate again at a greater rpm and for a longer time.

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



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

Notes

Run the Experiment

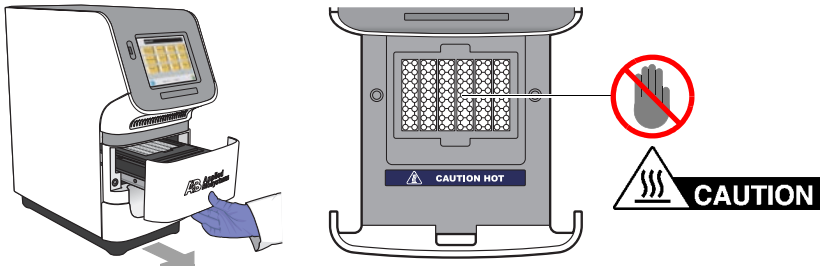
After preparing the TaqMan® RNase P Fast Instrument Verification Plate, load the plate into the instrument and start the run.

Load the Instrument

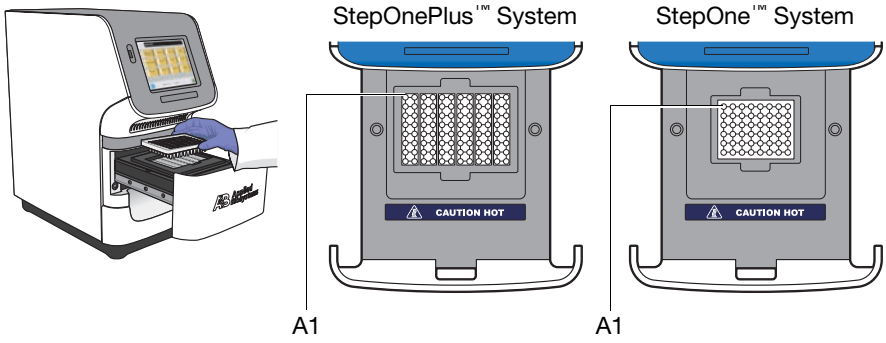
CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reach room temperature.

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

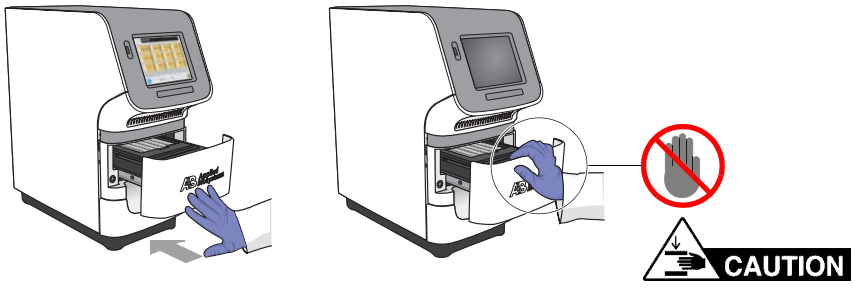
1. Open the instrument drawer.



2. Place the RNase P plate in the sample block(s) so that the A1 position is at the back-left corner of the sample block(s).



3. Close the instrument drawer carefully.

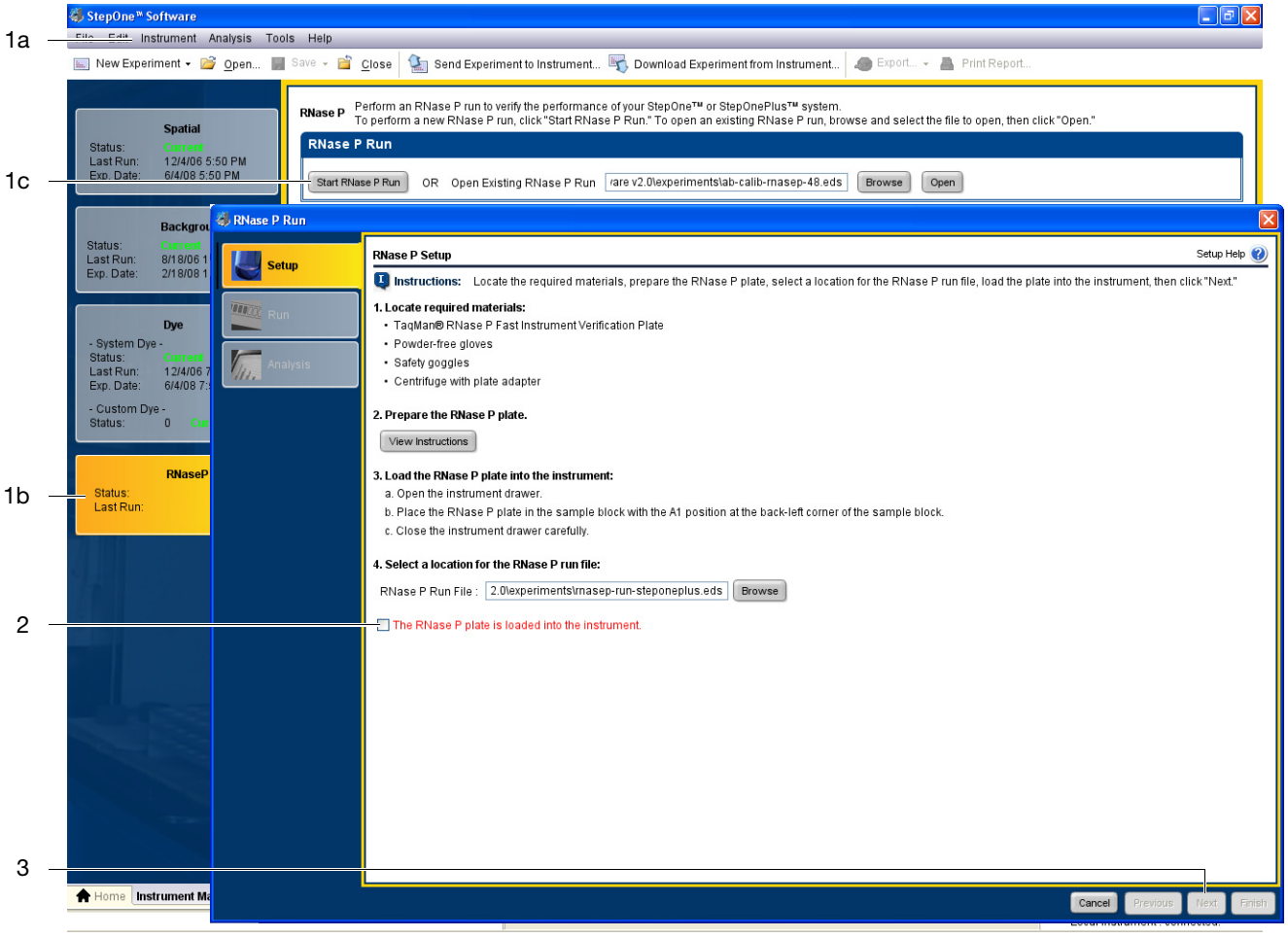


Notes

Start the Run

1. If the StepOne™ software automatically opened the RNase P Run dialog box, go to step 2. Otherwise, display the wizard as follows:
   a. Select **Instrument ▸ Instrument Maintenance Manager**.
   b. In the Instrument Maintenance Manager, select **RNase P** in the navigation column.
   c. Click **Start RNase P Run**.
2. In the Setup screen of the RNase P Run wizard, select **The RNase P plate is loaded into the instrument**.
3. Click **Next**.
4. In the Run screen of the RNase P Run wizard, click **Start Run** ▶.

Note: Before starting the run, the instrument may take up to 15 min to heat the heated cover to the correct temperature.



Notes

Analyze the Experiment

Review the data to confirm the results of the experiment.

Confirm the Results of the Analysis

Note: After the StepOne™ software completes the RNase P run, it automatically analyzes the run and displays the results in the Analysis screen.

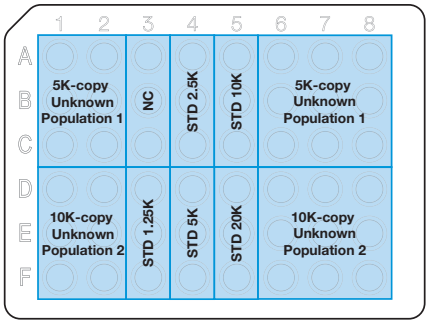
1. In the Analysis screen of the RNase P Run wizard, confirm the status of the run:
   - **Passed** – The instrument passed the RNase P run. Go to step 5 on page 72.
   - **Failed** – The instrument failed the RNase P run. Go to step 2 to screen the experiment for outliers.

   If the run fails, the automated analysis may have included outliers that caused the initial analysis to fail. 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.

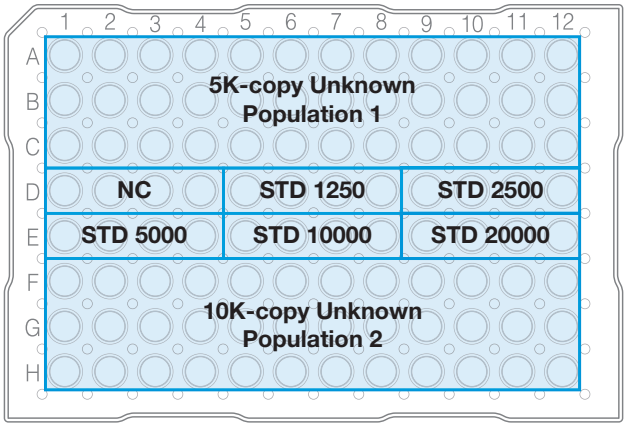
2. In the Amplification Plot, select **Ct vs. Well** from the Plot Type dropdown menu.

3. Confirm the uniformity of each replicate population on the reaction plate (controls, standards, and unknowns) by comparing the groupings of C_T values:

   a. In the plate layout, select the wells containing the 10,000-copy unknown population:
      - StepOne™ System – Wells from columns 1, 2, 6, 7, and 8 in rows D, E, and F
      - StepOnePlus™ System – Wells rows F, G, and H



TaqMan® 48-well RNase P Plate for the StepOne™ System



TaqMan® 96-well RNase P Plate for the StepOnePlus™ System

   b. In the plot, confirm that the C_Ts of the replicate population are equivalent.

Note: The numbers on the X-axis of the plot correspond to the wells of the reaction plate. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.

Notes

c. If an outlier is present in the population, select the corresponding well in the plate layout, then click **Omit** to remove the well from the analysis.

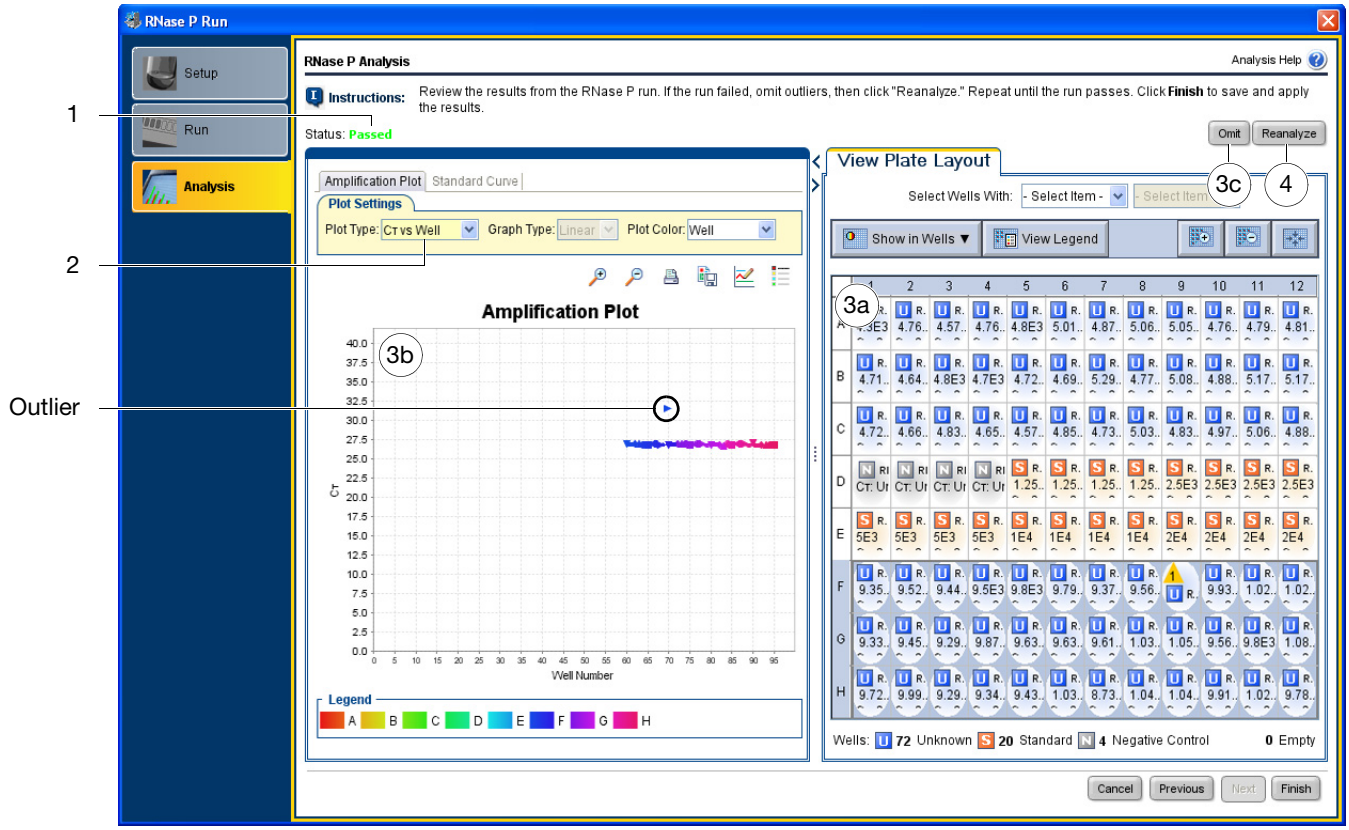
Instrument	Maximum number of outlier wells that can be removed from each…			Total
	Unknown Population	Standard (STD)	Negative Control (NC)	
StepOnePlus™ System	6	0	0	12
StepOne™ System	2	0	0	4

IMPORTANT! If too many outliers are present, order another RNase P plate and repeat the experiment.

d. Repeat steps 3a through 3c for each replicate population (unknowns, standards, and negative controls) on the reaction plate.

4. Click **Reanalyze** to analyze the run without the outliers.

   If the status of the RNase P Run is "**Failed**" after performing steps 2 through 4, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support as explained in "How to Obtain Support" on page 14.



Notes

5. Review the standard curve:

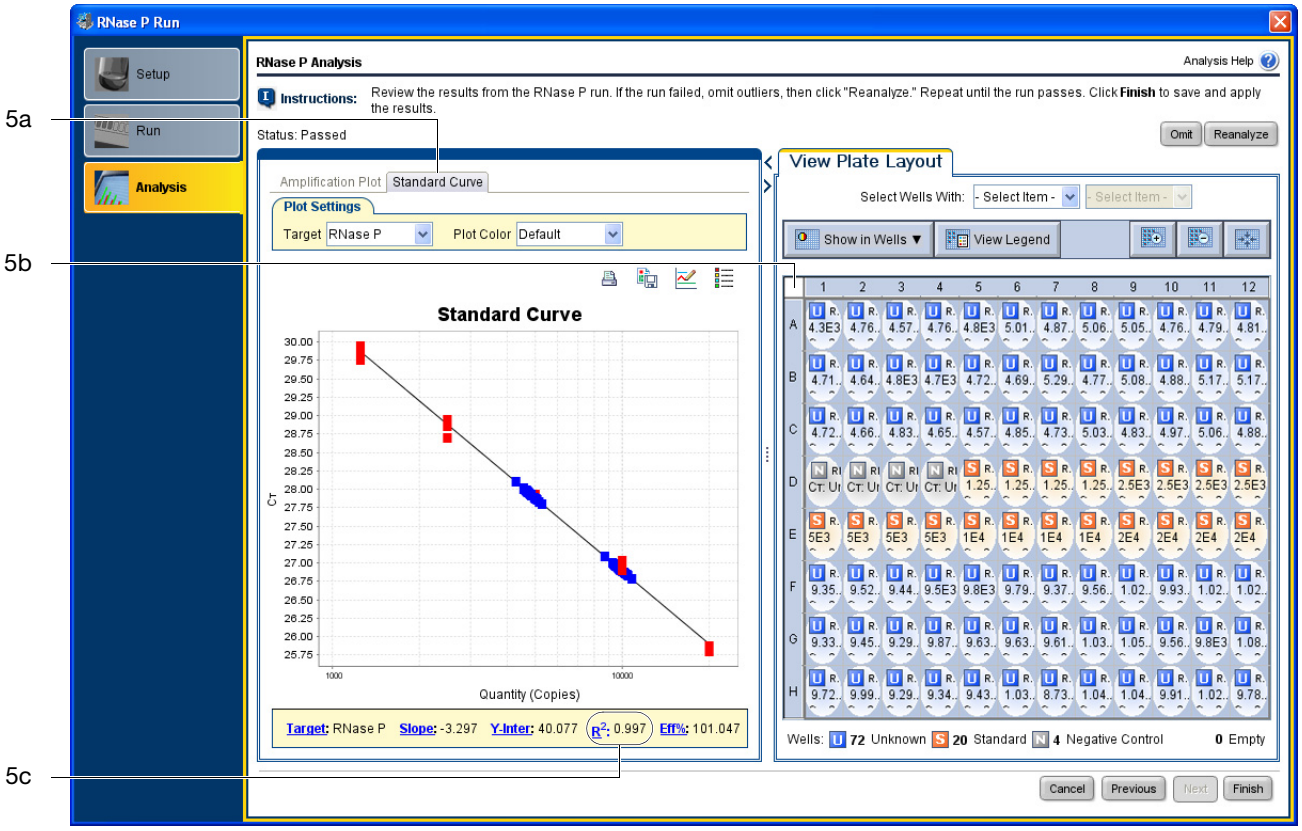
   a. Select the **Standard Curve** tab.

   b. Click the upper-right corner of the Plate Layout to select all wells.

   c. Confirm that the R2 value is greater than or equal to 0.990.

   If the R2 value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support as explained in "How to Obtain Support" on page 14.

6. Click **Finish**, click **Yes** when prompted to save the experiment.



Notes

Unload the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reach room temperature.

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

1. Open the instrument drawer.
2. Remove the RNase P plate from the sample block(s).
3. Close the instrument drawer carefully.

4. Dispose of the RNase P plate.

IMPORTANT! Do not power off the instrument following a run. The instrument automatically enters a hibernation mode when not in use. Power off the instrument only when it will not be used for an extended period.

Notes

After the Installation

After verifying the performance of the system by completing the RNase P experiment, you can install additional software to the computer and/or connect the instrument to a network.

Install Additional Software

We recommend installing the following types of software to improve the function and security of the system computer:

- Antivirus software
- Archival or file compression software
- Security software (firewall and encryption utilities)
- Performance optimizing software

Note: For more information, see "Select Protective Hardware and Software" on page 31.

To confirm that third-party software does not interfere with the StepOne™ software:

1. Install the software to the computer that contains the StepOne™ software.
2. Perform several test experiments using "dummy" plates (plates that do not contain reagents).

   Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.

3. Confirm that the system performs each test experiment without producing errors.

   If the system performs the tests successfully, perform experiments normally. If the system encounters errors during the test runs, the software may not be compatible with the StepOne™ software.

Network the System

You can expand the functionality of the colocated instrument by connecting it to an Ethernet network. When the colocated instrument is part of a network, computers on the network that are running the StepOne™ software can:

- Monitor the status of a run in progress
- Send and download experiments to and from the instrument

IMPORTANT! Computers linked to a colocated instrument over a network cannot control the instrument.

To connect the instrument to a network, go to Chapter 5, "Connect the System to a Network," on page 101.

Notes

4 Install the Standalone Layout

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

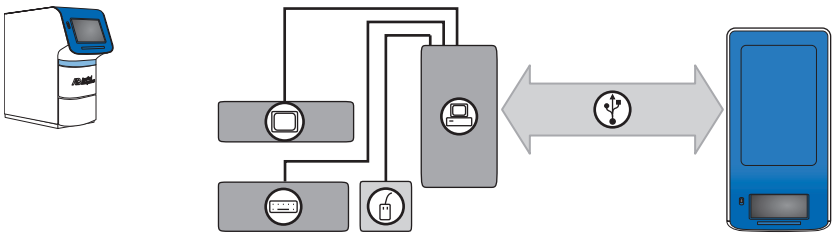
Notes

About the Standalone Installation

This chapter explains how to install the system for standalone operation.

When to Perform a Standalone Installation

In this layout, the instrument is *not* connected to the computer running the StepOne™ software. Instead, a USB drive ( ) is used to transfer data between the system components. Install the system in the standalone layout when the computer and the instrument must be placed in separate locations. See “Standalone Layout” on page 34 for complete descriptions of the layouts.



Note: If you want to connect the computer to the instrument, perform the colocated installation as described in Chapter 3, “Install the Colocated Layout.”

Notes

Standalone Installation Workflow

Chapter 2, Set Up the Instrument

Set Up the Computer

1. Install the computer.
2. Install the StepOne™ software.

Note: Perform step 2 only if you did not order a computer from us.

Check for Software Updates

Prepare the Software for Standalone Use

Perform the RNase P Experiment

1. Set up the experiment.
2. Run the experiment.

Analyze the RNase P Experiment

1. Transfer the experiment data.
2. Analyze the experiment.

Chapter 5, Connect the System to a Network

Notes

Set Up the Computer

After you install the StepOne™ or StepOnePlus™ instrument, set up the standalone computer.

IMPORTANT! If you ordered a computer from us, you need only to unpack the computer as explained on page 79. Computers shipped by us are ready for use with the instrument and already contain the StepOne™ software.

Materials Required

- CD, Applied Biosystems StepOne™ Real-Time PCR System Software
- Computer

  IMPORTANT! If you did not order a computer from us, provide a computer that satisfies the requirements listed in "Minimum Computer Requirements" on page 30.

- Screwdrivers, flathead and Phillips
- (Optional) Protective hardware to install to the computer

  We recommend that you install one or more of the following electrical devices to the computer to prevent loss of data and to protect the computer from damage resulting from electrical hazards:

  - Power line regulator
  - Uninterruptible power supply (UPS)
  - Surge protector
  - Backup storage device

  For more information, see "Select Protective Hardware and Software" on page 31.

Notes

Install the Computer

After obtaining the required materials, install the computer.

Confirm the Site Requirements

Confirm that the installation site meets the physical and environmental requirements for the computer. See the documentation for your computer for the site requirements.

Place the Computer

CAUTION **PHYSICAL INJURY HAZARD**. Improper lifting can cause painful and sometimes permanent back injury. Use proper lifting technique when lifting or moving the computer. We recommend safety training for proper lifting techniques. Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer, this may require two or more people.

1. If you have not done so already, unpack the monitor, computer, keyboard, and mouse, and assemble the computer components as described in the computer installation guide.

   Guidelines for lifting and moving the computer:

   - Make sure that you have a secure grip on the computer when lifting.
   - Make sure that the pathway from the beginning of the lift to the final location is clear of obstructions.
   - Do not lift an object and twist your torso at the same time.
   - Keep your spine in a neutral position while lifting with your legs.
   - Coordinate lift and movement with all participants before lifting and carrying the computer.
   - Instead of lifting the computer/monitor from the packing box, tilt the box on its side, then slide the contents out of the box.

2. Make the following connections:

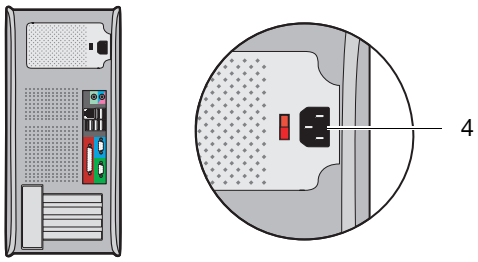
Connect				Do Not Connect
Power	Mouse	Keyboard	Monitor	Ethernet
	or	or	or	

3. If you ordered a laptop computer from us, install the PCMCIA Network Card as described in the documentation accompanying the card.

   After installing the PCMCIA Network Card, wait several minutes to allow the computer to enable the device. The computer may require time to initialize the drivers and register the network card with the operating system before it is available for use.

Notes

4. If you ordered a tower computer from us, set the voltage appropriately for your region as described in the computer installation guide.



5. (Optional) Install electrical protective devices to the computer.

Note: For more information, see "Select Protective Hardware and Software" on page 31.

Power On the Computer

1. Power on the computer and monitor.
2. Log onto the operating system:
   - If you are using a computer that you provided, log on to the operating system as a member of the Administrators user group.
   - If you are using a computer from us, enter **Administrator** in the User field, then click **Login**. Leave the Password field blank.

IMPORTANT! If you are installing a computer that you provided, log onto the Windows® operating system using a user account that belongs to the Administrators user group.

3. In the Getting Started with Windows® XP window, deselect **Show this screen at startup**, then click **Exit**.
4. Determine the next step:
   - If you are installing a computer that you provided, go to "Install the StepOne™ Software" on page 81.
   - If you are installing a computer from us, go to "Check for StepOne™ Software Updates" on page 85.

Notes

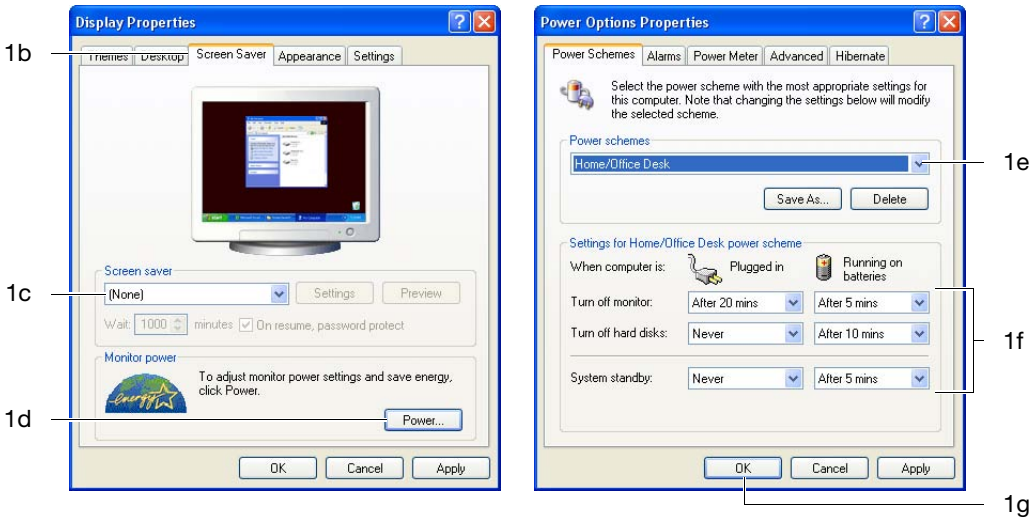
Install the StepOne™ Software

After setting up the computer, define the display properties, computer name, and the date and time settings, then install the StepOne™ software.

IMPORTANT! If you ordered a computer from us, skip "Install the StepOne™ Software" and go to "Check for StepOne™ Software Updates" on page 85. Computers that are supplied by us already contain the StepOne™ software, and are ready for use with the instrument.

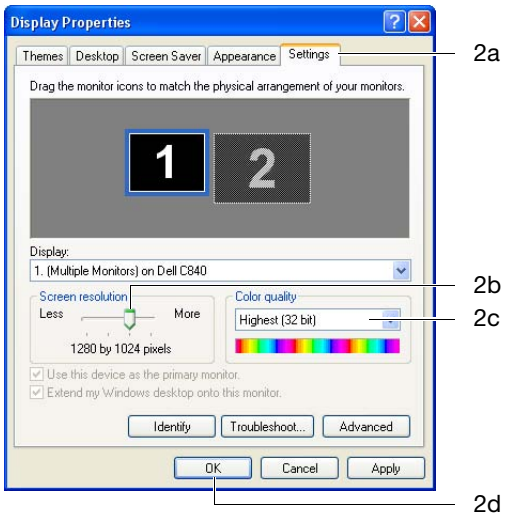
Prepare the Operating System

1. Change the screen saver and power options settings:
   a. Right-click the desktop, then select **Properties**.
   b. In the Display Properties dialog box, select the **Screen Saver** tab.
   c. In the Screen saver group box, select (**None)** in the dropdown menu.
   d. Click **Power**.
   e. In the Power Options Properties dialog box, select **Home\Office Desk** from the Power schemes dropdown list.
   f. Define the Plugged in power scheme settings:
      - Monitor – Select **After 20 mins** from the Plugged in dropdown menu.
      - Hard Disks – Select **Never** from the Plugged in dropdown menu.
      - System standby – Select **Never** from the Plugged in dropdown menu.
   g. Click **OK** to save the settings.



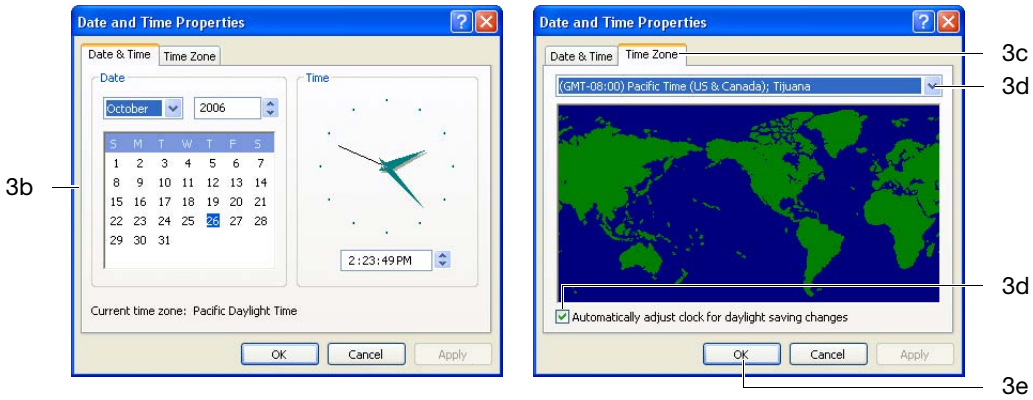
Notes

2. Change the screen resolution:
   a. In the Display Properties dialog box, select the **Settings** tab.
   b. In the Screen Area group box, use the slider to select **1280 by 1024 pixels**.
   c. In the Colors group box, select **Highest (32 bit)** in the dropdown menu.
   d. Click **OK** twice, then click **Yes** to accept the new resolution.



3. Define the date and time settings:
   a. Right-click the time readout in the toolbar, then select **Adjust Date/Time**.
   b. In the Date & Time tab, define the date and time settings.
   c. Select the **Time Zone** tab.
   d. Select a time zone from the dropdown menu, then select **Automatically adjust clock for daylight saving changes** if necessary.
   e. Click **OK**.

Note: Computers on a network synchronize their date/time settings with the server.



Notes

4. Change the computer name:

   a. In the desktop, right-click **My Computer,** then select **Properties**.

   b. In the System Properties dialog box, select the **Computer Name** tab.

   c. Click **Change**.

   d. In the Computer Name field, enter the computer name.

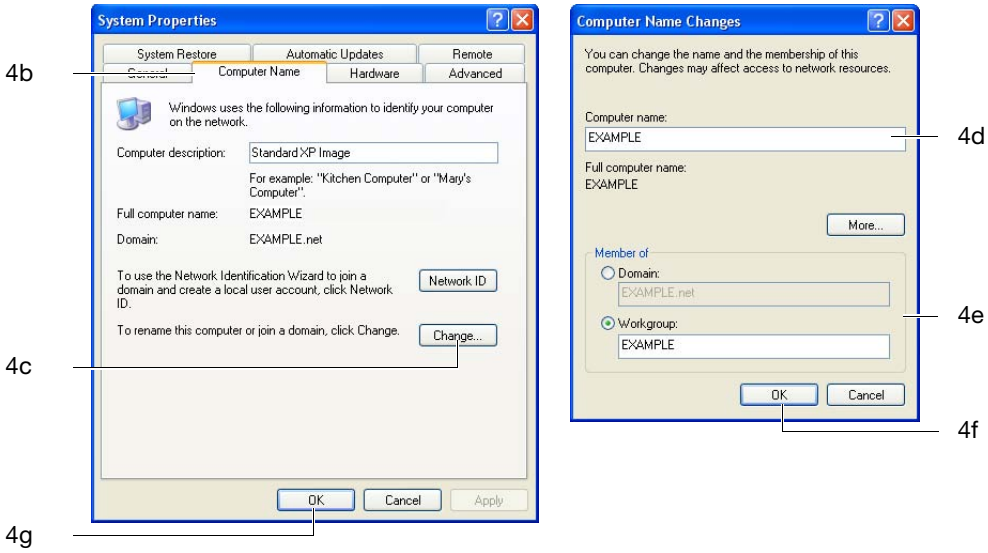
   CAUTION Do not give the computer the same name as the instrument. A shared name could lead to network conflicts if you install both devices to an Ethernet network.

   e. If necessary, define the Domain or Workgroup settings if you are going to connect the computer to a network.

   f. Click **OK** twice.

   g. In the Network Identification dialog box, click **OK**.

Note: The computer name identifies the computer on the network and must be different than that of the instrument. After you install the system, you can change the computer name without disrupting the function of the StepOne™ software.



5. In the Systems Settings Change dialog box, click **Yes** to restart the computer.

6. Log onto the operating system as a member of the Administrators user group.

Notes

Install the Software

IMPORTANT! You must be logged into the Windows® operating system as an administrator to install the StepOne™ software.

Note: If you encounter errors during the installation of the StepOne™ software, uninstall the software as explained in "Uninstall the StepOne™ Software" on page 160.

1. Insert the Applied Biosystems StepOne™ Real-Time PCR System Software CD into the CD drive of the computer and wait for the installer to start.

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

2. In the Welcome page of the InstallShield Wizard, click **Next**.

3. In the License Agreement page, click **Yes** to accept the agreement.

4. In the Choose Destination Location page, click **Next** to accept the default location.

   Note: We recommend installing the StepOne™ software to the D drive; however, you can install the software to another location.

5. In the Default Instrument Type page, select the type of instrument that you are installing (**StepOnePlus™ Instrument** or **StepOne™ Instrument**), then click **Next**.

6. In the Start Copying Files page, confirm that the installation location displayed in the Current Settings field is correct, then click **Next** to begin installing the StepOne™ software.

7. When the StepOne™ software completes the installation, click **Finish**.

Notes

Check for StepOne™ Software Updates

Updates and/or patches may be available for the StepOne software. We recommend that you check for software updates before you proceed with the installation.

Note: To periodically check for software updates after the StepOne or StepOnePlus system is installed (as part of routine maintenance), see "Update the StepOne™ Software or the Operating System" on page 154.

To check for software updates:

1. Go to: **http://www.lifetechnologies.com/support** and click the **Instrument Software, Patches & Updates** link.
2. In the Instrument Software, Patches & Updates page, click the link for the appropriate software.
3. Under Software Download, view the available downloads.
4. If there are updates and/or patches for StepOne software v2.1 or later, select the appropriate option, then follow the prompts to begin the download.

   You may be asked for your instrument serial number. The serial number is on the back panel of your instrument, or can be obtained from the instrument touchscreen.

Notes

Prepare the StepOne™ Software for Standalone Use

After installing the StepOne™ software, set the software preferences so that the software does not attempt to connect to the instrument at startup or confirm the status of the RNase P experiment.

Start the Software

1. In the desktop, double-click (StepOne software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ StepOne Software ▸ <*software name*>**

   where <*software name*> is the current version of the StepOne software.

2. In the Login dialog box, create a user name.

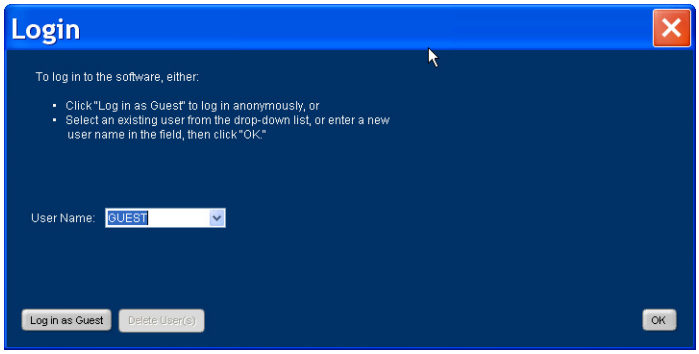
   a. In the User Name field, enter a user name.

   Note: You cannot use the following characters in the User Name field: space, forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (|), colon (:), or semicolon (;).

   b. Click **OK**.

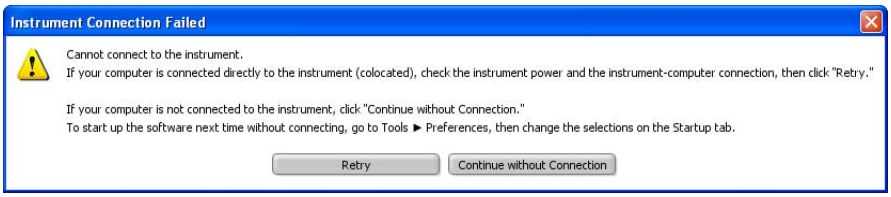
We recommend that you log in with a user name. If you log in with a user name, you can set preferences in the software. The next time you log in to the software with the same user name, the software uses the preferences you set as the defaults.

IMPORTANT! If you log in to the software as a Guest, you cannot set preferences. You will not be able to perform the steps in "Set the Preferences" on page 87.



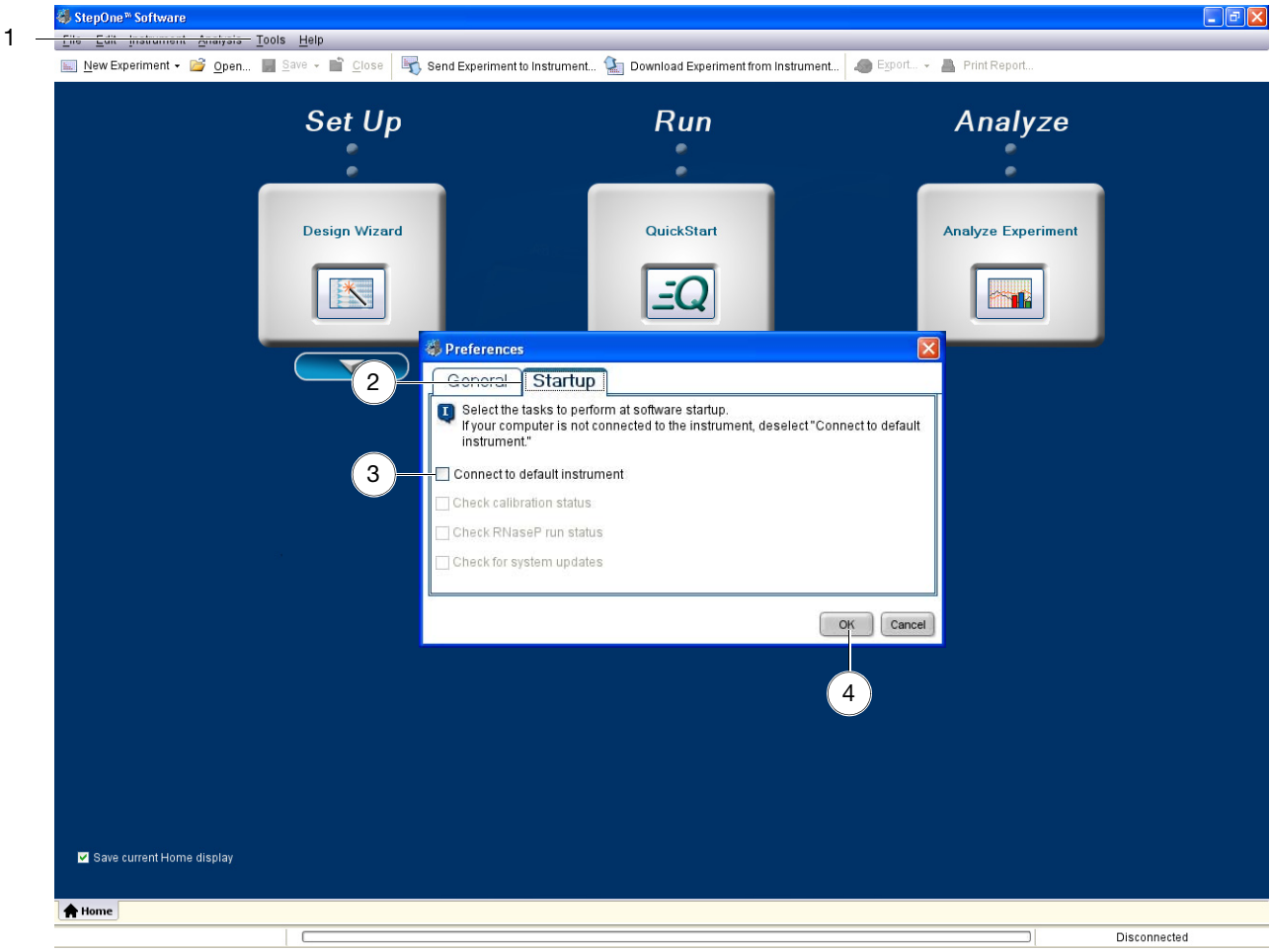
Notes

3. When the Instrument Connection Failed dialog box appears, click **Continue without connection**.



Set the Preferences

1. In the Main Screen, select **Tools** ▸ **Preferences**.
2. In the Preferences dialog box, select the **Startup** tab.
3. Deselect **Connect to default instrument**.
4. Click **OK**.



Notes

Perform the RNase P Experiment

After you install the instrument, confirm the function of the system by running a TaqMan® RNase P Fast Instrument Verification Plate.

Materials Required

- TaqMan® RNase P Fast Instrument Verification Plate
- Powder-free gloves
- Safety glasses
- Centrifuge with plate adapter

When to Perform the RNase P Experiment

We recommend performing an RNase P experiment:

- After installing the system.
- After moving the instrument to another location.
- As needed to confirm the function of the instrument, depending on your laboratory and regulatory requirements.

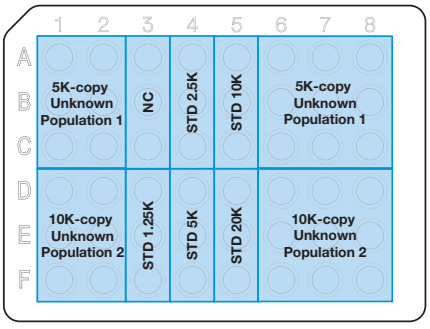
Purpose of the Experiment

The TaqMan® RNase P Fast Instrument Verification Plate experiment verifies the performance of the instrument. The RNase P plate is preloaded with the reagents necessary for the detection and quantitation of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme).

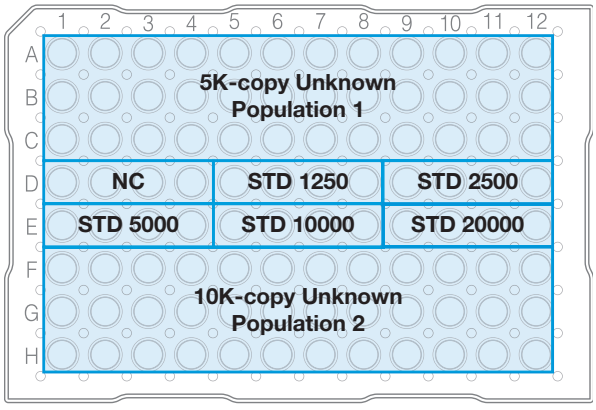
Each well contains:

- 1✕ TaqMan® Fast Universal PCR Master Mix, No AmpErase® UNG
- RNase P primers
- FAM™ dye-labeled probe
- Known concentration of human genomic DNA template

The figure below illustrates 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 negative control wells.



TaqMan® 48-well RNase P Plate for the StepOne™ System



TaqMan® 96-well RNase P Plate for the StepOnePlus™ System

Notes

After the run, the StepOne™ software:

1. Generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards.
2. Calculates the concentration of the two unknown populations using the standard curve.
3. Calculates the following to assess the instrument performance:

   $$[(CopyUnk_2) - 3(\sigma_{CopyUnk2})] > [(CopyUnk_1) + 3(\sigma_{CopyUnk1})]$$

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

Installation Specification

The instrument passes the RNase P experiment if the inequality holds and the instrument successfully distinguishes between 5,000 and 10,000 copies with greater than 99.7% confidence.

To meet the installation specification, you can omit a limited number of outlier wells from the 5,000- and 10,000-copy unknown populations. The number of wells that you can remove depends on the instrument that you are installing.

Instrument	Maximum number of outlier wells that can be removed from each…			Total
	Unknown Population	Standard	Negative Controls (NC)	
StepOnePlus™ System	6	0	0	12
StepOne™ System	2	0	0	4

Notes

Set Up the Experiment

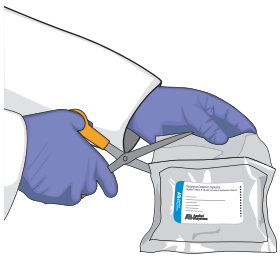
Prepare the TaqMan® RNase P Fast Instrument Verification Plate for the run.

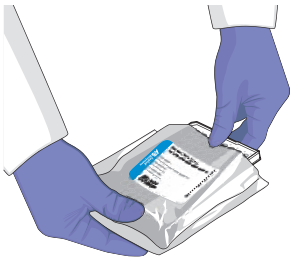
Prepare the RNase P Plate

IMPORTANT! Do not use an RNase P plate for another Thermo Fisher Scientific instrument to verify the performance of the StepOne™ system. RNase P plates for other instruments contain the TAMRA™ dye, which is not supported by the StepOne™ system.

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

1. Get the TaqMan® RNase P Fast Instrument Verification Plate from the freezer, then allow the reaction plate to warm to room temperature (for approximately 5 min).
2. Remove the RNase P plate from its packaging.



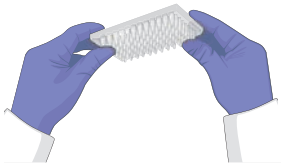


3. Vortex the RNase P plate for 5 sec.
4. Centrifuge the RNase P plate for 2 min at less than 1500 rpm.

IMPORTANT! The RNase P plate must be well mixed and centrifuged.

5. Confirm that the liquid is at the bottom of each well of the RNase P plate. If not, centrifuge the plate again at a greater rpm and for a longer time.

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



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

Notes

Run the Experiment

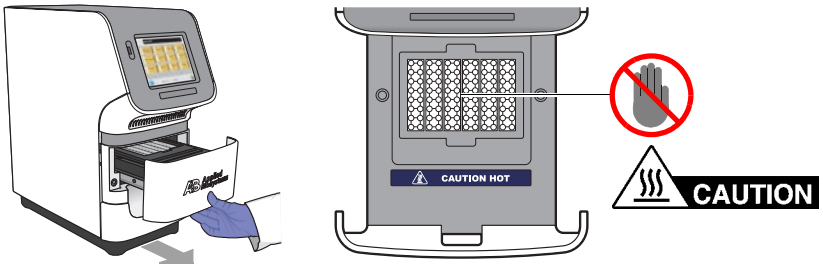
After preparing the TaqMan® RNase P Fast Instrument Verification Plate, load the plate into the instrument and start the run.

Load the Instrument

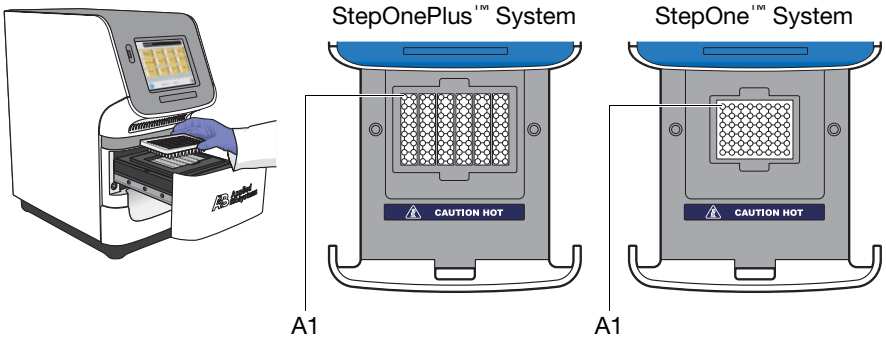
CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

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

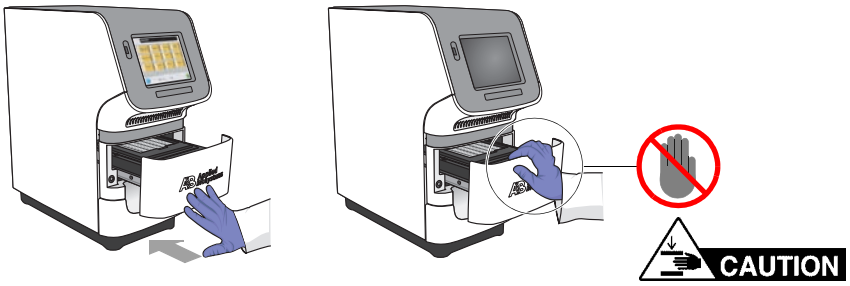
1. Open the instrument drawer.



2. Place the RNase P plate in the sample block(s) so that the A1 position is at the back-left corner of the sample block(s).



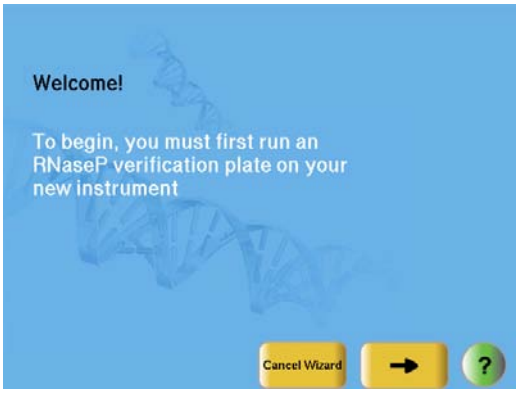
3. Close the instrument drawer carefully.



Notes

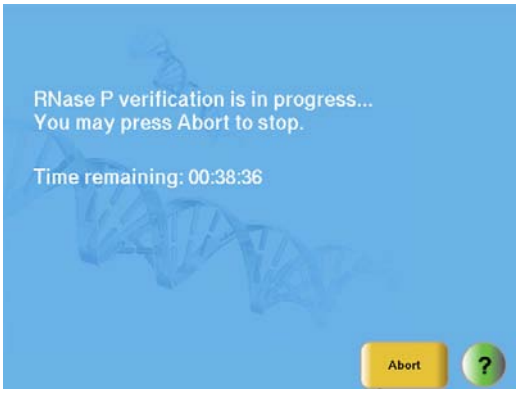
Start the Run

1. Touch the instrument touchscreen to awaken it, then touch  .
2. In the Main Menu, touch **Tools Menu**, then touch **RNase P Wizard**.
3. In the Welcome screen, touch **Continue**, then touch → four times to bypass the instructions screens.



4. In the Start Run screen, touch **Start** to begin the run.

Note: Before starting the run, the instrument may take up to 15 min to heat the heated cover to the correct temperature.



Notes

Analyze the RNase P Experiment

After setting up the computer and the instrument completes the run, analyze the RNase P experiment.

Materials Required

- Powder-free gloves
- Safety glasses
- USB Drive (from the system packing kit)

Unload the Instrument

Unload the RNase P plate from the instrument.

Remove the RNase P Plate

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

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

1. Open the instrument drawer.
2. Remove the RNase P plate from the sample block(s).
3. Close the instrument drawer carefully.

4. Dispose of the RNase P plate.

IMPORTANT! Do not power off the instrument following a run. The instrument automatically enters a hibernation mode when not in use. Power off the instrument only when it will not be used for an extended period.

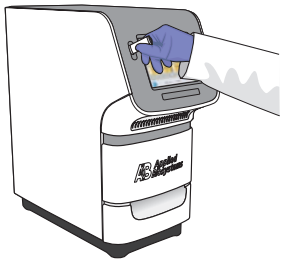
Notes

Transfer the Experiment

After unloading the instrument, transfer the experiment data to the computer for analysis.

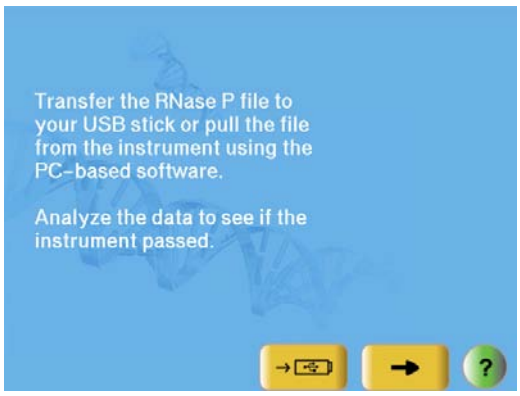
Transfer the Data to the USB Drive

1. Connect a USB drive to the USB port.

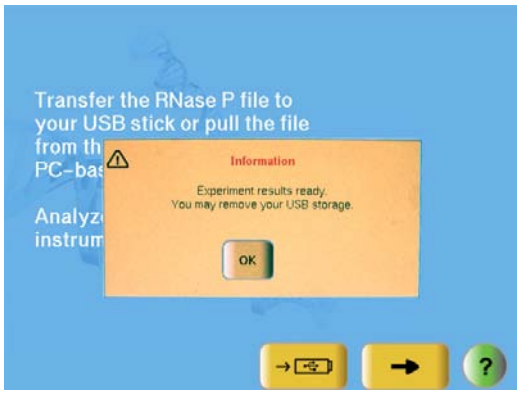


2. In the RNase P Wizard, touch →  .

   If the instrument cannot find the USB drive, touch **OK**, wait for 30 sec, then touch **Collect Results** again.



3. When prompted that the data has been transferred successfully, touch **OK**, then touch ➡.



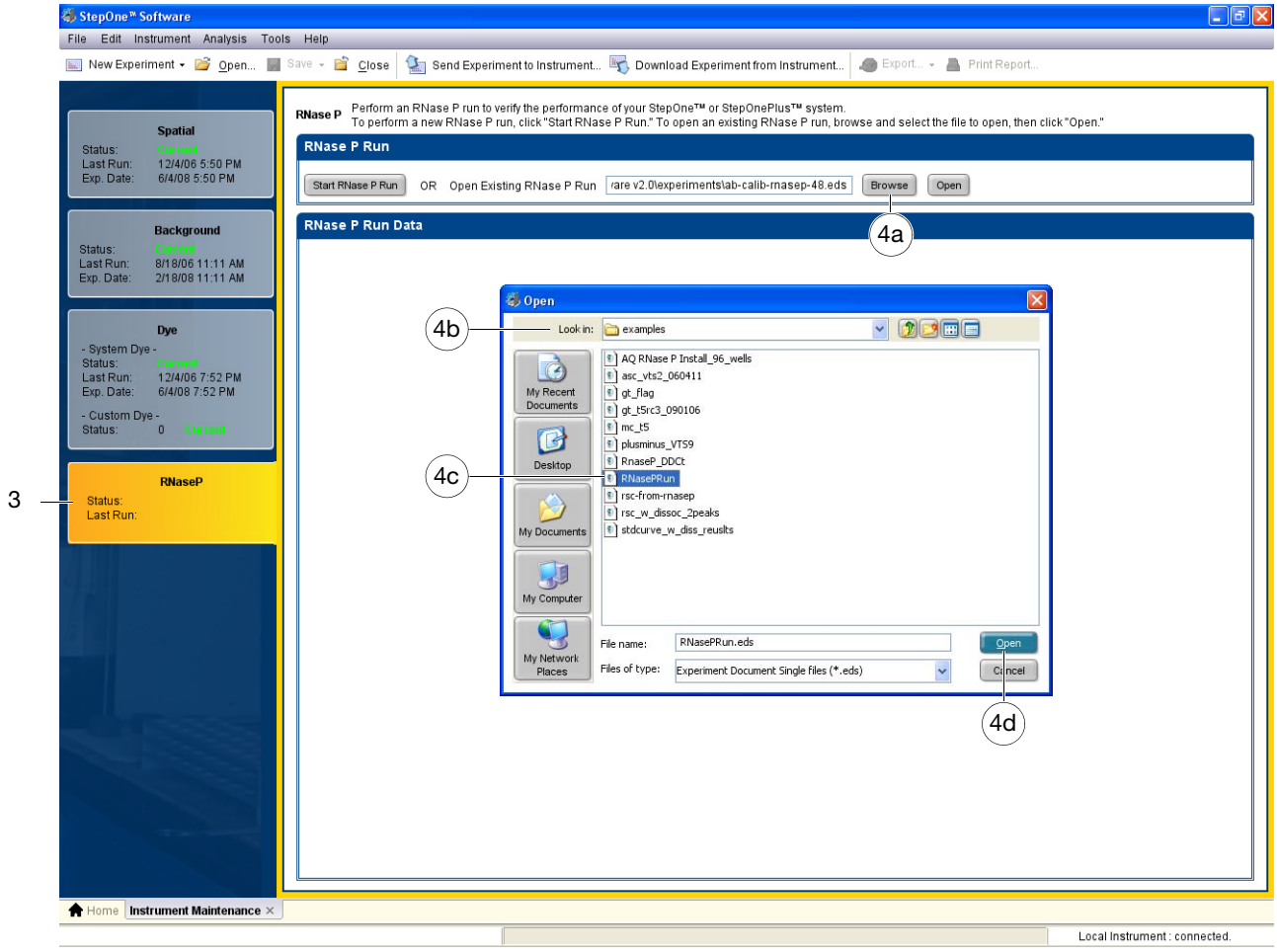
4. Remove the USB drive from the instrument.

Notes

Transfer the Data to the Computer

1. Connect the USB drive to one of the USB ports on the computer.
2. In the StepOne™ software, select **Instrument ▸ Instrument Maintenance Manager**.
3. In the Instrument Maintenance Manager, click **RNase P**.
4. Select the RNase P Run:
   a. In the RNase P Run screen, click **Browse**.
   b. In the Open dialog box, navigate to the USB drive.
   c. In the file name field, select the experiment.
   d. Click **Open**.

   The StepOne™ software automatically analyzes the run and displays the results in the RNase P Run screen.



Notes

Analyze the Experiment

Review the data to confirm the results of the experiment.

Confirm the Results of the Analysis

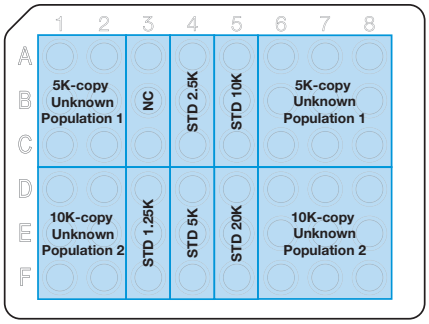
1. In the Analysis screen of the RNase P Run wizard, confirm the status of the run:
   - **Passed** – The instrument passed the RNase P run. Go to step 5 on page 98.
   - **Failed** – The instrument failed the RNase P run. Go to step 2 to screen the experiment for outliers.

   If the run fails, the automated analysis may have included outliers that caused the initial analysis to fail. 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.

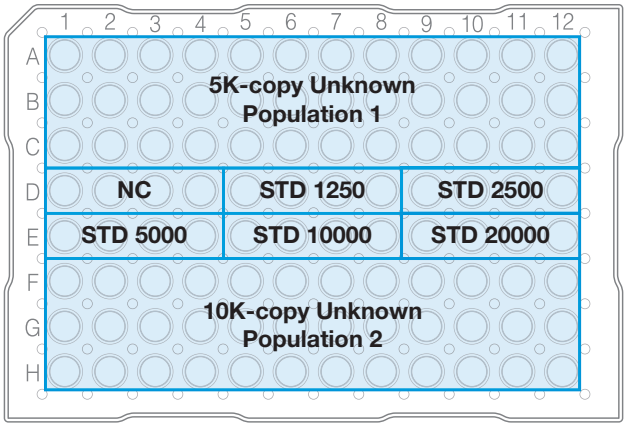
2. In the Amplification Plot, select **Ct vs. Well** from the Plot Type dropdown menu.

3. Confirm the uniformity of each replicate population on the RNase P plate (controls, standards, and unknowns) by comparing the groupings of C_T values:

   a. In the plate layout, select the wells containing the 10,000-copy unknown population:
      - StepOne™ System – Wells from columns 1, 2, 6, 7, and 8 in rows D, E, and F
      - StepOnePlus™ System – Wells rows F, G, and H



TaqMan® 48-well RNase P Plate for the StepOne™ System



TaqMan® 96-well RNase P Plate for the StepOnePlus™ System

   b. In the plot, confirm that the C_Ts of the replicate population are equivalent.

> **Note:** The numbers on the X-axis of the plot correspond to the wells of the reaction plate. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.

Notes

c. If an outlier is present in the population, select the corresponding well in the plate layout, then click **Omit** to remove the well from the analysis.

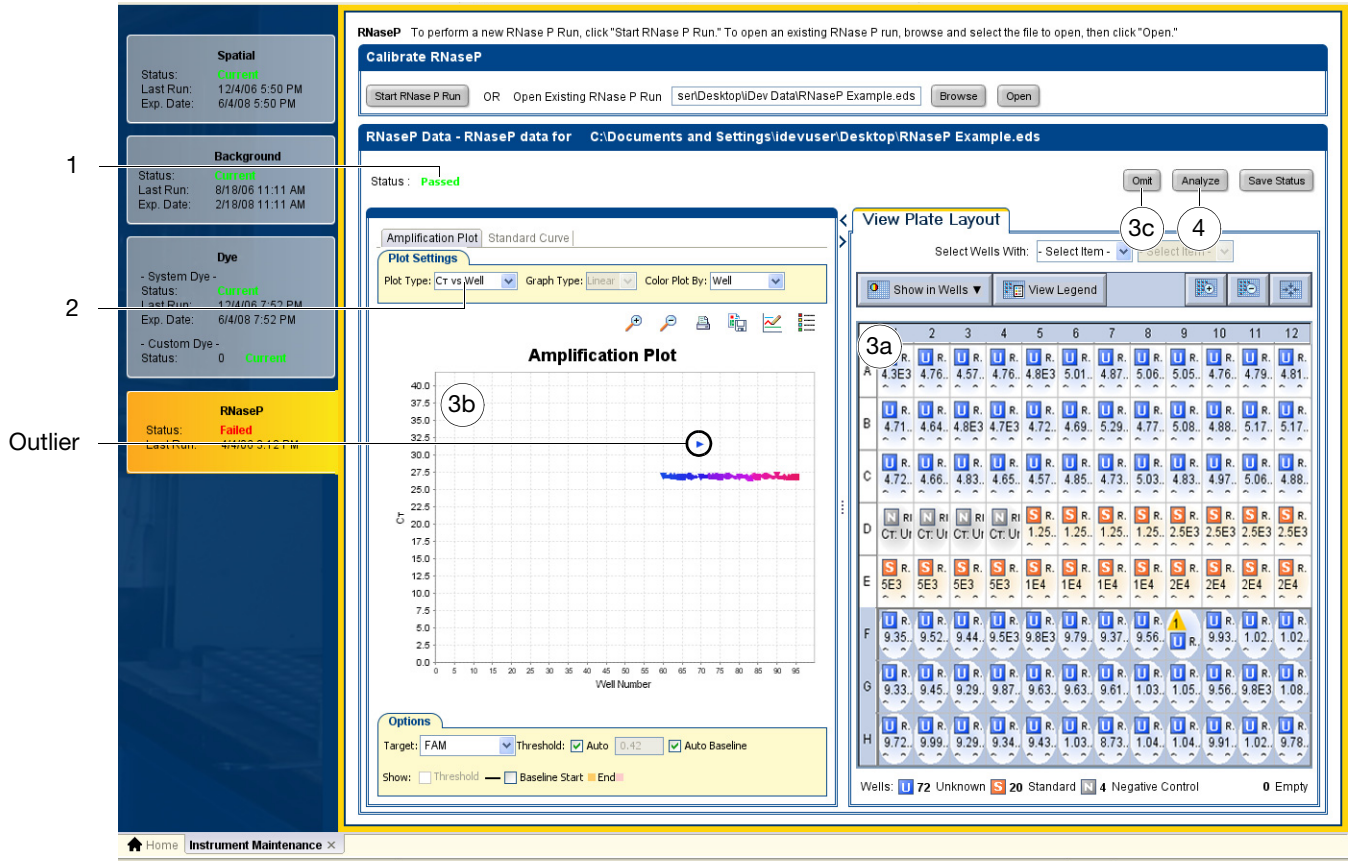
Instrument	Maximum number of outlier wells that can be removed from each…			Total
	Unknown Population	Standard (STD)	Negative Control (NC)	
StepOnePlus™ System	6	0	0	12
StepOne™ System	2	0	0	4

IMPORTANT! If too many outliers are present, order another RNase P plate and repeat the experiment.

d. Repeat steps 3a through 3c for each replicate population (unknowns, standards, and negative controls) on the reaction plate.

4. Click **Reanalyze** to analyze the run without the outliers.

   If the status of the RNase P Run is "**Failed**" after performing steps 2 through 4, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support as explained in "How to Obtain Support" on page 14.



Notes

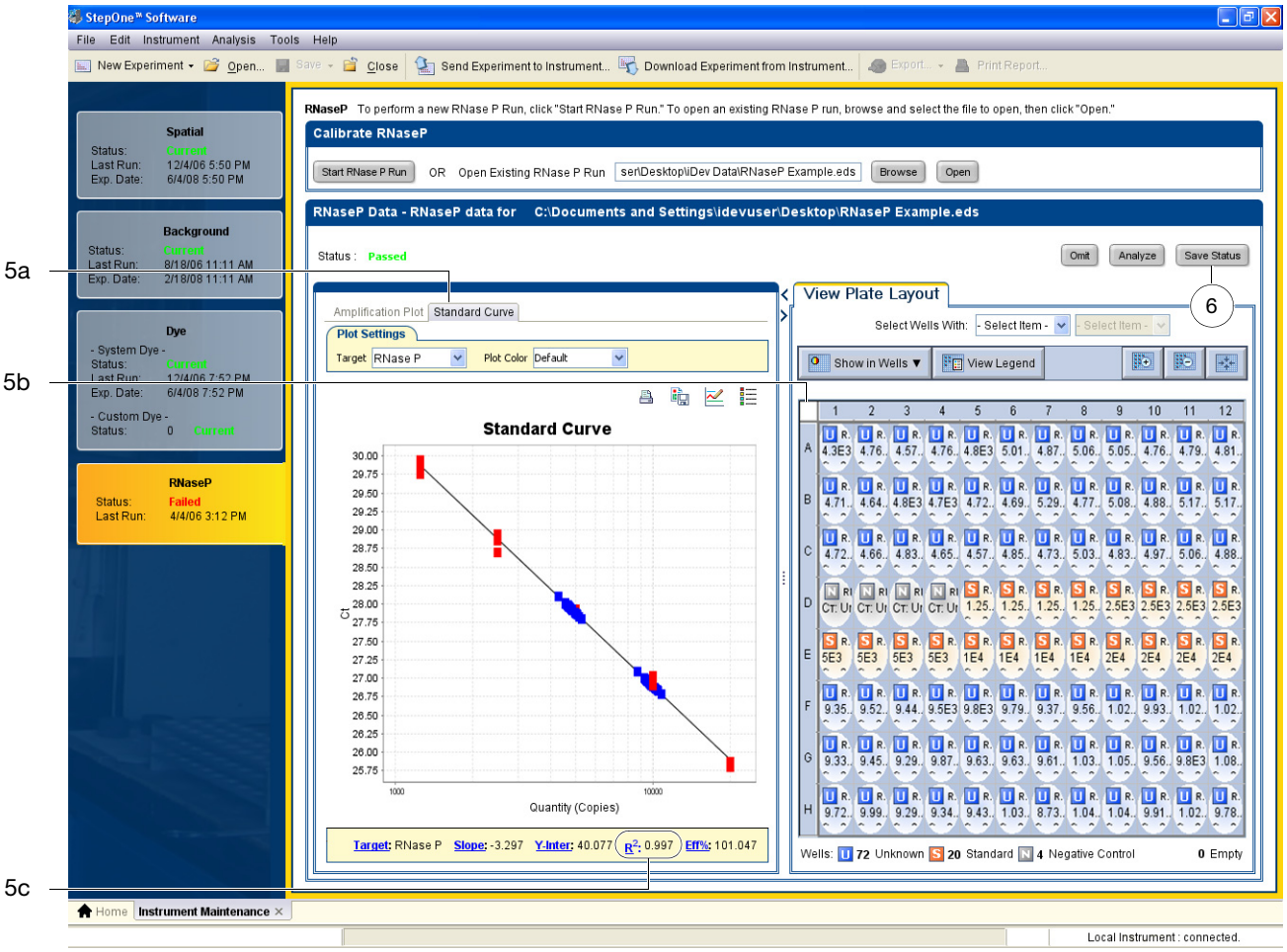
5. Review the standard curve:

   a. Select the **Standard Curve** tab.

   b. Click the upper-right corner of the Plate Layout to select all wells.

   c. Confirm that the R2 value is greater than or equal to 0.990.

   If the R2 value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support as explained in "How to Obtain Support" on page 14.



6. Click **Save Status**, then click **Yes** when prompted.

7. Touch the instrument touchscreen to awaken it, then touch **Pass** in the RNase P wizard.

Notes

After the Installation

After verifying the performance of the system, you can install additional software to the computer and/or connect the instrument to an Ethernet network.

Install Additional Software

We recommend installing the following types of software to improve the function and security of the system computer:

- Antivirus software
- Archival or file compression software
- Security software (firewall and encryption utilities)
- Performance optimizing software

Note: For more information, see "Select Protective Hardware and Software" on page 31.

To confirm that third-party software does not interfere with the StepOne™ software:

1. Install the software to the computer that contains the StepOne™ software.
2. Perform several test experiments using "dummy" plates (plates that do not contain reagents).

   Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.

3. Confirm that the system performs each test experiment without producing errors.

   If the system performs the tests successfully, perform experiments normally. If the system encounters errors during the test runs, the software may not be compatible with the StepOne™ software.

Network the System

If the standalone instrument is connected to a network, then computers on the network that are running the StepOne™ software can:

- Monitor the status of the run in progress
- Send and download experiments to and from the instrument instead of using the USB drive

To connect the instrument to a network, go to Chapter 5, "Connect the System to a Network," on page 101.

Notes

Notes

5 Connect the System to a Network

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

IMPORTANT! This chapter *does not* provide adequate detail to integrate the Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR Instrument into all possible network architectures. Because your network may contain advanced features (such as a firewall or network domains), we recommend that you consult a network administrator before connecting the Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR System to your laboratory network.

Notes

Overview

After installing the Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR System, you can connect the instrument to a local area network to enhance its functionality.

This chapter describes how to:

- Set up the instrument for use on a network.
- Set up a computer for remote monitoring.
- Test the network connection by engaging the remote monitoring feature.

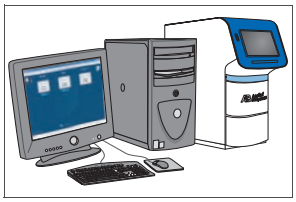
About Remote Monitoring

Once the instrument is connected to a network, any computer on the network can use the remote monitoring feature of the StepOne™ software to monitor the instrument. During a run, the remote monitoring feature can provide run status, temperature, and amplification data as the instrument collects them. See “Monitor the Instrument” on page 110 for more information on remote monitoring.

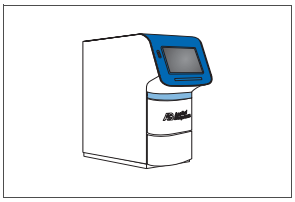
IMPORTANT! The remote monitoring feature does not allow you to control of the instrument. Control of the instrument is limited to the colocated computer when the system is in a colocated layout, or the instrument touchscreen when the system is in a standalone layout.

Network Setup Workflow

Chapter 3, Install the Colocated Layout



Chapter 4, Install the Standalone Layout



Connect the instrument to a network

1. Define the LAN Port IP settings.

Set up the computer for remote monitoring

1. Connect the computer to the network.
2. Install the StepOne™ software.

Test the network connection

1. Enable remote monitoring.

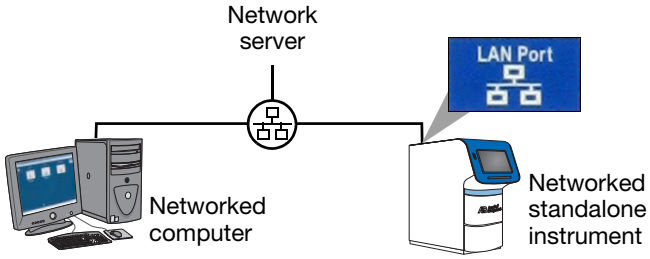
Notes

Example Network Layouts

The LAN and Computer Ports of the instrument allow you to add the system to a network in several ways. The following examples describe two basic network layouts of the system.

Networked Standalone Layout

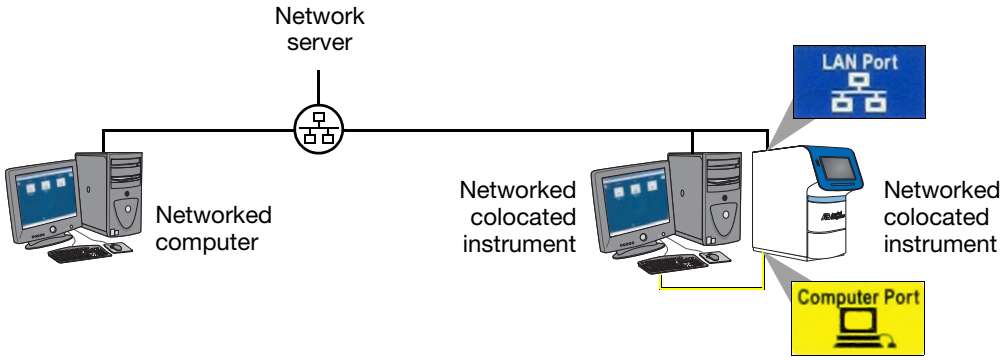
In this example, a standalone instrument is connected to a network by the instrument LAN Port (), which has been configured for dynamic host configuration protocol (DHCP) or static IP operation. In this layout, any computer on the network can monitor and exchange experiment data with the instrument; however, computers on the network cannot control the instrument. Runs must be started from the instrument touchscreen.



Networked Colocated Layout

In this example, a colocated instrument is connected to a networked colocated computer by the Computer Port (), and to a network by the LAN Port () that has been configured for DHCP or static IP operation. In this layout, computers on the network can monitor and exchange experiment data with the instrument; however, the instrument is controlled locally by the colocated computer.

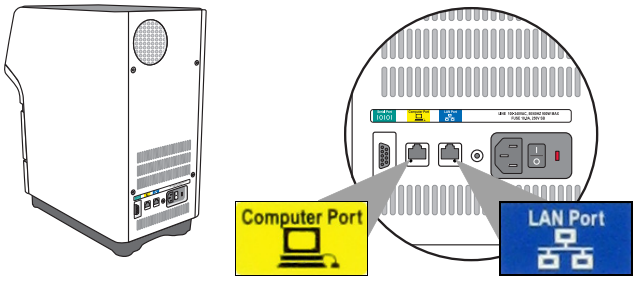
IMPORTANT! Consult your IT department to network a colocated computer. To enable the full functionality of the StepOne™ software, the colocated computer requires a network connection.



Notes

Instrument Ports

The instrument supports two 10Base-T Ethernet connections: a Computer Port for communication with the colocated computer, and a Local Area Network (LAN) Port for network communication.



Port	Purpose	Supports
Computer Port	The connection for the colocated computer, which controls the instrument. **Note:** We do not support colocated layouts in which the Computer Port does not directly connect the instrument to the colocated computer.	Static IP network service only with subnet mask and default gateway settings
LAN Port	The connection for a local area network. When the instrument is connected to a network, computers on the network that run the StepOne™ software can: • Remote monitor the instrument as it performs runs. • Send experiments to, and download experiments from the instrument. **IMPORTANT!** Computers connected to the instrument through the LAN port *cannot* control the instrument.	• Static IP network service with subnet mask and default gateway settings *or* • Dynamic host configuration protocol (DHCP) network service • Managed data network service (mDNS/DNS) for local domains‡ • IPv4 link-local (IPV4LL) in the RFC§

‡ Because managed data network service (mDNS) is limited to direct network connections, a instrument set for mDNS may not be visible to other nodes that are separated by a router, hub, or another network device.

§ Also known as Automatic Private IP Addressing (APIPA) or Internet Protocol Automatic Configuration (IPAC). When the instrument is set for DHCP, Automatic Private IP Addressing (APIPA) is automatically enabled, and the instrument provides an IP address when no address is supplied by the DHCP server.

Notes

Networking Guidelines and Best Practices

- **Consult a Network Administrator**
  - We recommend that you consult a network administrator before connecting the system to your laboratory network.
  - To enable the full functionality of the StepOne™ software, the colocated computer requires a network connection.
- **Confirm the Instrument Connections**

  Confirm that the instrument is connected to the network by the blue LAN Port (🖧). If the system is installed in a colocated layout, confirm that the instrument is connected to the computer by the yellow Computer Port (💻).
- **Limit Remote Monitoring to One Computer**

  Avoid using more than one computer to simultaneously remote monitor the instrument. Although the system supports remote monitoring from multiple computers, each connection taxes the instrument microprocessor. Too many connections can overburden the instrument and result in instrument errors.

  Note: The effects of an overburdened instrument are evident in the Temperature Plot during a run. Symptoms can include extended hold times or brief, unexpected plateaus in the instrument Temperature Plot.
- **Observe Restrictions to mDNS and Auto Discovery**

  The instrument supports multicast domain name service (mDNS) but only when the instrument and computer share a direct network connection and are within the same subnet. Consequently, computers on the network that are separated from the instrument by a router, hub, or other network device may not be able to access the instrument by its host name.

  The instrument also does not support auto discovery for mDNS. However, the instrument does support name resolution but the instrument name must be unique within the subnet.

  Note: Confirm the uniqueness of the instrument name. The instrument does not test the uniqueness of the instrument name when it is set.
- **Add Instruments Using Lower Case Letters**

  When you add the instrument for remote monitoring (see “Enable Remote Monitoring” on page 110), enter the instrument name using lower case letters only.

Notes

Connect the Instrument to a Network

After deciding how to connect the instrument to a network, set up the instrument according to your network policies.

Materials Required

Ethernet cable with RJ45 connectors

Collect Required Information

- Network policy for obtaining IP addresses: DHCP *or* static IP.

  IMPORTANT! When the instrument is set for DHCP, Automatic Private IP Addressing (APIPA) is automatically enabled and the instrument will provide an IP address when no address is supplied by a DHCP server.

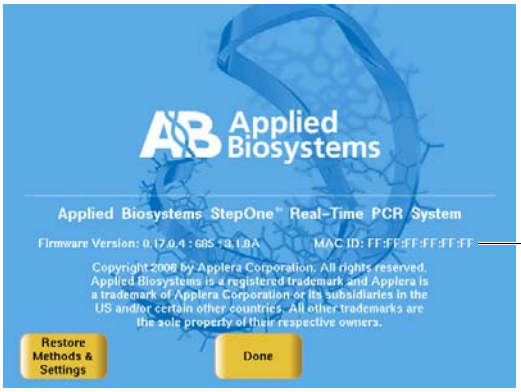
- If the network requires a static IP address, obtain the IP address, subnet mask, and gateway address for the instrument.

How to Obtain the MAC Address of the Instrument

If your systems administrator requests the Media Access Control (MAC) address for the instrument, obtain the MAC address as follows:

1. Touch the instrument touchscreen to awaken it, then touch  .
2. In the Main Menu, touch **Settings Menu**, then touch **About the Instrument**.

   The MAC address for the instrument appears in the rights side of the About Instrument screen.

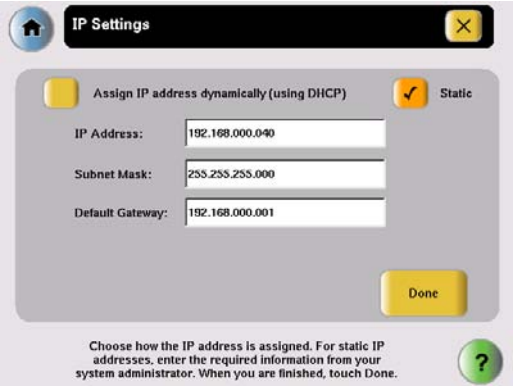


3. When finished, touch **Done**.
4. In the Settings Menu, touch  to return to the Main Menu.

Notes

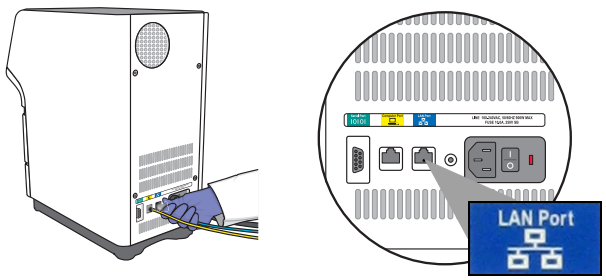
Define the LAN Port IP Settings

1. Touch the instrument touchscreen to awaken it, then touch .
2. In the Main Menu, touch **Settings Menu**, touch **Admin Menu**, then touch **Set IP Address**.
3. Enter the IP settings for the network connection.

Network Service	Action
DHCP	Touch **Assign IP address dynamically (using DHCP)**, touch **Done**, then touch **OK** when prompted.
Static IP	1. Touch **Static**. 2. Enter the IP address, subnet mask, and default gateway settings. For each setting, touch the field, enter the address, then touch **Done**. 3. Touch **Done** to save the settings, then touch **OK** when prompted. The following figure shows an example of static IP network settings for the LAN port of the instrument. 

4. Touch to return to the Main Menu.
5. Connect the Ethernet cable between the:
   - *Blue* LAN Port ( ) of the instrument, and the
   - Ethernet port for your network

IMPORTANT! Do not connect the Ethernet cable to the *yellow* Computer Port ( ); this port is reserved for the colocated computer connection.



Notes

Set Up a Computer for Remote Monitoring

After connecting the instrument to the network, connect the computer to the network and install the StepOne™ software for remote monitoring.

Materials Required

Ethernet cable with RJ45 connectors

Computer Requirement

If you are connecting a computer that you provided to a network, confirm that the computer contains a network interface card (NIC) or a free network port.

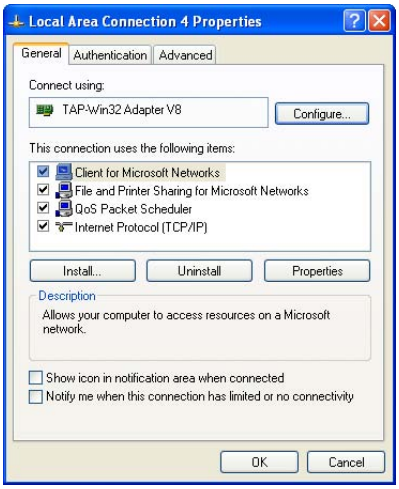
Collect Required Information

- Network policy for obtaining IP addresses: DHCP *or* static IP
- If the network requires a static IP address, obtain the IP address, subnet mask, and gateway address for the computer

Connect the Computer to the Network

IMPORTANT! We recommend that you arrange for a network administrator to connect your computer to the network. The following procedure does not provide adequate detail for all network architectures.

1. Use the Ethernet cable to connect the computer to the nearest network port.
2. Power on the computer, then log in using an account that belongs to the Administrators user group.
3. In the computer desktop, right-click **My Network Places**, then select **Properties**.
4. Right-click **Local Area Connection**, then select **Properties**.
5. Select **Internet Protocol (TCP/IP)**, then click **Properties**.



Notes

6. Set the Internet Protocol (TCP/IP) Properties:

Network Configuration	Action
DHCP	1. Select **Obtain an IP address automatically**. 2. If the computer obtains DNS addresses: • Automatically – Select **Obtain DNS server address automatically**. • Statically – Select **Use the following DNS address**, enter the address of the preferred and alternate DNS servers if available.
Static IP	1. Select **Use the following IP address**. 2. In the IP Address field, enter the static IP address. 3. If necessary, enter a subnet mask. 4. If necessary, enter a static gateway address in the Default Gateway field.

7. If your network requires advanced TCP/IP settings (such as WINS settings):
   a. Click **Advanced** in the Internet Protocol (TCP/IP) Properties dialog box.
   b. Define the IP Settings, DNS, and WINS tabs as instructed by your systems administrator, then click **OK**.
8. Close all dialog boxes by clicking **OK**.
9. Restart the computer.

Note: The computer is now visible to other computers on the network.

Install the StepOne™ Software

1. If you have not already done so, install the StepOne™ software to the networked computer (see "Install the Software" pages 59 or 84).

Note: The StepOne™ software is required to monitor the instrument over the network.

2. (Optional) Install protective software to the computer (see "Select Protective Hardware and Software" on page 31).

Notes

Monitor the Instrument

After connecting the instrument and a computer to the network, test the network connection by enabling remote monitoring.

About Remote Monitoring

When the instrument is connected to the network, any computer on the network that is running the StepOne™ software can:

- Monitor the status of the instrument during and between runs
- Send an experiment to the instrument (see page 112)
- Download an experiment from the instrument (see page 112)
- Enable or change email notification settings for the instrument (see page 113)

Guidelines for Remote Monitoring

To ensure optimal performance of the remote monitoring feature, observe the following:

- Although the StepOne™ software can add multiple instruments for remote monitoring, it can monitor only one instrument at a time.
- We do not recommend that an instrument be monitored by more than one computer simultaneously.
- Unless you are sure that your instrument and computer exist on the same subnet, we recommend that you use the IP address of the instrument to add it for remote monitoring.

Enable Remote Monitoring

1. In the StepOne™ software, select **Instrument ▸ Remote Monitor**.
2. In the Manage Instrument screen, click **Add Instrument**.
3. Add the instrument in the Add Instrument dialog box:
   a. In the Profile Name field, enter the name of your instrument (from step 1b on page 45).
   b. In the Instrument Name, Host Name, or IP Address field, enter the host name or IP Address of the instrument that you want to monitor.
   c. Click **Save & Exit**.

   The StepOne™ software displays an entry for the instrument in the left-most column of the Manage Instruments screen.
4. Enable remote monitoring for the instrument:
   a. In the Manage Instrument pane of the StepOne™ software, select the instrument that you want to monitor.
   b. In the entry for the instrument, click  .

Note: You can also monitor the instrument by selecting your instrument from the dropdown menu, then clicking **Monitor**.

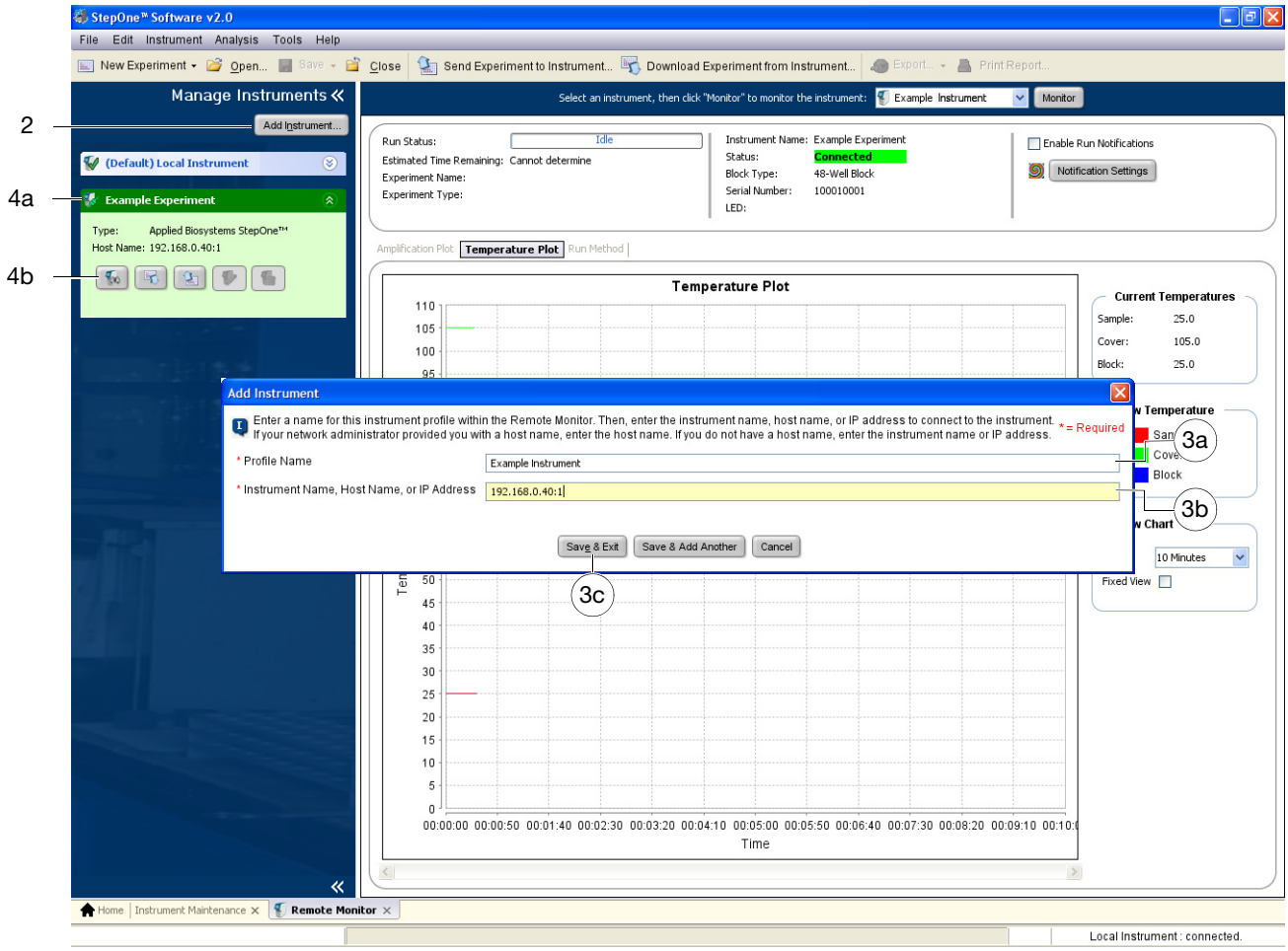
Notes

The StepOne™ software displays the status, attributes, and plot data for the selected instrument in real time. If a communications warning appears, troubleshoot the problem as explained in “Network Connection Problems” on page 161.

You will lose the software connection to the colocated instrument if you:

- Change the instrument that is connected directly to your computer
- Use the colocated instrument touchscreen to change the instrument name or IP address

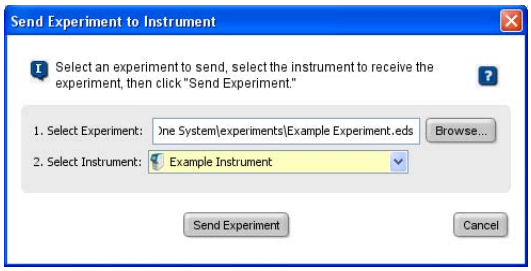
To reestablish the colocated connection, restart the StepOne™ software.



Notes

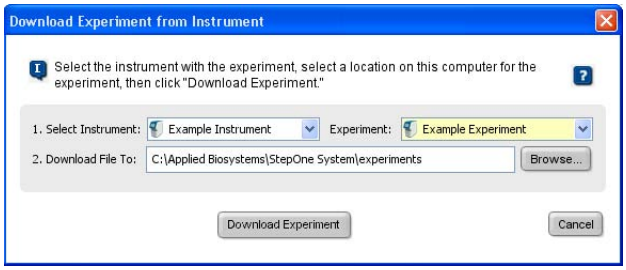
Send an Experiment to a Monitored Instrument

1. In the entry for your instrument, click  .
2. Click **Browse**, then navigate to and select the experiment that you want send to the instrument, then click **Open**.
3. Select your instrument from the Select Instrument dropdown menu.
4. Click **Send Experiment**, then click **OK** when prompted.



Download an Experiment from a Monitored Instrument

1. In the entry for your instrument, click  .
2. Select your instrument from the Instrument dropdown menu.
3. Select your experiment from the Experiment dropdown menu.
4. Click **Browse**, then navigate to and select the folder to which you want to send the experiment, then click **Select**.
5. Click **Download Experiment**, then click **OK** when prompted



Notes

Enable or Change Notification Settings

The notification settings allow you to configure the StepOne™ software to alert you by email when the instrument begins and completes a run, or if an error occurs during a run. You can also set up the software to attach a completed run file to the Run Completed email notification. The notifications settings feature is optional and does not affect system performance.

IMPORTANT! The StepOne™ software will transmit email only while the instrument is monitored. If the network connection is interrupted, the StepOne™ software will stop transmitting updates.

To modify the notification settings:

1. Contact your systems administrator or information technology department for the:
   - Network address of a Simple Mail Transfer Protocol (SMTP) server.
   - A user name and password for the server, if required for access.
   - The Secure Sockets Layer (SSL) setting of the server (on or off).
2. While monitoring an instrument, select **Enable Run Notifications** in the top-right corner of the screen.
3. Click **Change Notifications**, change the Notification Settings as desired, then click the close box.
4. Select **Enable Notifications**.
5. Select the events that generate notifications:
   - **Instrument Error** – Causes the system to email recipients all errors encountered by the instrument during each run.
   - **Run Started** – Causes the system to email the recipients every time the instrument starts a run.
   - **Run Completed** – Causes the system to email the recipients every time the instrument completes a run.
6. Click the **Enter e-mail addresses for notifications** field, then enter the email address(es) that you want to receive email notifications.
7. Click the Outgoing Mail Server (SMTP) field, then enter the address of the SMTP server.
8. Select whether or not to attach completed run files to the Run Completed email notifications.

   Note: This option applies only to email notifications that are generated when the instrument completes a run (that is, **Run Completed** is selected in step 5).

Notes

9. Set the authentication settings as needed.

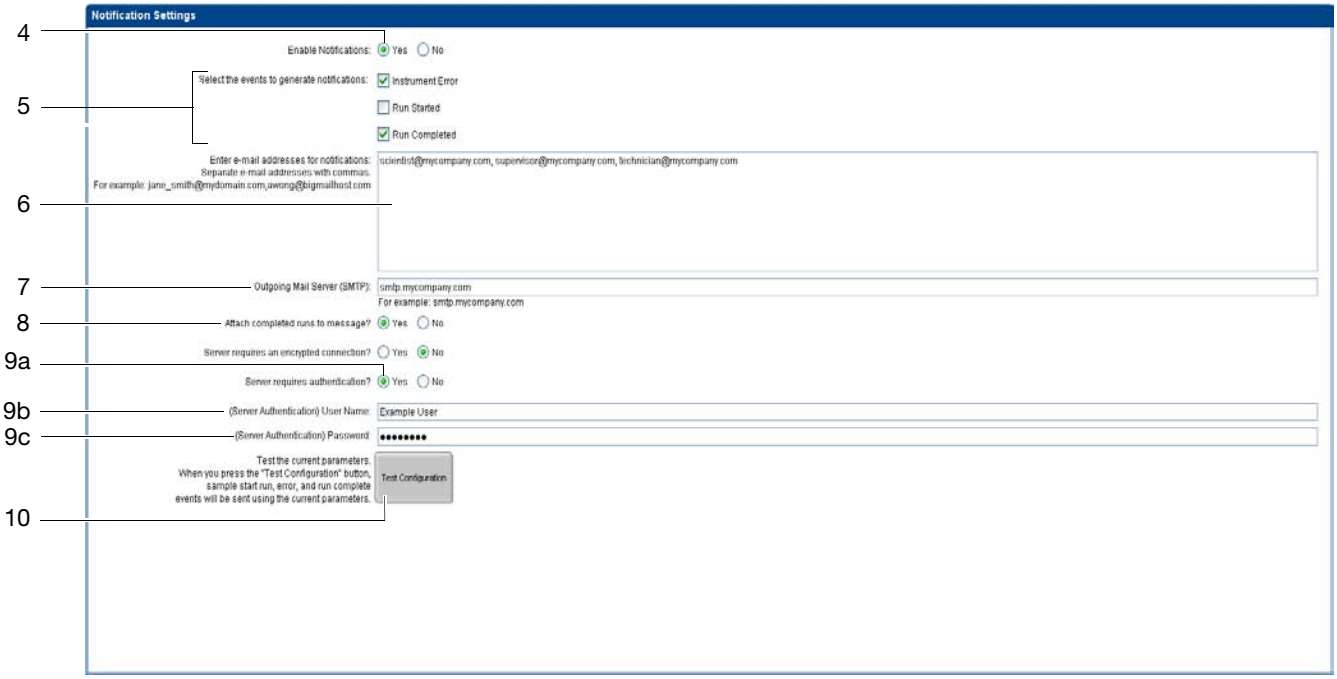
   If the SMTP server requires authentication:

   a. Click **Yes**.

   b. Click the User Name field, then enter the user name to access the server.

   c. Click the Password field, then enter the password for the user account.

10. Click **Test Configuration**. If the notification settings are set up correctly, sample emails are sent to the addresses entered in step 6.



Notes

6 Maintain the System

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

Notes

Regular Maintenance

Maintenance Schedule

Perform routine maintenance of your Applied Biosystems StepOne™ or StepOnePlus™ Real-Time PCR Instrument and computer to ensure proper operation. The following table displays the recommended maintenance schedule.

Perform every...	Task	See Page
Week	Check the computer disk space. If necessary, archive or back up your experiment files.	142
	Power off the computer controlling the instrument, then after 30 sec power on the computer.	—
	Clean the surface of the instrument with a lint-free cloth.	—
	Defragment the computer hard drive.	—
Month	Perform a background calibration.‡	122
1.5 Years	Perform a spatial calibration.	118
	Perform a dye calibration.	128

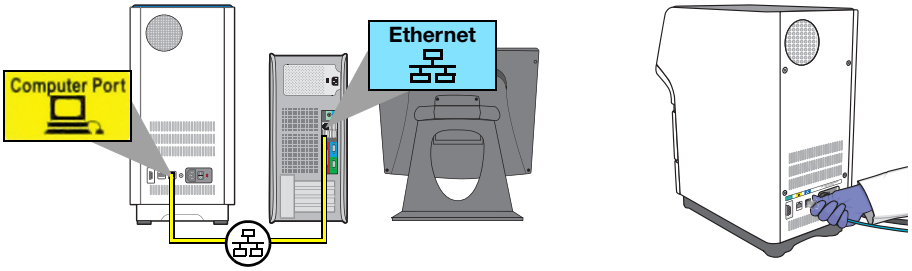
‡ You can run a background calibration to check for contamination. Also, you must run a background calibration and a dye calibration if any part of the instrument optics are replaced or moved.

Connection Required for Maintenance

The spatial, background, and dye calibrations must be performed while the system is in a colocated layout. To set up the system for maintenance, connect the yellow StepOne system cable between the:

- *Yellow* Ethernet port (💻) of the instrument, and
- Ethernet port (🖧) of the computer running the StepOne™ software

IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port (🖧), which is reserved for a network connection.



Maintenance Notifications

When a required maintenance task such as a calibration is overdue, the StepOne™ software and instrument display messages that prompt you to perform the necessary procedure.

Notes

How to View Maintenance Information

You can use the StepOne™ software to view a summary of maintenance data for your instrument. To view the maintenance information, select **Instrument ▸ Instrument Maintenance Manager** in the StepOne™ software. For each calibration (spatial, background, and dye), the software lists the following information:

- **Status** – Condition of the current calibration where:
  - **Current** indicates that the calibration is up to date.
  - **Expired** indicates that the calibration must be performed at the earliest possible convenience.
  - **Not Run** indicates that the calibration has not been run (RNase P only).
- **Last Run** – Date when current calibration was run.
- **Expiry Date** – Date when current calibration will expire.

Instrument Tools Menu Tests

The Tools Menu of the instrument provides several features that test the temperature of several instrument components against the product specification.

Test	Description
Run Cycle Performance Test	Verifies that the sample block(s) achieves the temperature accuracy specification for the instrument.
Optics Verification	Verifies that the instrument optics is operating to the specifications of the instrument.
Statistics	Displays the usage data for the instrument, including: • Thermal cycles performed by the sample block(s). • Degrees Celsius heated or cooled by the sample block(s). • Hours of operation for the instrument LED. • RNase P verification run data.

Temperature Verification Service

We recommend that you perform an annual temperature verification of the instrument depending on your usage and laboratory requirements. The temperature verification service can be performed by us when the instrument is shipped for an annual service, or by the service engineer at an additional cost. See “How to Obtain Support” on page 14 to contact Support for pricing and further information.

Notes

Perform a Spatial Calibration

Perform a spatial calibration every 18 months, or as often as necessary, depending on instrument use.

Materials Required

- Spectral calibration plate 1 from the spectral calibration kit for your instrument:
  - StepOne™ Real-Time PCR System Spectral Calibration Kit (PN 4371433)
  - StepOnePlus™ Real-Time PCR System Spectral Calibration Kit (PN 4371435)
- Safety glasses
- Powder-free gloves
- Centrifuge with reaction plate adapter
- StepOne system cable, yellow (from the system packing kit)

Purpose of the Calibration

A spatial calibration maps the positions of the wells on the sample block(s) so that the StepOne™ software can associate increases in fluorescence during a run with specific wells of the reaction plate.

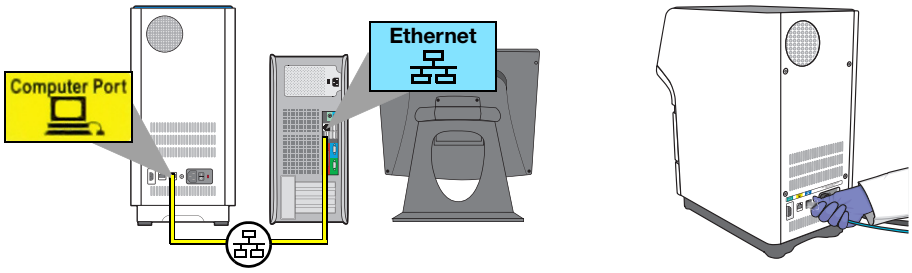
Prepare for the Calibration

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

IMPORTANT! Wear powder-free gloves when you handle calibration plates.

1. If you have not already done so, use the StepOne system cable to connect the:
   - *Yellow* Ethernet port ( ) of the instrument to the
   - Ethernet port ( ) of the computer running the StepOne™ software

   IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port ( ).



2. Get calibration plate 1 from the spectral calibration kit in the freezer.
3. Allow calibration plate 1 to warm to room temperature (approximately 5 min).

   IMPORTANT! Do not remove calibration plate 1 from its packaging until you are ready to run it. The fluorescent dyes in the wells of both spectral calibration plates are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the dyes.

Notes

4. Remove calibration plate 1 from its packaging.

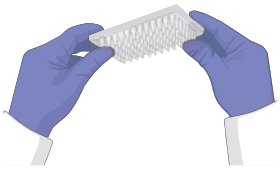
5. Vortex calibration plate 1 for 5 sec.

6. Centrifuge calibration plate 1 for 2 min at less than 1500 rpm.

IMPORTANT! The calibration plate must be well mixed and centrifuged.

7. Confirm that the liquid is at the bottom of each well of calibration plate 1. If not, centrifuge the calibration plate again at a greater rpm and for a longer time.

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



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

8. Load calibration plate 1 into the instrument:

   a. Open the instrument drawer.

   b. Place the calibration plate in the sample block(s) so that the A1 position is at the back-left corner.

   c. Close the instrument drawer carefully.

Notes

Perform the Calibration

1. In the main screen of the StepOne™ software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the navigation pane of the Instrument Maintenance Manager, click **Spatial**.
3. In the Instrument Maintenance Manager, click **Start Calibration**.
4. In the Setup screen of the Spatial Calibration dialog box, select **The calibration plate is loaded into the instrument**, then click **Next**.
5. In the Run screen, click **START RUN**, then wait for the instrument to complete the spatial calibration.

Remove the Calibration Plate

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

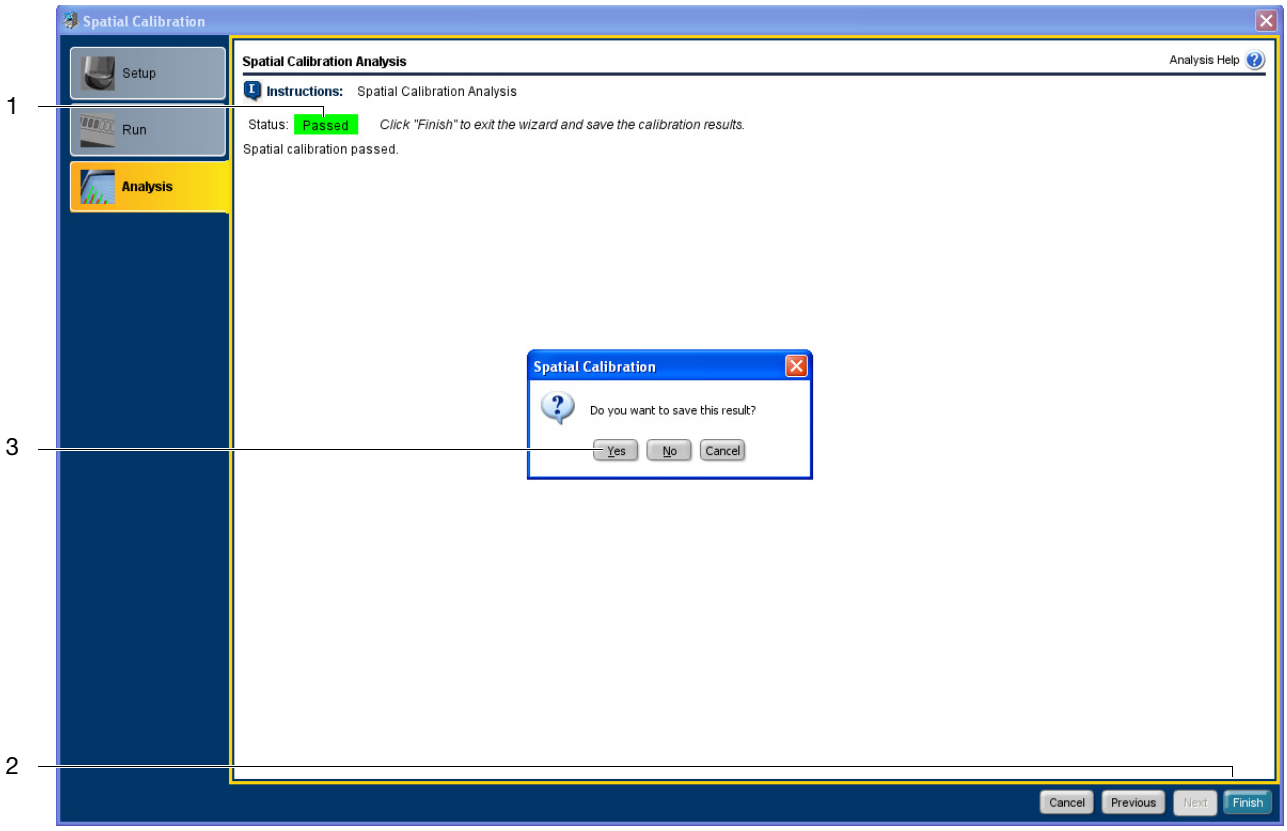
IMPORTANT! Wear powder-free gloves when you handle calibration plate.

1. Open the instrument drawer.
2. Remove calibration plate 1 from the sample block(s), place it inside its packaging sleeve, and return it to the spectral calibration kit in the freezer.
3. Close the instrument drawer carefully.

Notes

Analyze the Calibration Data

1. In the Analysis screen of the Spatial Calibration dialog box, confirm the status of the calibration:
   - **Passed** – The instrument passed the calibration. Go to step 2.
   - **Failed** – The instrument failed the calibration. Contact Support as described in "How to Obtain Support" on page 14.
2. Click **Finish**.
3. When prompted to save the calibration, click **Yes**.



Notes

Perform a Background Calibration

Perform a background calibration monthly, or as often as necessary, depending on instrument use.

Materials Required

- Background plate from the spectral calibration kit for your instrument:
  - StepOne™ Real-Time PCR System Spectral Calibration Kit (PN 4371433)
  - StepOnePlus™ Real-Time PCR System Spectral Calibration Kit (PN 4371435)

  Note: Alternatively, create a background plate as described in "How to Create a Background Plate" on page 127.
- Powder-free gloves
- Safety glasses
- Centrifuge with reaction plate adapter
- StepOne system cable, yellow (from the system packing kit)

Calibration Guidelines

- 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. To avoid contaminating the reaction plate, do not place plates on a lab bench. Always return the calibration plate to its original bag.

Purpose of the 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 min at 60°C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

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

Background Fluorescence

Fluorescence data collected by the system includes a fluorescent spectral component inherent to the system, commonly referred to as background fluorescence. This background fluorescence is a composite signal found in all signal data that consists of fluorescence from several sources, including:

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

Notes

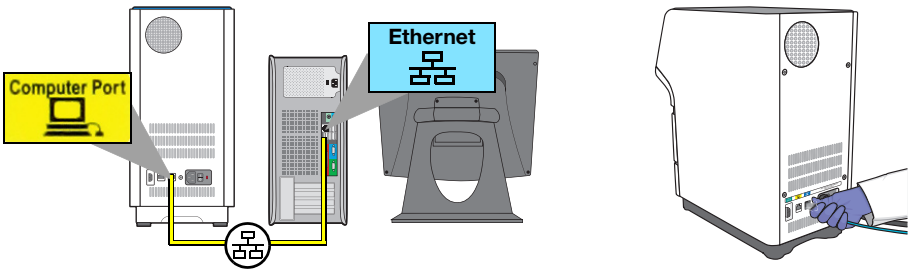
Prepare for the Calibration

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

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

1. If you have not already done so, use the StepOne system cable to connect the:
   - *Yellow* Ethernet port ( ) of the instrument to the
   - Ethernet port ( ) of the computer running the StepOne™ software

   IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port ( ).



2. Get the background plate from the spectral calibration kit in the freezer.
3. Allow the background plate to warm to room temperature (at least 5 min).
4. Remove the background plate from its packaging.

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

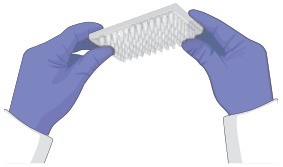
5. Vortex the background plate for 5 sec.
6. Centrifuge the background plate for 2 min at less than 1500 rpm.

   IMPORTANT! The background plate must be well mixed and centrifuged.

Notes

7. Confirm that the liquid is at the bottom of each well of the background plate. If not, centrifuge the background plate again at a greater rpm and for a longer 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 background plate can contaminate the sample block(s) and cause an abnormally high background signal.



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

8. Load the background plate into the instrument:
   a. Open the instrument drawer.
   b. Place the background plate in the sample block(s) so that the A1 position is at the back-left corner.
   c. Close the instrument drawer carefully.

Perform the Calibration

1. In the main screen of the StepOne™ software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the navigation pane of the Instrument Maintenance Manager, click **Background**.
3. Click **Start Calibration**.
4. In the Setup screen of the Background Calibration dialog box, select **The background plate is loaded into the instrument**, then click **Next**.
5. In the Run screen, click **START RUN**, then wait for the instrument to complete the background calibration.

Note: If the StepOne™ software displays messages during the run, troubleshoot the errors as described in “Background Calibration Failure” on page 164.

Notes

Remove the Background Plate

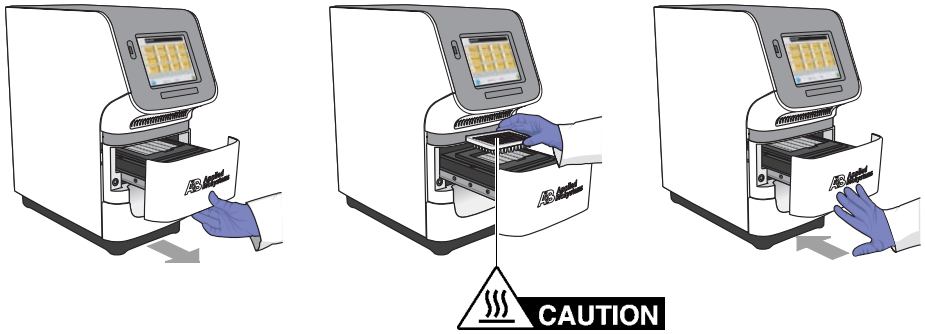
CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

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

1. Open the instrument drawer.
2. Remove the background plate from the sample block(s), place it inside its packaging sleeve, and return it to the spectral calibration kit in the freezer.

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

3. Close the instrument drawer carefully.

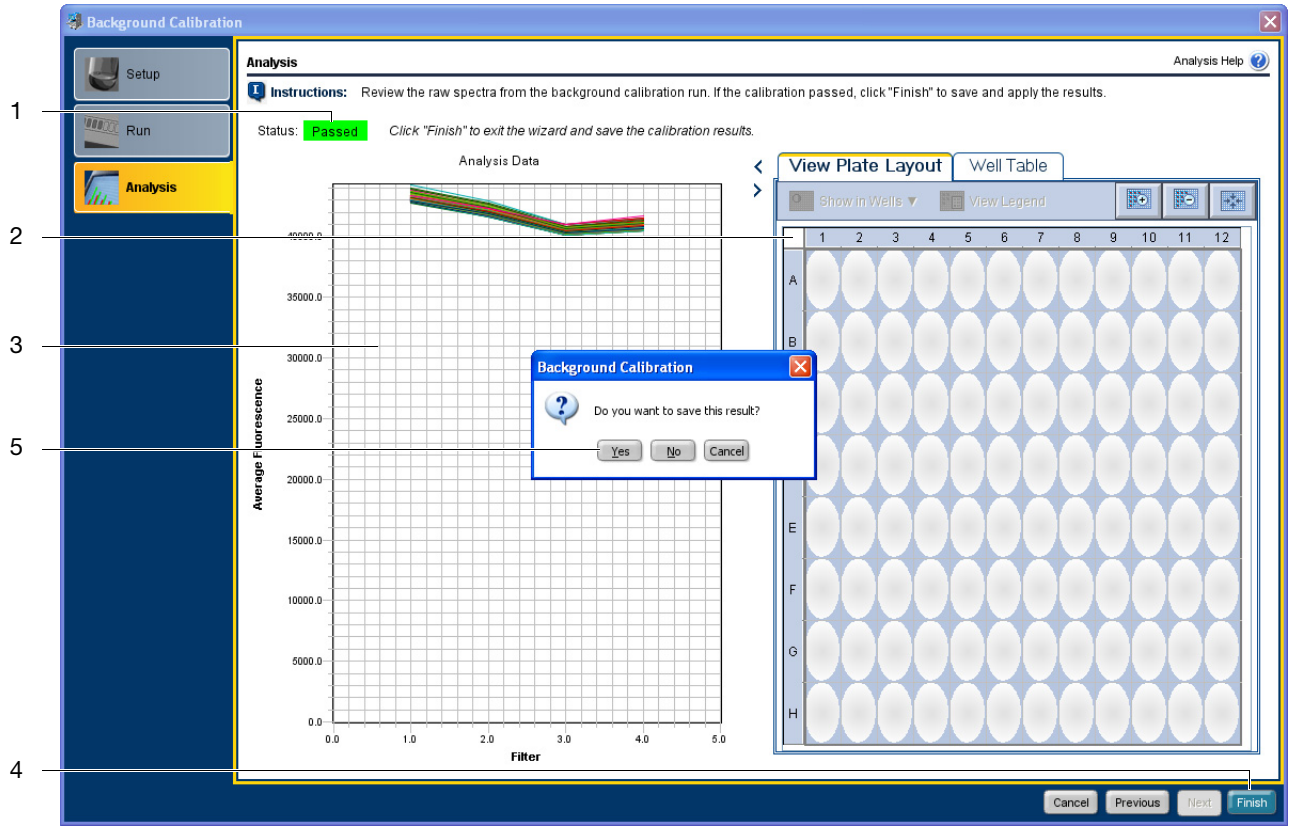


Notes

Analyze the Calibration Data

1. In the Analysis screen of the Background Calibration dialog box, confirm the status of the calibration:
   - **Passed** – The instrument passed the calibration. Go to step 2.
   - **Failed** – The instrument failed the calibration. Troubleshoot the error as described in “Background Calibration Failure” on page 164.
2. Select all wells of the plate layout.
3. Inspect the raw data for irregular spectral peaks.

   If one or more wells produce raw spectra that diverge significantly from that of the neighboring wells, the background plate or the sample block(s) could contain a fluorescent contaminant. If you suspect contamination, troubleshoot the irregular signal as explained in “Background Calibration Failure” on page 164.
4. If all spectra are acceptable, click **Finish**.
5. When prompted to save the background calibration, click **Yes**.



Notes

How to Create a Background Plate

Whenever possible, use a background plate included with the spectral calibration kit. The background plate contains a buffer that accurately models the reagents used for PCR, and produces high-quality calibration data. However, if a background plate from a spectral calibration kit is not available, you can create one as explained below.

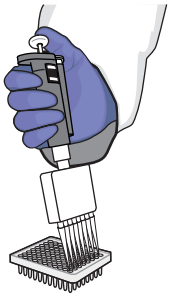
Materials Required

- Deionized water
- MicroAmp® Optical Adhesive Film or MicroAmp® 8-Cap Strip (Flat Caps only)
- MicroAmp® Optical Reaction Plate
- Pipettor, 200-µL and pipette tips
- Powder-free gloves
- Safety glasses

Create the Background Plate

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

1. Remove a reaction plate from its box and place it on a clean, dry surface.
2. Pipette 30 µL of deionized water to each well of the reaction plate.



3. Seal the reaction plate using an optical adhesive film or optical flat caps.

4. Use the reaction plate to perform the background calibration.

Notes

Perform a Dye Calibration

Perform a dye calibration at least every 18 months, or as often as necessary depending on instrument use. Because the age and use of the instrument can affect spectral readings, you may need to perform the calibration more frequently.

IMPORTANT! Perform a background calibration before performing a dye calibration (see "Perform a Background Calibration" on page 122).

Materials Required

- Spectral Calibration Plates from the spectral calibration kit for your instrument:
  - StepOne™ Real-Time PCR System Spectral Calibration Kit (PN 4371433)
  - StepOnePlus™ Real-Time PCR System Spectral Calibration Kit (PN 4371435)
- Safety glasses
- Powder-free gloves
- Centrifuge with reaction plate adapter
- StepOne system cable, yellow (from the system packing kit)

Custom Dye Plates

The StepOne™ and StepOnePlus™ instrument supports the use of custom dyes (dyes not supplied by Thermo Fisher Scientific) that are excited with a light source at 470 nm and fluoresce within the spectral range it supports (500 to 650 nm). See "How to Calibrate a Custom Dye" on page 139 for more information.

Purpose of the Calibration

During a dye calibration run, the system:

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

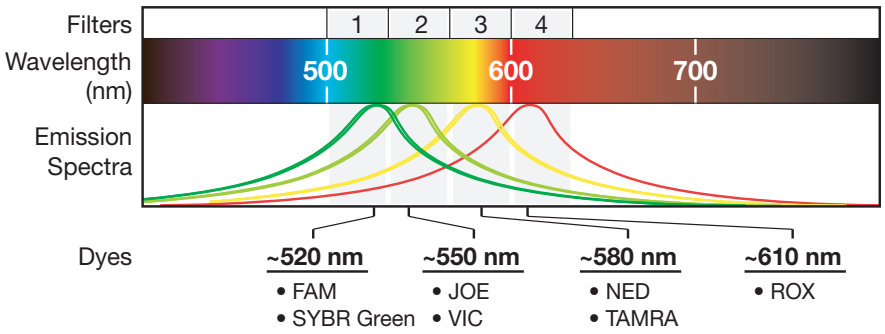
The StepOne™ software uses spectral data during subsequent runs to characterize dyes and distinguish the individual contribution of each dye in the collective fluorescence collected by the instrument during a run. After each run, the StepOne™ software stores run data in the form of a raw spectral signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the spectral file. After analysis, the StepOne™ software stores the spectral data with the collected fluorescence data for each experiment.

Notes

Supported Dyes

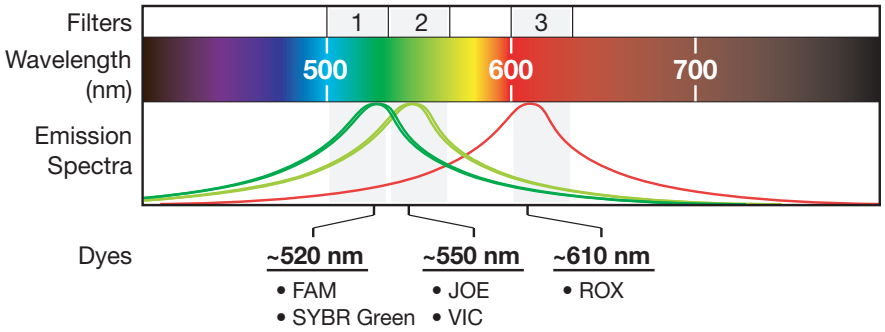
StepOnePlus™ System Dyes

The StepOnePlus™ instrument uses the following dye set for calibration: FAM™ dye, JOE™ dye, NED® dye, ROX™ dye, TAMRA® dye, VIC® dye, and SYBR® Green dye. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



StepOne™ System Dyes

The StepOne™ instrument uses the following dye set for calibration: FAM™ dye, JOE™ dye, ROX™ dye, VIC® dye, and SYBR® Green dye. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



Notes

Custom Dyes

The system can be used to run assays designed with custom dyes (dyes not supplied by Thermo Fisher Scientific). However, before using custom dyes with the instrument, you must create and run a custom calibration plate. The purpose of the custom plate is similar to that of a spectral calibration plate. The StepOne™ software uses the custom calibration plate to create a spectral standard to distinguish the custom dye in the fluorescence data collected during the run.

IMPORTANT! To use a custom dye on your system, it must be excited with light at 470 nm and fluoresce within the 500 to 650 nm spectral range measured by the StepOne™ and StepOnePlus™ instrument.

IMPORTANT! We do not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA™ dye may be used as a reporter or quencher with the StepOnePlus™ system.

Notes

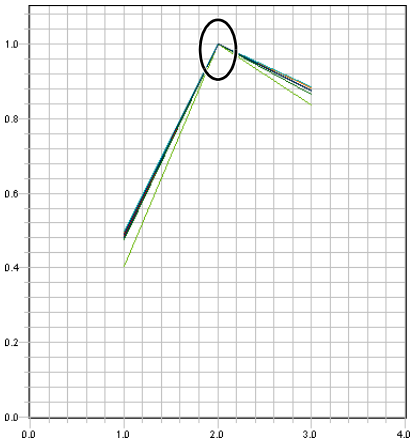
About the Analysis

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

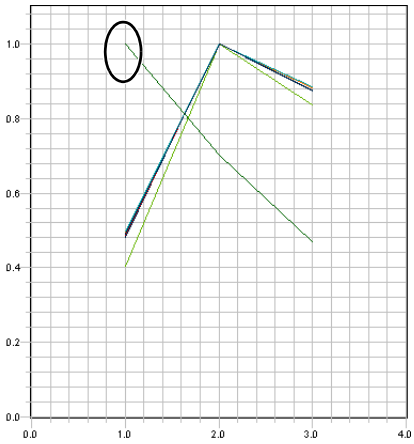
When the StepOne™ software extracts the calibration data from a dye run, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire calibration plate. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths (see below).

The StepOne™ software can compensate for some differences in a spectral profile by replacing (auto-repairing) the spectra of unacceptable wells with the spectra of other wells on the reaction plate. 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 calibration plate contain dyes at identical concentrations, the resulting signals for the wells containing each dye should be similar. Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical and excitation energy between the individual wells.



Acceptable Spectra
Spectra peak at the same wavelength and do not diverge significantly



Unacceptable Spectra
Spectra peak at the different wavelengths

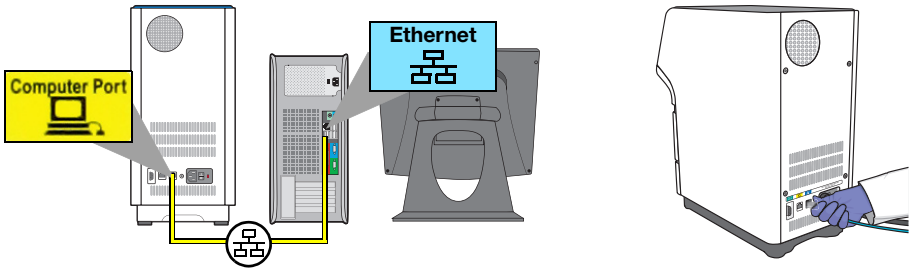
Notes

Prepare for the Calibration

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

1. If you have not already done so, use the StepOne system cable to connect the:
   - *Yellow* Ethernet port ( ) of the instrument to the
   - Ethernet port ( ) of the computer running the StepOne™ software

   IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port ( ).



2. Get calibration plate 1 from the spectral calibration kit in the freezer.
3. Allow calibration plate 1 to warm to room temperature (~5 min).

   IMPORTANT! Do not remove calibration plate 1 from its packaging until you are ready to run it. The fluorescent dye in the wells of each calibration plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the dyes.

4. Remove calibration plate 1 from its packaging.

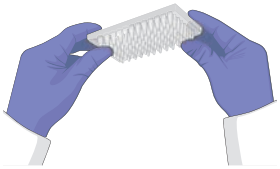
5. Vortex calibration plate 1 for 5 sec.
6. Centrifuge calibration plate 1 for 2 min at less than 1500 rpm.

   IMPORTANT! The calibration plate must be well mixed and centrifuged.

Notes

7. Confirm that the liquid is at the bottom of each well of the calibration plate. If not, centrifuge the plate again at a greater rpm and for a longer time.

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



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

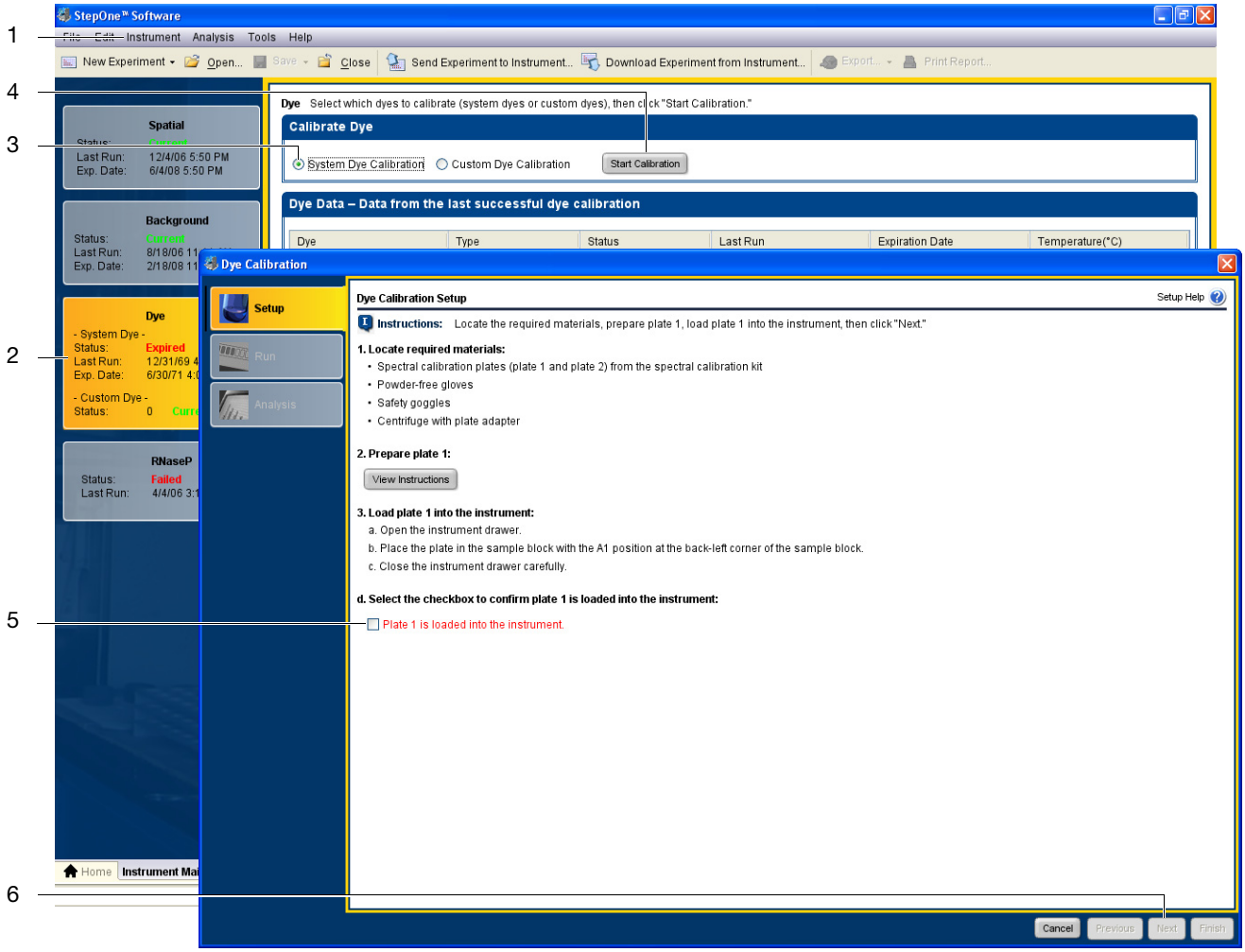
8. Load calibration plate 1 into the instrument:
   a. Open the instrument drawer.
   b. Place calibration plate 1 in the sample block(s) so that the A1 position is at the back-left corner.
   c. Close the instrument drawer carefully.

Notes

Perform the Calibration

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

1. In the main menu of the StepOne™ software, select **Instrument** ▸ **Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, click **Dye** in the navigation pane.
3. In the Dye screen, select **System Dye Calibration**.
4. Click **Start Calibration**.
5. In the Setup screen, select **Plate 1 is loaded into the instrument**.
6. Click **Next**.
7. In the Run screen, click **Start Run** ▶.



Notes

8. While the instrument is running calibration plate 1, prepare calibration plate 2 by performing steps 4 through 7 on page 133.

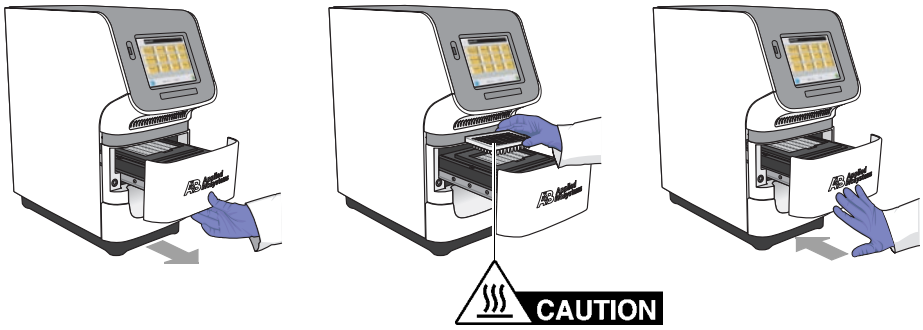
9. When the wizard prompts you to do so, load and run calibration plate 2:

   a. Open the instrument drawer.

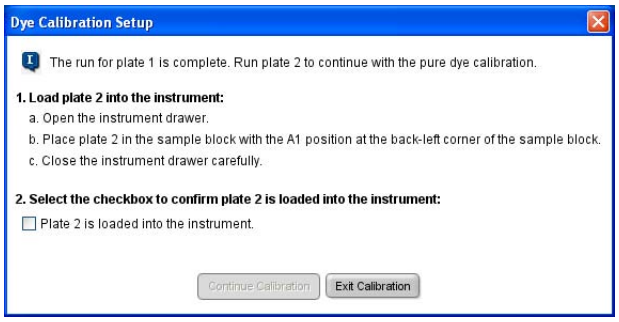
   b. Remove the calibration plate 1 from the sample block(s) and discard it.

   c. Place calibration plate 2 in the sample block(s) so that the A1 position is at the back-left corner.

   d. Close the instrument drawer carefully.



   e. In the Dye Calibration Setup wizard, select **Plate 2 is loaded in the instrument**, click **Continue Calibration**.



10. When the instrument completes the calibration, unload calibration plate 2:

    a. Open the instrument drawer.

    b. Remove the calibration plate 2 from the sample block(s) and discard it.

    c. Close the instrument drawer carefully.

Analyze the Calibration Data

1. After the calibration is complete, confirm the status of the calibration:
   - **Passed** – The instrument passed the calibration. Go to step 2a.
   - **Failed** – The instrument failed the calibration. Troubleshoot the error as described in “Dye Calibration Failure” on page 166.

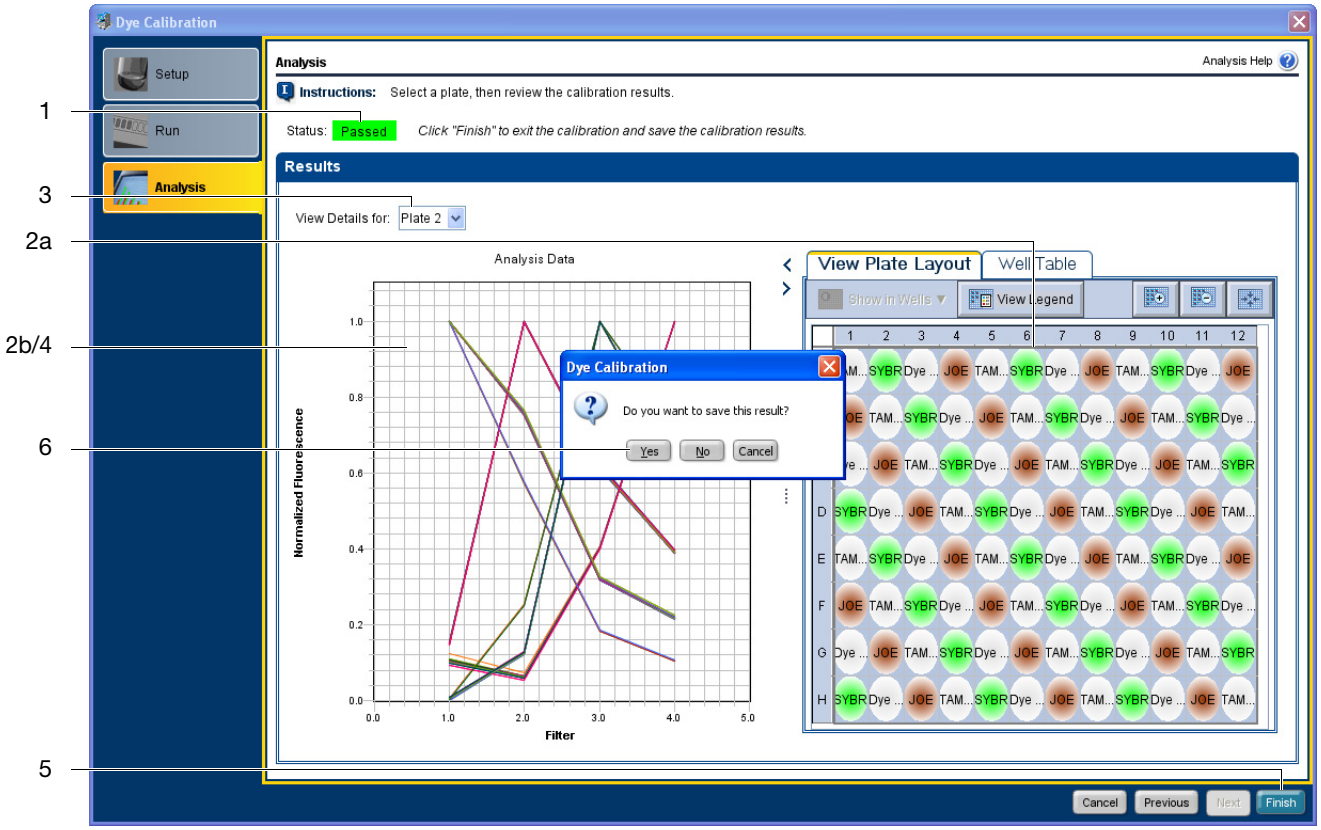
Notes

2. For each dye present on the plate, confirm the grouping of the dye spectra:
   a. In the plate layout, press **Ctrl**, then select the wells that contain the same dye.
   b. Inspect the raw data. For each spectrum, confirm that the peak is:
      - Within the detectable range for the instrument.
      - Free of irregular spectral peaks.
      - Present in the correct channel for the dye (see “StepOne™ System Dye Spectra” on page 137 or “StepOnePlus™ System Dye Spectra” on page 138).

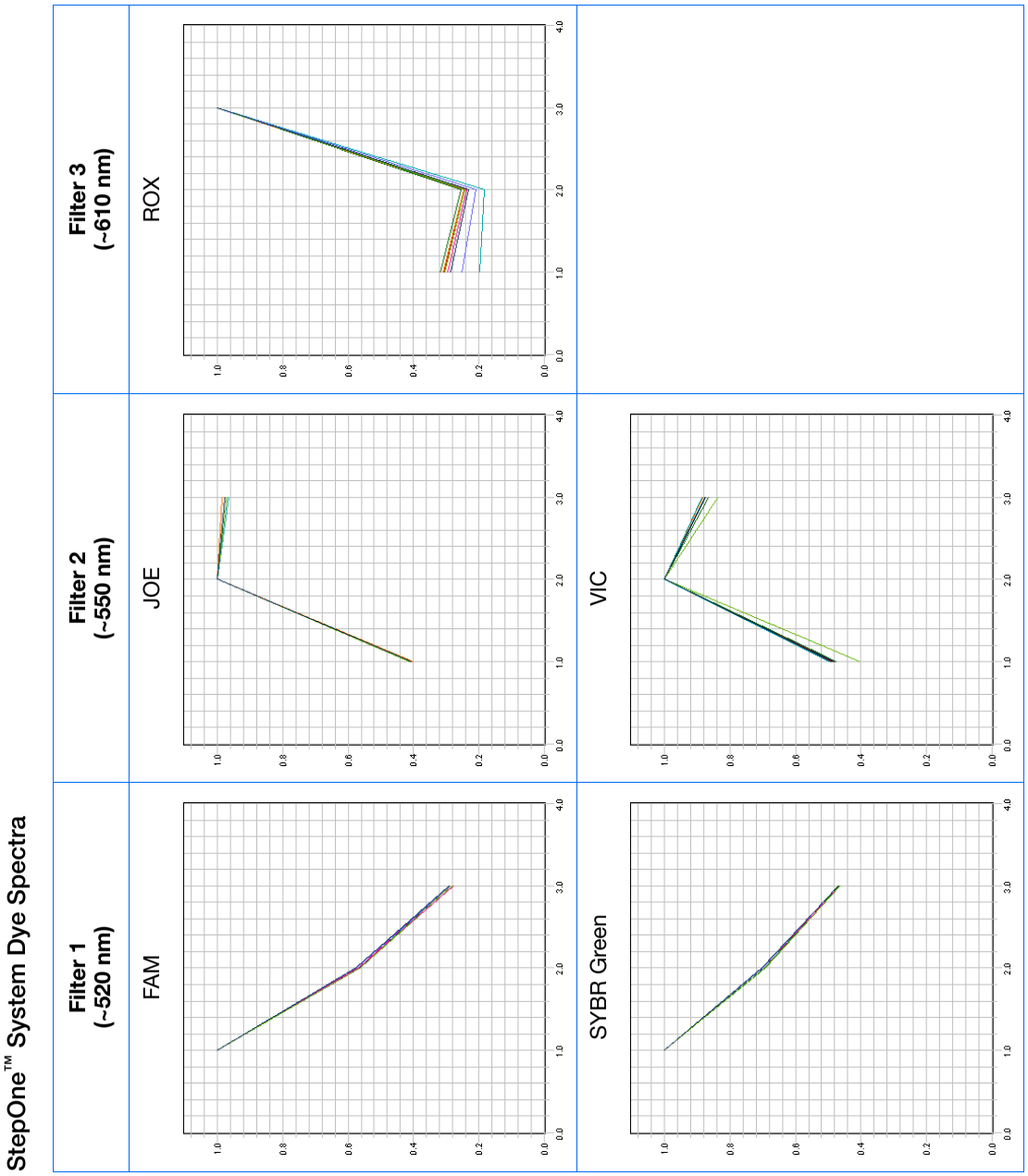
   If a spectrum does not comply with the criteria above, troubleshoot the problem as described in “Dye Calibration Failure” on page 166.

   Note: Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical and excitation energy between the individual wells.

3. If all spectra are acceptable, click **Plate 2** from the View Details menu.
4. Inspect the raw data for the as described in step 2b.
5. If all spectra are acceptable, click **Finish**.
6. When prompted to save the dye calibration, click **Yes**.

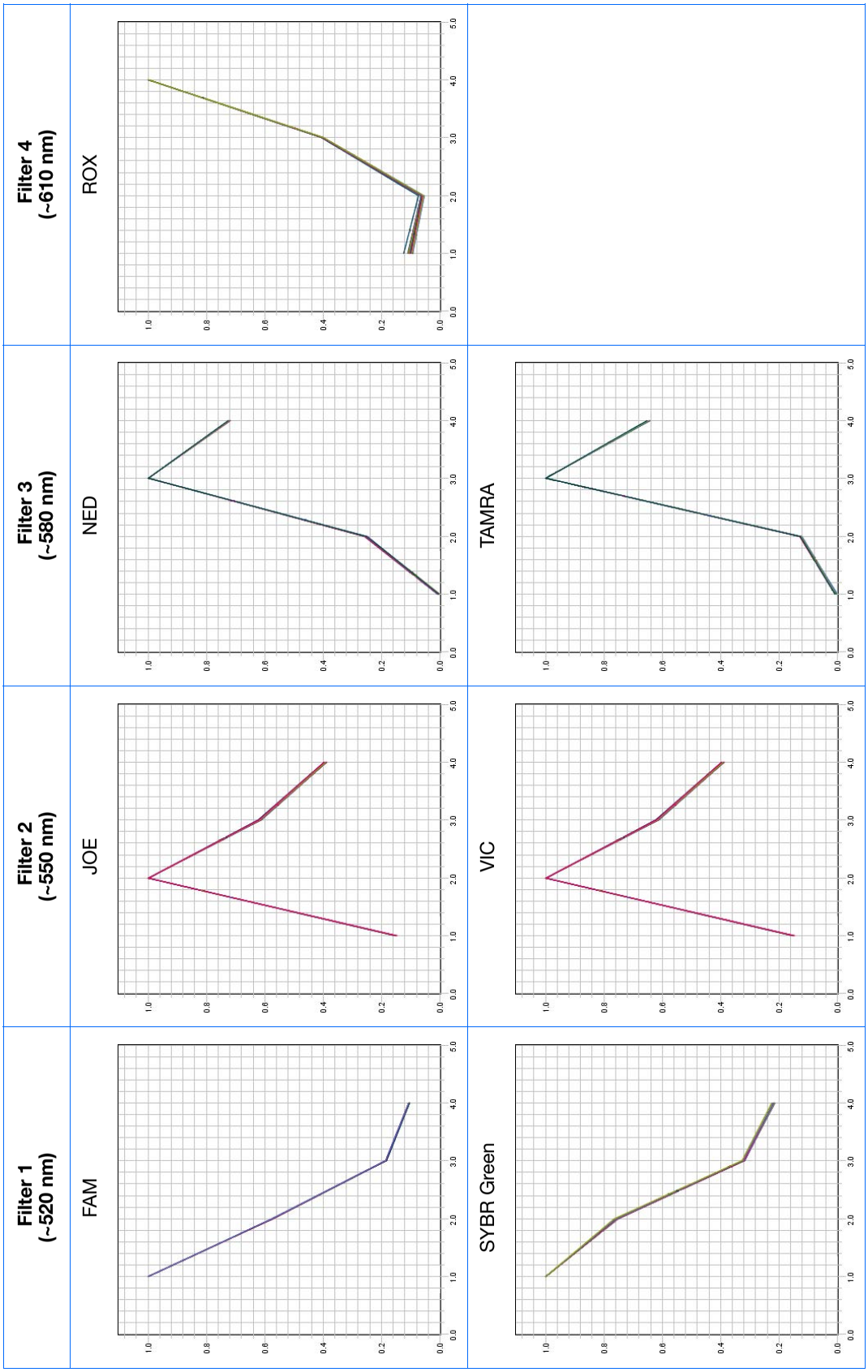


Notes


StepOne™ System Dye Spectra
Filter 1 (~520 nm)
Filter 2 (~550 nm)
Filter 3 (~610 nm)
FAM
JOE
ROX
SYBR Green
VIC

Notes

StepOnePlus™ System Dye Spectra



Notes

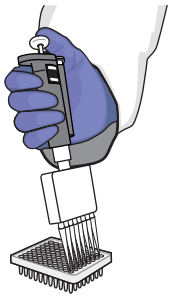
How to Calibrate a Custom Dye

Materials Required

- Centrifuge with reaction plate adaptor
- Custom dye(s)
- Deionized water
- MicroAmp® Optical Adhesive Film or MicroAmp® 8-Cap Strip (Flat Caps only)
- MicroAmp® Optical Reaction Plate of the size appropriate for your instrument
- Pipettors and pipette tips (200-µL and 1000-µL)
- Powder-free gloves
- Safety glasses
- Tubes (2-ml and 10-ml)

Prepare the Custom Spectral Calibration Plate

1. Create a calibration plate for the custom dye:
   a. Prepare 1.5 mL of the custom dye at the desired concentration.
   b. Pipette 30 µL of the diluted custom dye to all wells of a reaction plate.



   c. Seal the wells of the reaction plate using an adhesive film or optical flat caps.

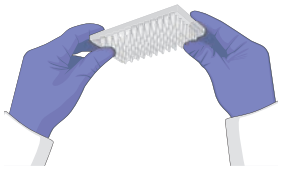
   d. Vortex the calibration plate for 5 sec.
   e. Centrifuge calibration plate for 2 min at less than 1500 rpm.

IMPORTANT! The calibration plate must be well mixed and centrifuged.

Notes

f. Confirm that the liquid in each well of the calibration plate is at the bottom of the well. If not, centrifuge the calibration plate again at a greater rpm and for a longer time.

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



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

2. Load the custom calibration plate into the instrument:
   a. Open the instrument drawer.
   b. Place the custom calibration plate in the sample block(s) so that the A1 position is at the back-left corner.
   c. Close the instrument drawer carefully.

Run the Custom Spectral Calibration Plate

1. In the main screen of the StepOne™ software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager:
   a. In the navigation pane, click **Dye**.
   b. In the Dye screen, select **Custom Dye Calibration**.
   c. Click **Start Calibration**.

Notes

3. In the Setup screen of the Dye Calibration dialog box, select a custom dye from the list or create the custom dye as follows:

   a. Click **New Dye**.

   b. In the Dye Manager dialog box, click **New**.

   c. Complete the New Dye dialog box, then click **OK**.

Field/Option	Action
Name	Enter a name for the custom dye.
Wavelength	Enter the wavelength at which the dye fluoresces.
Type	Select: • **Reporter** if the dye works in conjunction with a quencher dye to report an increase of PCR product. • **Quencher** if the dye suppresses the fluorescence of a reporter dye until amplification of PCR product. • **Both** if the dye reports an increase of PCR product without the aid of a quencher dye.

   d. Click **Close**.

4. In the Setup screen of the Dye Calibration dialog box, enter a temperature setting for the calibration.

   Note: Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Thermo Fisher Scientific system dyes is 60°C because data collection for TaqMan® reagents occurs during the 60°C extension step of the PCR.

5. Select **The custom dye plate is loaded in the instrument**, then click **Next**.

6. In the Run screen, click **Start Run**, then wait for the instrument to complete the dye calibration.

   Note: If the StepOne™ software displays messages during the run, troubleshoot the errors as described in “Dye Calibration Failure” on page 166.

7. When the instrument displays the Main Menu, unload the custom calibration plate.

8. Analyze the custom spectral calibration plate as explained in “Analyze the Calibration Data” on page 135.

Notes

Archive and Back Up Data

Develop a strategy that addresses the following data management issues for the instrument.

Check Disk Space Regularly

If you perform real-time experiments on your system, or collect real-time data for genotyping experiments, check the 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.

Archive Older Experiments

Conserve space on the computer hard drive by using a data compression utility to archive unused or older experiments. Several commercially available compression utilities are available, such as the PKZIP and ARC archive formats common to the Microsoft® Windows® operating system.

Back Up Experiments Regularly

Back up the experiments generated by your system to:

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

See “Select Protective Hardware and Software” on page 31 for more information on backup storage devices.

Notes

Notes

Infrequent Maintenance

Maintenance

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

Notes

Decontaminate the Sample Block(s)

If you perform a background calibration and observe high fluorescent background that you suspect to be contamination, decontaminate the sample block(s).

Materials Required

- Bleach solution (10%)
- Cotton or nylon swabs and lint-free cloths
- Deionized water
- EtOH solution (95%)
- Pipettor and pipette tips (100-µL)
- Powder-free gloves
- StepOne system cable, yellow (from the system packing kit)

Guidelines for Bleach Solution Use

CAUTION We recommend the use of 10% bleach solution for resolving fluorescent contamination on the instrument sample block(s); however, excessive use of the solution can corrode the block material.

To prevent block degradation:

- Use bleach solution to decontaminate the sample block(s) as a last resort. Rinse the sample block(s) with bleach only after treatments of deionized water and 95% ethanol fail to remove the contamination.
- Use deionized water to rinse the sample block(s) thoroughly after treating the block with bleach solution. Thoroughly removing residual bleach from the metal surfaces with water minimizes the long term effects of bleach.

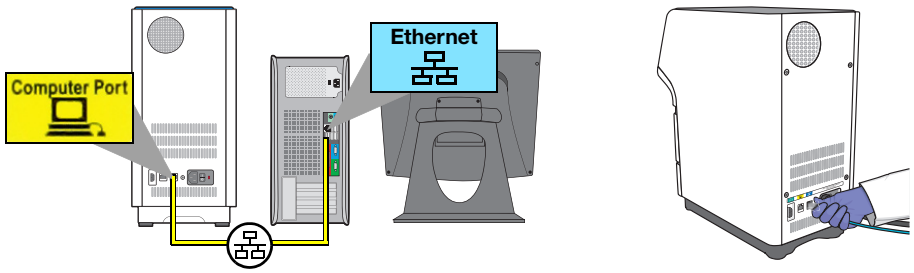
Clean the Contaminated Wells

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100°C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

Prepare the Instrument

1. If you have not already done so, use the StepOne system cable to connect the:
   - *Yellow* Ethernet port ( ) of the instrument to the
   - Ethernet port ( ) of the computer running the StepOne™ software

   IMPORTANT! Do not connect the StepOne system cable to the *blue* LAN Port ( ).

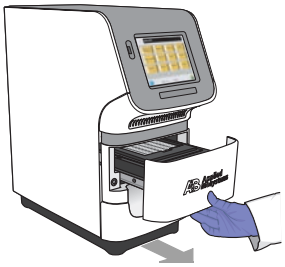


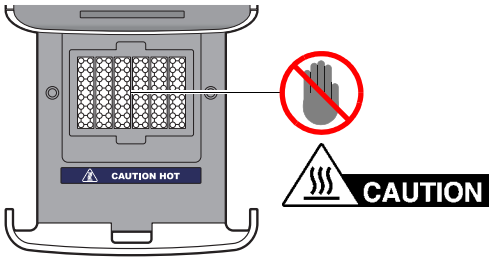
Notes

2. Using the data from the background calibration, identify the location of the contaminated wells on the sample block(s).

3. Turn off and unplug the system. Allow it to cool for 15 min.

4. Open the instrument drawer.





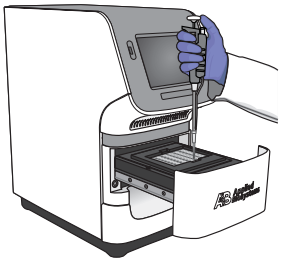
5. Clean each contaminated well of the sample block(s):

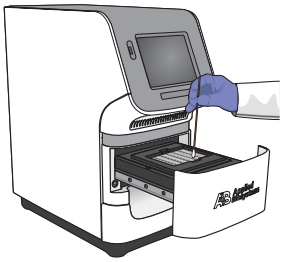
   a. Pipette a small volume of water into the contaminated well, slowly pipette the water up and down several times, then expel the water into a waste beaker.

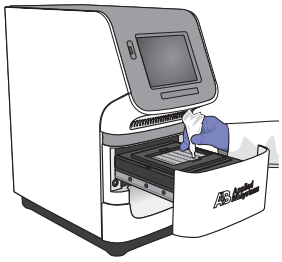
   b. Use a cotton swab to scrub inside of the contaminated well.

   c. Use a lint-free cloth to absorb the excess deionized water in the well.

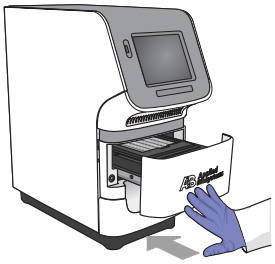
   d. Repeat steps 5a through 5d.







6. Close the instrument drawer, then plug in and power on the instrument.



7. Perform a background calibration to confirm that you have eliminated the contamination.

Notes

8. If the contamination persists, clean the wells with a 95% EtOH solution:

   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 SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

   a. Perform steps 2 through 5, substituting a 95% EtOH solution in place of the deionized water.
   b. Repeat step 5 using deionized water to rinse the wells of the sample block(s).
   c. Perform steps 6 and 7 to confirm that you have eliminated the contamination.

   IMPORTANT! Use deionized water to rinse wells after they have been treated with bleach or EtOH solution.

9. If the contamination persists, clean the wells with a 10% bleach 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 SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and powder-free gloves.

   a. Perform steps 2 through 5, substituting a 10% bleach solution in place of the deionized water.
   b. Repeat step 5 using deionized water to rinse the wells of the sample block(s).
   c. Perform steps 6 and 7 to confirm that you have eliminated the contamination.

   IMPORTANT! Use deionized water to rinse wells after they have been treated with bleach or EtOH solution.

10. If the contamination is still present, contact technical support.

Notes

Move the Instrument

Perform this procedure to safely move the instrument short distances (for example, between laboratories in the same building).

Note: If you want to transport the instrument via ground or air shipping, package the instrument for shipping as explained in "Ship the Instrument for Service" on page 152.

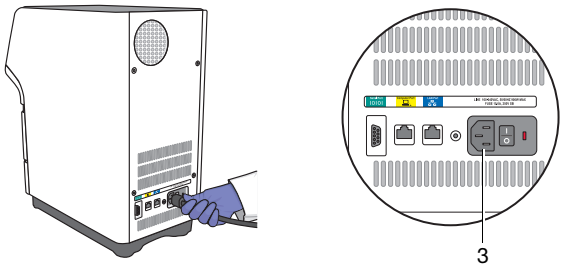
Materials Required

Original packing plate or a MicroAmp® Optical Reaction Plate

Prepare the Instrument

1. Load the packing plate or empty reaction plate into the instrument:
   a. Open the instrument drawer.
   b. Place the packing plate or empty reaction plate onto the sample block(s).
   c. Close the instrument drawer.

2. Raise the sample block(s) to secure it for transport:
   a. Touch the instrument touchscreen to awaken it, then touch  .
   b. In the Main Menu, touch **Tools Menu**, touch **Ship Prep**, then touch **Ship Prep**.
   c. Wait for the instrument to raise the sample block(s), then power off the instrument when prompted.
3. Disconnect the power cord from the back of the instrument.



Notes

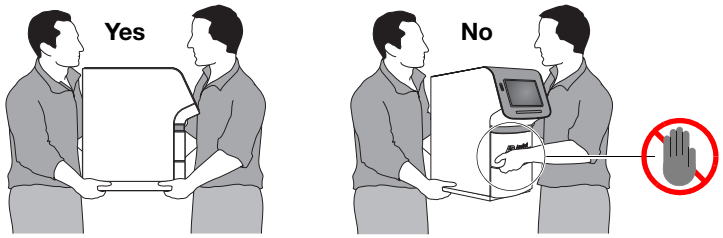
Move and Reconnect the Instrument

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 two people are required to lift the instrument.

IMPORTANT! Moving your instrument can create subtle changes in the alignment of the instrument optics. We recommend that you run a TaqMan® RNase P Fast Instrument Verification Plate after you move the instrument to confirm the performance of the system.

1. Move your instrument according to the guidelines in step 6 on page 40.

   CAUTION Do not carry the instrument by the instrument drawer. Carrying the instrument by the drawer can damage the instrument optics.



2. Install the components of the system according the chosen layout.

Layout	Description	Perform the installation described in…
Colocated	In this layout, the instrument is connected to the colocated computer by the yellow StepOne system cable.	Chapter 3, “Install the Colocated Layout,” on page 49
Standalone	In this layout, the instrument is not directly connected to the computer running the StepOne™ software.	Chapter 4, “Install the Standalone Layout,” on page 75

If the RNase P experiment performed at the end of the installation:

- **Passes**, then you have successfully moved the instrument.
- **Fails**, recalibrate the instrument:
  a. Perform a spatial calibration (see “Perform a Spatial Calibration” on page 118).
  b. Perform a background calibration (see “Perform a Background Calibration” on page 122).
  c. Perform a dye calibration (see “Perform a Dye Calibration” on page 128).
  d. Perform another RNase P experiment (see “Perform the RNase P Experiment” on page 65 or page 88).

Notes

Replace the External Fuses

Replace the fuses of the instrument as needed.

Materials Required

- Fuses, 10 A 250 VAC Slo-Blo®
- Small flatblade screwdriver

About the Fuses

The instrument uses two 10 A 250 VAC fuses to protect the system electronics from power surges. When replacing one fuse, we recommend that you replace the companion fuse, regardless of its condition.

Note: Acceptable AC line voltage tolerances are 100, 120, 220, 230 ±10%; 240 VAC +6%/–10%, 50/60 Hz ± 1%.

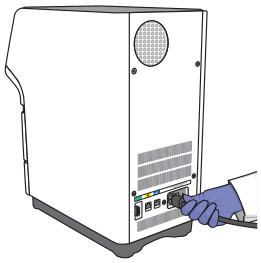
WARNING **FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the same type and rating as those currently in the instrument.

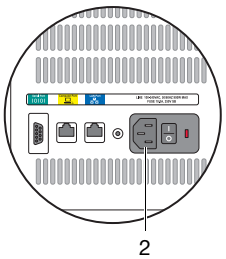
DANGER **SHOCK HAZARD.** To protect yourself against shock hazards, use a properly wired three-terminal outlet. Do not use an adapter to a two-terminal outlet since this does not provide positive ground protection.

Replace the Fuses

DANGER **ELECTRICAL SHOCK HAZARD.** Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 min before working on the instrument.

1. Power off the instrument.
2. Disconnect the power cord from the back of the instrument.





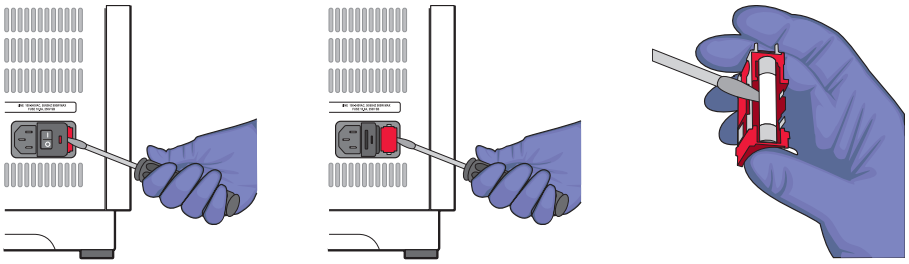
Notes

3. Remove the power entry module and fuses from the power entry module:

   a. Insert a small flat-tip screwdriver into one of two slots in the right side of the power entry module containing the fuses, and pry open the door.

   b. Insert the screwdriver into either side slot on the fuse compartment and eject the power entry module.

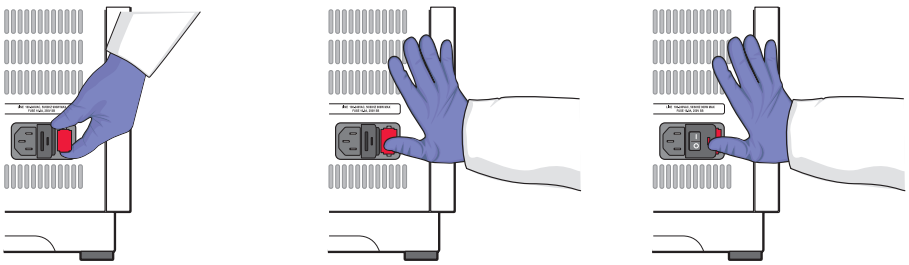
   c. Replace the burnt fuse(s) in the power entry module with a new 10 A fast-acting 250 VAC fuse.



4. Replace the power entry module:

   a. Lightly squeeze both mounted fuses between your thumb and forefinger and insert the power entry module into the fuse compartment until it is fully seated.

   b. Press the power entry module door until it locks in place.



5. Connect the power cord to the back of the instrument.

6. Power on the instrument, then wait for it to perform a diagnostic of all major system components. If the diagnostic is successful, the instrument displays the main screen.

   If the instrument detects a problem, it displays an error code for the related failed test. Repeat the procedure above without replacing the fuses to reseat the fuse holder. If the problem persists, contact Support as explained in “How to Obtain Support” on page 14.

Notes

Ship the Instrument for Service

If the instrument requires the attention of a service representative, decontaminate the instrument and package it for transportation to Thermo Fisher Scientific.

Materials Required

MicroAmp® Optical Reaction Plate

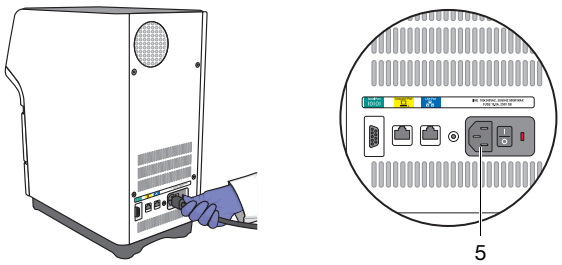
Prepare for Shipment

1. Contact the Customer Care Center (see "How to Obtain Support" on page 14) and request a Certificate of Decontamination.
2. Decontaminate the instrument according to the guidelines in the Certificate of Decontamination.
3. Sign the completed Certificate of Decontamination and fax or e-mail it to the Customer Care Center.

   IMPORTANT! Do not dispose the completed Certificate of Decontamination. You must include a copy in the package containing the instrument.

4. Raise the sample block(s) to secure it for transport:
   a. Load the empty reaction plate into the instrument.

   b. Touch the instrument touchscreen to awaken it, then touch  .
   c. In the Main Menu, touch **Tools Menu**, touch **Ship Prep**, then touch **Ship Prep**.
   d. Wait for the instrument to raise the sample block(s), then power off the instrument when prompted.
5. Disconnect the power cord from the back of the instrument.



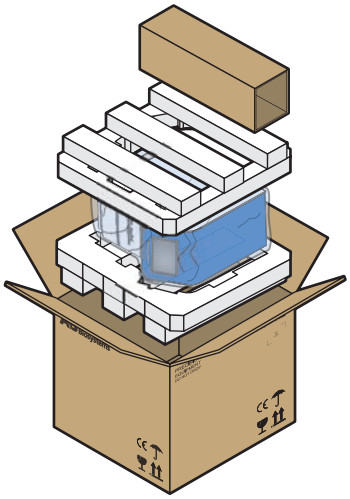
Notes

6. Using the packaging material provided by Thermo Fisher Scientific, create a package to ship the instrument that includes the:
   - StepOne™ or StepOnePlus™ instrument
   - Certificate of Decontamination (from step 3b on page 152)

     IMPORTANT! Shipping an instrument swithout a completed Certificate of Decontamination will delay service of the instrument.

   - Payment for the service in one of the following forms:
     - Purchase order, *or*
     - Company letterhead with the words “verbal purchase order,” *or*
     - Visa or MasterCard credit card information
   - Your address and contact information, including:
     - Billing address
     - Return shipping address
     - Name and phone number of a contact

   IMPORTANT! Do not include accessories or cords in the package.



7. Affix the postage provided to the package.
8. Inform the courier to arrange pickup, then ship the package to the location specified by the Customer Care Center.

Install the Serviced Instrument

When you receive the serviced instrument, install it as you would a new instrument by completing Chapters 1 through 5 of this guide.

Notes

Update the StepOne™ Software or the Operating System

Visit the StepOne™ and StepOnePlus™ System Website

For the latest services and support information for the StepOne™ and StepOnePlus™ systems, go to:

info.appliedbiosystems.com/stepone

The website contains the latest software and documentation updates for the instrument and analysis software.

At the system support page, you can:

- Submit a question directly to Technical Support
- Order user documents, SDSs, certificates of analysis, and other related documents
- Download documents in Adobe portable document format (PDF)
- Obtain information about customer training
- Download software updates and patches for the instrument

Update the StepOne™ Software

Prepare for the Update

If you want to update the StepOne™ Software, prepare your computer by exporting the application libraries and backing up your experiment files.

1. Back up the application libraries:
   a. In the main menu of the StepOne™ software, select **Tools** ▸ **<*desired library*>**.
   b. When the library dialog box opens, select the element(s) that you want to export, then click **Export**.
   c. In the Export dialog box, click **Save** to archive the selected records.
   d. Repeat steps 1 through 3 for the remaining libraries to archive them.
2. Back up all experiment files by creating a copy of the directory that you are using to store files.

   The default directory for StepOne or StepOnePlus™ experiments is:

   D:\Applied Biosystems\<*software name*>\experiments

   where <*software name*> is the current version of the StepOne software.
3. Back up the instrument data:
   a. Connect a USB drive to the USB port.

Notes

b. Touch the instrument touchscreen to awaken it, then touch  .

c. In the Main Menu, touch **Settings Menu**, then touch **Admin Menu**, touch **Admin Menu**, then touch **Back Up Experiments and Settings**.

d. Touch **Back Up**, then wait for the instrument to transfer the data.

e. When the transfer is complete, touch **Done**, then remove the USB drive.

Check for Updates

1. Go to:

   http://info.appliedbiosystems.com/stepone

   to open the StepOne and StepOnePlus system product page.

2. Under Software Download, view the available downloads.

3. If there are updates and/or patches for v2.1 or later, select the appropriate option, then follow the prompts to begin the download.

   You may be asked for your instrument serial number. The serial number is on the back panel of your instrument, or can be obtained from the instrument touchscreen.

Determine the Compatibility of an Operating System Upgrade or Update

Do not upgrade or update the Microsoft Windows® operating system of the computer that runs the StepOne™ software without first consulting the software release notes or the Thermo Fisher Scientific website. Future versions of the Windows® operating system and updates to the current operating system may conflict with the StepOne™ software and render the software inoperable.

1. From the desktop, browse to and open:

   <*drive*>:\Applied Biosystems\<*software name*>

   where:

   – <*drive*> is the computer hard drive on which the StepOne software is installed. The default installation drive for the software is the D drive.

   – <*software name*> is the current version of the StepOne software.

2. Double-click **release-notes.html**, then read the *StepOne™ Software Release Notes* for the compatibility of interest.

3. If the release notes do not mention the compatibility:

   a. Go to:

   info.appliedbiosystems.com/stepone

   b. Search the system website for the compatibility of interest.

4. If the website does not contain the information of interest, contact Support for the information (see “How to Obtain Support” on page 14).

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How to Troubleshoot the Installation

This appendix covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within the Applied Biosystems StepOne™ Real-Time PCR System Software by pressing **F1**, or clicking in the toolbar, or selecting **Help ▸ StepOne Software Help**.

Notes

StepOne™ Software Problems

If you encounter errors during the installation of the StepOne™ software, uninstall the software before reinstalling it.

Uninstall the StepOne™ Software

1. Log onto the operating system as Administrator, or as a member of the Administrators user group.

   IMPORTANT! If you are installing the StepOne™ software, you must log onto the Windows® operating system using a user account that belongs to the Administrators user group.

2. Remove the StepOne™ software:
   a. In the desktop, select **Start** ▸ **Control Panel** ▸ **Add/Remove Programs**.
   b. In the Add/Remove Programs dialog box, select **StepOne™** software, then click **Change/Remove**.
   c. In the Welcome page of the InstallShield Wizard dialog box, select **Remove**.
   d. In the Confirm Uninstall dialog box, click **OK**.
   e. When the uninstallation is complete, then click **Finish**.
   f. Close the Add/Remove Programs dialog box.
3. Restart the computer and log in as Administrator, or as a member of the Administrators user group.
4. Reinstall the StepOne™ software.

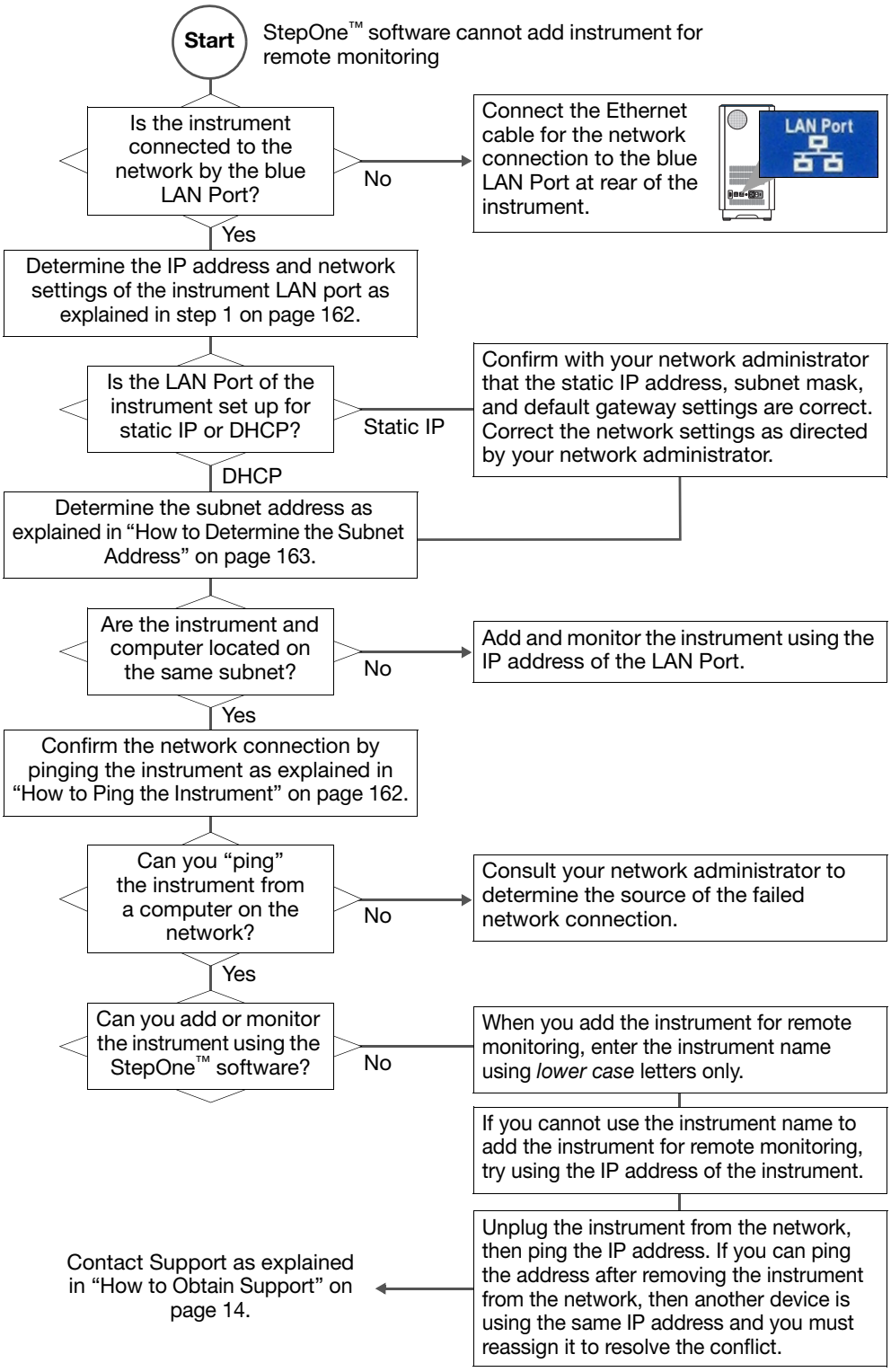
   If the StepOne™ software installation continues to fail, reimage the computer and repeat the installation.

Notes

Network Connection Problems

Use this topic to resolve problems when setting up the instrument for remote monitoring.

Troubleshooting Workflow

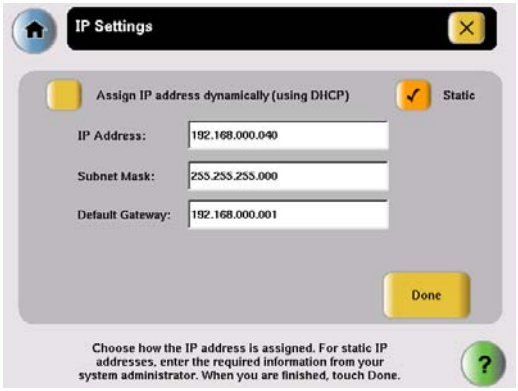


Notes

How to Ping the Instrument

You can confirm the connection between a network computer and instrument by pinging the instrument name or IP address from the computer. The ping command issued from the DOS command shell causes the computer to send a series of 32-byte data packets to the instrument. If the network connection between the computer and the instrument is unobstructed, the instrument sends data packets in reply.

1. Determine the IP address of the instrument:
   a. Touch the instrument touchscreen to awaken it, then touch  .
   b. In the Main Menu, touch **Settings Menu** ▸ **Admin Menu** ▸ **Set IP Address**.
   c. Record the IP address in the IP Address field.



2. Ping the instrument:
   a. Power on the instrument and wait for the instrument to boot.
   b. In the computer desktop, select **Start** ▸ **Programs** ▸ **Accessories** ▸ **Command Prompt**.
   c. In the Command Prompt window, enter **`ping`** `<IP address>`, then press **Enter**.

If the DOS prompt…	Then…
replies to the pings	the computer can communicate with the IP address.
displays “request timed out”	Wait a few minutes for the network to update the IP address of the instrument, then ping the instrument again. If you still cannot ping the instrument after two attempts, consult your network administrator to determine the source of the failed network connection.

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Resolving an IP Address Conflict

If you suspect that the IP address used by either port of the instrument may conflict with another device on your network, you can use the following procedure to test for the conflict.

1. Unplug the instrument from the network.
2. Ping the IP address of the instrument LAN port as explained above.

   If you can ping the IP address, then another device on the network is already using the address and you must reassign the IP address of the instrument to resolve the conflict.

How to Determine the Subnet Address

1. Touch the instrument touchscreen to awaken it, then touch  .
2. In the Main Menu, touch **Settings Menu ▸ Admin Menu ▸ Set IP Address**.
3. Record the IP address in the IP Address field. The first three numbers of the IP address are the subnet address.

   For example, the *italicized numbers* in the following IP address are the subnet address:

   192.168.100.140

If the subnet address for the instrument is different than that of the computer used for remote monitoring, then you cannot use the instrument name to add the instrument for remote monitoring. The mDNS limitation explained in “Networking Guidelines and Best Practices” on page 105 restricts the use of the instrument name as an identifier to network devices on the same subnet.

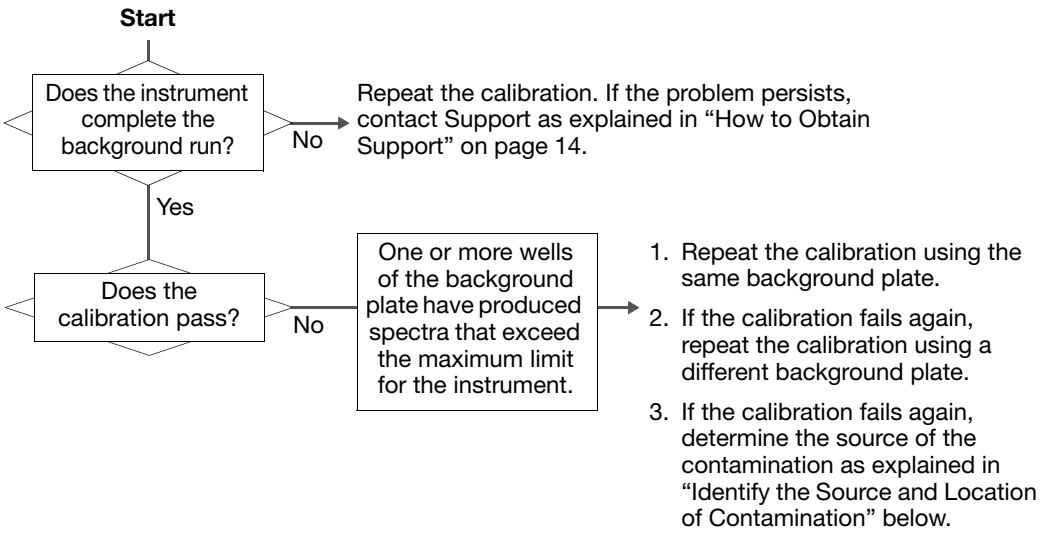
Note: To determine the subnet address of the computer that you want to use to monitor the instrument, select **Start ▸ Help and Support** in the computer desktop, then use the Microsoft Windows Help System search for the procedure appropriate for the operating system.

Notes

Background Calibration Failure

Use this topic to troubleshoot failed background calibrations.

Troubleshooting Workflow



Identify the Source and Location of Contamination

Signals that exceed the limit of normal background fluorescence may indicate the presence of fluorescent contamination on the calibration plate or the sample block(s). Common contaminants include: ink residue from permanent pens, powder from disposable gloves, and dust.

To determine the source and location of the contamination:

1. While viewing the raw spectra, locate the contaminated well position(s) by selecting successively smaller regions of the plate layout.
2. Rotate the background plate 180°, then perform the background calibration again.
3. Repeat step 1 to locate the contamination. If the position(s) of the contamination in steps 1 and 3 are:
   - **Identical** – The sample block(s) are contaminated. Decontaminate the sample block(s) as explained on page 145.
   - **Reversed** – The background plate is contaminated. Discard the background plate and perform the background run using a new background plate.

If the calibration fails after you have replaced the background plate and decontaminated the sample block(s), perform the following test:

1. Load a plate covered by a piece of black paper into the instrument.
2. Perform the background run as explained in "Perform a Background Calibration" on page 122.
3. After the run is complete, select all wells of the plate layout.

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4. View the Spectral plot for the peak(s). If the peak is:
   - **Visible** – The optics of your instrument may be contaminated. Contact Support as explained in "How to Obtain Support" on page 14.
   - **Absent** – The sample block(s) are contaminated. Decontaminate the sample block(s) as explained in "Decontaminate the Sample Block(s)" on page 145.

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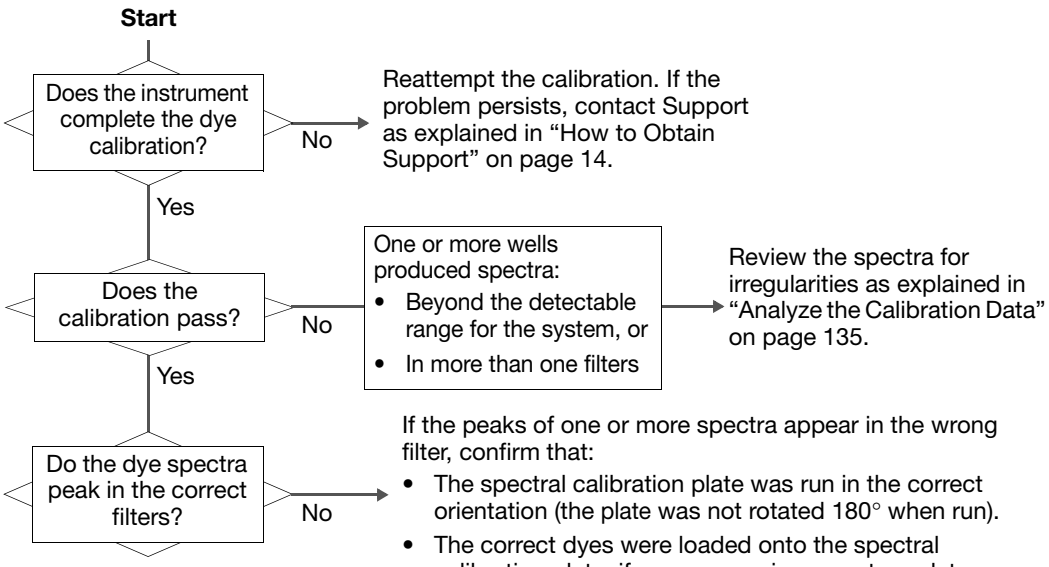
Dye Calibration Failure

Use this topic to troubleshoot failed dye calibrations.

Troubleshooting Workflow



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Common Irregularities

The following table displays irregular spectra common to dye calibrations.

Spectra	Possible Cause	Action
One or more raw spectra are at or below the detectable threshold for the calibration.	• The spectral calibration plate has been centrifuged insufficiently. • The spectral calibration plate contains old or insufficient reagents. • If you are running a custom spectral calibration plate, the dye may not be present at a sufficient concentration.	1. Unload the instrument and view the wells of the spectral calibration plate. If the liquid in the wells are not: – At the bottom of the wells, centrifuge the plate for a longer time, then repeat the calibration. – Equivalent in volume, the plate is not sealed and the reagents have evaporated. Discard it and run another. 2. If the spectral calibration plate appears to be normal, discard the plate and run another. 3. If the problem persists, contact Support as explained in "How to Obtain Support" on page 14. **Note:** If you are running a custom spectral calibration plate, create another plate but increase the concentration of the dye that produced insufficient signal.
One or more raw spectra exceed the maximum limit for the instrument.	• Fluorescent contamination is present on the sample block(s) or spectral calibration plate. • If you are running a custom spectral calibration plate, the dye may be too concentrated.	Confirm that the presence of contamination by performing a background calibration as explained in "Perform a Background Calibration" on page 122. If the background calibration does not show sample block contamination, the spectral calibration plate may be contaminated. **Note:** If you are running a custom spectral calibration plate, create another plate but decrease the concentration of the dye that exceeds the detectable limit.
The spectra contain peaks in more than one filter.	Fluorescent contamination is present on the sample block(s) or spectral calibration plate.	

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Glossary

Advanced Setup — In the StepOne™ software, a feature that allows you to set up your experiment according to your experiment design. Advanced Setup provides you with maximum flexibility in the design and setup of your experiment.

AIF — See assay information file (AIF).

allele — For a given target, any of the different sequences that occurs in the population.

allelic discrimination plot — Display of data collected during the post-PCR read. The allelic discrimination plot is a graph of the normalized reporter signal from the allele 1 probe plotted against the normalized reporter signal from the allele 2 probe.

amplicon — A segment of DNA amplified during PCR.

amplification — Part of the instrument run in which PCR produces amplification of the target. For quantitation experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data are used to calculate results. For genotyping or presence/absence experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data can be used for troubleshooting.

amplification efficiency (EFF%) — Calculation of efficiency of the PCR amplification. The amplification efficiency is calculated using the slope of the regression line in the standard curve. A slope close to −3.32 indicates optimal, 100% PCR amplification efficiency. Factors that affect amplification efficiency:

- **Range of standard quantities** – To increase the accuracy and precision of the efficiency measurement, use a broad range of standard quantities, 5 to 6 logs (10^5 to 10^6 fold).
- **Number of standard replicates** – To increase the precision of the standard quantities and decrease the effects of pipetting inaccuracies, include replicates.
- **PCR inhibitors** – PCR inhibitors in the reaction can reduce amplification and alter measurements of the efficiency.

amplification plot — Display of data collected during the cycling stage of PCR amplification. Can be viewed as:

- Baseline-corrected normalized reporter (ΔRn) vs. cycle
- Normalized reporter (Rn) vs. cycle
- Threshold cycle (C_T) vs. well

amplification stage Part of the instrument run in which PCR produces amplification of the target. The amplification stage is called a cycling stage in the thermal profile and consists of denaturing, primer annealing, and polymerization steps that are repeated.

For quantitation experiments, fluorescence data collected during the amplification stage are displayed in an amplification plot, and the data are used to calculate results. For genotyping or presence/absence experiments, fluorescence data collected during the amplification stage are displayed in an amplification plot, and the data can be used for troubleshooting. See also cycling stage.

assay In the StepOne™ and StepOnePlus™ systems, a PCR reaction mix that contains primers to amplify a target and a reagent to detect the amplified target.

Assay ID Identifier assigned by Thermo Fisher Scientific to TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays.

assay information file (AIF) Data file on a CD shipped with each assay order. The file name includes the number from the barcode on the plate. The information in the AIF is provided in a tab-delimited format.

assay mix PCR reaction component in Thermo Fisher Scientific TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays. The assay mix contains primers designed to amplify a target and a TaqMan® probe designed to detect amplification of the target.

AutoDelta In the run method, a setting to increase or decrease the temperature and/or time for a step with each subsequent cycle in a cycling stage. When AutoDelta is enabled for a cycling stage, the settings are indicated by an icon in the thermal profile:

- AutoDelta on: ▲
- AutoDelta off: ▲

automatic baseline An analysis setting in which the software calculates the baseline start and end values for the amplification plot. You can apply the automatic baseline setting to specific wells in the reaction plate. See also baseline.

automatic C_T An analysis setting in which the software calculates the baseline start and end values and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle (C_T). See also threshold cycle (CT).

baseline In the amplification plot, a line fit to the fluorescence levels during the initial stages of PCR, when there is little change in fluorescence signal.

baseline-corrected normalized reporter (ΔRn)

The magnitude of normalized fluorescence signal generated by the reporter:

1. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. In the ΔRn vs. Cycle amplification plot, ΔRn is calculated at each cycle as:

   ΔRn (cycle) = Rn (cycle) − Rn (baseline), where Rn = normalized reporter

2. In genotyping experiments and presence/absence experiments, the difference in normalized fluorescence signal generated by the reporter between the pre-PCR read and the post-PCR read. In the allelic discrimination plot (genotyping experiments) and the presence/absence plot (presence/absence experiments), ΔRn is calculated as:

   ΔRn = Rn (post-PCR read) − Rn (pre-PCR read), where Rn = normalized reporter

See also normalized reporter (Rn).

biological replicate

Reactions that contain identical components and volumes, but evaluate separate samples of the same biological source (for example, samples from three different mice of the same strain, or separate extractions of the same cell line or tissue sample).

When using biological replicate groups in a comparative C_T study, the values displayed in the Biological Replicates tab are calculated by combining the results of the separate biological samples and treating this collection as a single population (that is, as one sample). For ΔC_T computations (normalizing by the endogenous control) in a singleplex experiment, the separate biological samples are treated as unpaired data when computing variability estimates of the single biological replicate. You can observe individual contributions of the separate biological samples to the single biological replicate results in the Technical Replicates tab.

Note: To view the Biological Replicates and Technical Replicates tabs, from the Study Menu pane, select **Analysis** ▸ **Gene Expression**.

blocked IPC

In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the StepOne™ software, the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells.

calibrator

See reference sample.

chemistry

See reagents.

colocated layout

A system layout in which the StepOne™ or StepOnePlus™ instrument is directly connected to a colocated computer by the yellow cable. In this layout, you can control the instrument with the StepOne™ software on the colocated computer or with the instrument touchscreen.

comparative C_T ($\Delta\Delta C_T$) method

Method for determining relative target quantity in samples. With the comparative C_T ($\Delta\Delta C_T$) method, the StepOne™ software measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. The software determines the relative quantity of target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample.

C_T

See threshold cycle (CT).

custom dye

Dye that is not supplied by Thermo Fisher Scientific. Custom dyes may be adapted for use in experiments on the StepOne™ and StepOnePlus™ systems. When using custom dyes, the custom dye should be added to the Dye Library and a custom dye calibration performed.

IMPORTANT! We do not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus™ system.

cycle threshold

See threshold cycle (CT).

cycling stage

In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. For cycling stages, you can enable AutoDelta settings. See also amplification stage.

data collection

A process during the instrument run in which an instrument component detects fluorescence data from each well of the reaction plate. The instrument transforms the signal to electronic data, and the data are saved in the experiment file. In the StepOne™ software, a data collection point is indicated by an icon in the thermal profile:

- Data collection on: 
- Data collection off: 

delta Rn (ΔRn)

See baseline-corrected normalized reporter (DRn).

derivative reporter (–Rn′)

The negative first-derivative of the normalized fluorescence generated by the reporter during PCR amplification. In the derivative reporter (–Rn′) vs. temperature melt curve, the derivative reporter signal is displayed in the y-axis.

Design Wizard

A feature in the StepOne™ software that helps you set up your experiment by guiding you through best practices as you enter your experiment design.

diluent

A reagent used to dilute a sample or standard before adding it to the PCR reaction. The diluent can be water or buffer.

Diluted Sample Concentration (10× for Reaction Mix)

In the StepOne™ software, a field displayed on the Sample Dilution Calculations tab of the Reaction Setup screen. For this field, enter the sample concentration you want to use to add to the reaction mix for all samples in the experiment. “10× for Reaction Mix” indicates that the software assumes the sample or standard component of the reaction mix is at a 10× concentration. For example, if the diluted sample concentration is 50.0 ng/µL (10×), the final sample concentration in the reaction is 5 ng/µL (1×).

dilution factor See serial factor.

dissociation curve See melt curve.

EFF% See amplification efficiency (EFF%).

endogenous control A target or gene that should be expressed at similar levels in all samples you are testing. Endogenous controls are used in relative standard curve and comparative C_T ($\Delta\Delta C_T$) experiments to normalize fluorescence signals for the target you are quantifying. Housekeeping genes can be used as endogenous controls. See also housekeeping gene.

When using multiple endogenous controls, the software treats all endogenous controls as a single population, and calculates the experiment-appropriate mean to establish a single value against which the target of interest is normalized. In comparative C_T experiments, the mean calculated is the arithmetic mean of the C_T values. In relative standard curve experiments, the C_T values are converted to relative quantities prior to normalization; the mean calculated is subsequently the geometric mean of the relative quantities.

Note: Arithmetic and geometric means are related and equivalent due to logarithmic transformation of the data.

Variability estimates for multiple endogenous controls are computed separately. The final variability estimate is a pooled combination of the individual variability estimates (similar to computing pooled standard deviations).

endpoint read See post-PCR read.

experiment Refers to the entire process of performing a run using the StepOne™ or StepOnePlus™ systems, including setup, run, and analysis. The types of experiments you can perform using the StepOne™ and StepOnePlus™ systems:

- Quantitation - standard curve
- Quantitation - relative standard curve
- Quantitation - comparative C_T ($\Delta\Delta C_T$)
- Melt curve
- Genotyping
- Presence/absence

experiment name Entered during experiment setup, the name that is used to identify the experiment. Experiment names cannot exceed 100 characters and cannot include any of the following characters: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (|), colon (:), semicolon (;), and sign (&), percent sign (%), dollar sign ($), at sign (@), circumflex (^), left parenthesis (( ), right parenthesis ( )), or exclamation point (!).

IMPORTANT! If you run the instrument in standalone mode from the instrument touchscreen, you cannot enter more than 32 characters in the Experiment Name field and you cannot include spaces in the name.

experiment type The type of experiment you are performing using the StepOne™ or StepOnePlus™ system:

- Standard curve
- Comparative C_T ($\Delta\Delta C_T$)
- Relative standard curve
- Melt curve (not available in the Design Wizard)
- Genotyping
- Presence/absence

The experiment type you select affects the setup, run, and analysis.

forward primer Oligonucleotide that flanks the 5′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

holding stage In the thermal profile, a stage that includes one or more steps. You can add a holding stage to the thermal profile to activate enzymes, to inactivate enzymes, or to incubate a reaction.

housekeeping gene A gene that is involved in basic cellular functions and is constitutively expressed. Housekeeping genes can be used as endogenous controls. See also endogenous control.

internal positive control (IPC) In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. You can use the IPC to distinguish between true negative results (that is, the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.

inventoried assays TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays that have been previously manufactured, passed quality control specifications, and stored in inventory.

IPC In presence/absence experiments, abbreviation for internal positive control (IPC). In the StepOne™ software, the task for the IPC target in wells that contain the IPC and do not contain IPC blocking agent. See also internal positive control (IPC).

IPC blocking agent Reagent added to PCR reactions to block amplification of the internal positive control (IPC).

IPC+ See negative control-IPC wells.

made-to-order assays TaqMan® Gene Expression Assays or TaqMan® SNP Genotyping Assays that are manufactured at the time of order. Only assays that pass manufacturing quality control specifications are shipped.

manual baseline An analysis setting in which you enter the baseline start and end values for the amplification plot. You can apply the manual baseline setting to specific wells in the reaction plate.

manual C_T An analysis setting in which you enter the threshold value and select whether to use automatic baseline or manual baseline values. The software uses the baseline and the threshold values to calculate the threshold cycle (C_T).

melt curve	A plot of data collected during the melt curve stage. Peaks in the melt curve can indicate the melting temperature (Tm) of the target or can identify nonspecific PCR amplification. In the StepOne™ software, you can view the melt curve as normalized reporter (Rn) vs. temperature or as derivative reporter (−Rn′) vs. temperature. Also called dissociation curve.
melt curve stage	In the thermal profile, a stage with a temperature increment to generate a melt curve.
melting temperature (Tm)	In melt curve experiments, the temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. The Tm is displayed in the melt curve.
multicomponent plot	A plot of the complete spectral contribution of each dye for the selected well(s) over the duration of the PCR run.
negative control (NC)	In the StepOne™ software, the task for targets or SNP assays in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Previously called no template control (NTC).
negative control-blocked IPC wells	In presence/absence experiments, wells that contain IPC blocking agent instead of sample in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample and amplification of the IPC is blocked. Previously called no amplification control (NAC).
negative control-IPC wells	In presence/absence experiments, wells that contain IPC template and buffer or water instead of sample. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample. Previously called IPC+.
no amplification control (NAC)	See negative control-blocked IPC wells.
no template control (NTC)	See negative control (NC).
nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3′ end of TaqMan® probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence signal. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantitation. The minor groove binder (MGB) increases the melting temperature (Tm) without increasing probe length. It also allows the design of shorter probes.
normalized quantity	Quantity of target divided by the quantity of endogenous control.
normalized reporter (Rn)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.
omit well	An action that you perform before reanalysis to omit one or more wells from analysis. Because no algorithms are applied to omitted wells, omitted wells contain no results.
outlier	For a set of data, a datapoint that is significantly smaller or larger than the others.

passive reference A dye that produces fluorescence signal. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in concentrations or volume. Normalization to the passive reference signal allows for high data precision.

plate layout An illustration of the grid of wells and assigned content in the reaction plate. In StepOne™ systems, the grid contains 6 rows and 8 columns. In StepOnePlus™ systems, the grid contains 8 rows and 12 columns.

In the StepOne™ software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.

point One standard in a standard curve. The standard quantity for each point in the standard curve is calculated based on the starting quantity and serial factor.

positive control In genotyping experiments, a DNA sample with a known genotype, homozygous or heterozygous. In the StepOne™ software, the task for the SNP assay in wells that contain a sample with a known genotype.

post-PCR read Used in genotyping and presence/absence experiments, the part of the instrument run that occurs after amplification. In genotyping experiments, fluorescence data collected during the post-PCR read are displayed in the allelic discrimination plot and used to make allele calls. In presence/absence experiments, fluorescence data collected during the post-PCR read are displayed in the presence/absence plot and used to make detection calls. Also called endpoint read.

pre-PCR read Used in genotyping and presence/absence experiments, the part of the instrument run that occurs before amplification. The pre-PCR read is optional but recommended. Fluorescence data collected during the pre-PCR read can be used to normalize fluorescence data collected during the post-PCR read.

primer mix PCR reaction component that contains the forward primer and reverse primer designed to amplify the target.

primer/probe mix PCR reaction component that contains the primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.

pure dye See custom dye and system dye.

quantitation method In quantitation experiments, the method used to determine the quantity of target in the samples. In StepOne™ and StepOnePlus™ systems, there are three types of quantitation methods: standard curve, relative standard curve, and comparative C_T ($\Delta\Delta C_T$).

quantity In quantitation experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.

quencher A molecule attached to the 3′ end of TaqMan® probes to prevent the reporter from emitting fluorescence signal while the probe is intact. With TaqMan® reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher. With SYBR® Green reagents, no quencher is used.

IMPORTANT! We do not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus™ system.

QuickStart A feature in StepOne™ and StepOnePlus™ systems that allows you to run an experiment without entering plate setup information. QuickStart requires a colocated layout with the instrument powered on and an intact instrument-computer connection.

R^2 value Regression coefficient calculated from the regression line in the standard curve. The R^2 value indicates the closeness of fit between the standard curve regression line and the individual C_T data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.

ramp The rate at which the temperature changes during the instrument run. Except for the melt curve step, the ramp is defined as a percentage. For the melt curve step, the ramp is defined as a temperature increment. In the graphical view of the thermal profile, the ramp is indicated by a diagonal line.

ramp speed Speed at which the temperature ramp occurs during the instrument run. Available ramp speeds include fast and standard.

- For optimal results using the fast ramp speed, we recommend using fast reagents in your PCR reactions.
- For optimal results using the standard ramp speed, we recommend using standard reagents in your PCR reactions.

IMPORTANT! Fast reagents are not supported for presence/absence experiments.

raw data plot A plot of raw fluorescence signal (not normalized) for each optical filter.

reaction mix A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control).

reagents The PCR reaction components you are using to amplify the target and to detect amplification. Types of reagents used on the StepOne™ and StepOnePlus™ systems:

- TaqMan® reagents
- SYBR® Green reagents
- Other reagents

real-time PCR Process of collecting fluorescence data during PCR. Data from the real-time PCR are used to calculate results for quantitation experiments or to troubleshoot results for genotyping or presence/absence experiments.

reference sample In relative standard curve and comparative C_T ($\Delta\Delta C_T$) experiments, the sample used as the basis for relative quantitation results. Also called the calibrator.

refSNP ID Identifies the reference SNP (refSNP) cluster ID. Generated by the Single Nucleotide Polymorphism Database of Nucleotide Sequence Variation (dbSNP) at the National Center for Biotechnology Information (NCBI). The refSNP ID can be used to search the Thermo Fisher Scientific Store for an Thermo Fisher Scientific SNP Genotyping Assay. Also called an rs number.

regression coefficients Values calculated from the regression line in standard curves, including the R^2 value, slope, and y-intercept. You can use the regression coefficients to evaluate the quality of results from the standards. See also standard curve.

regression line In standard curve and relative standard curve experiments, the best-fit line from the standard curve. Regression line formula:

$C_T = m [\log (Qty)] + b$

where m is the slope, b is the y-intercept, and Qty is the standard quantity.

See also regression coefficients.

reject well An action that the software performs during analysis to remove one or more wells from further analysis if a specific flag is applied to the well. Rejected wells contain results calculated up to the point of rejection.

relative standard curve method Method for determining relative target quantity in samples. With the relative standard curve method, the StepOne™ software measures amplification of the target and of the endogenous control in samples, in a reference sample, and in a standard dilution series. Measurements are normalized using the endogenous control. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates target quantity in the samples and in the reference sample. The software determines the relative quantity of target in each sample by comparing target quantity in each sample to target quantity in the reference sample.

Remote Monitor A feature in the StepOne™ software that allows you to monitor a StepOne™ or StepOnePlus™ instrument over the network. With the Remote Monitor, you can monitor the instrument status, send an experiment to the instrument, monitor amplification plots and temperature plots in real time, and download the results to your computer. You cannot operate the StepOne™ or StepOnePlus™ instrument using the Remote Monitor.

replicate group A set of identical reactions in an experiment.

replicates Total number of identical reactions containing identical components and identical volumes.

reporter Fluorescent dye used to detect amplification. If you are using TaqMan® reagents, the reporter dye is attached to the 5′ end. If you are using SYBR® Green reagents, the reporter dye is SYBR® Green dye.

reverse primer An oligonucleotide that flanks the 3′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

reverse transcriptase	An enzyme that converts RNA to cDNA. Reverse transcriptase is added to the PCR reaction to perform 1-step RT-PCR.
Rn	See normalized reporter (Rn).
ROX™ dye	A dye supplied by Thermo Fisher Scientific and precalibrated on the StepOne™ and StepOnePlus™ systems. ROX dye is used as the passive reference.
rs number	See refSNP ID.
run method	Definition of the reaction volume and the thermal profile for the StepOne™ or StepOnePlus™ instrument run.
sample	The template that you are testing.
Sample DNA (10×)	In the StepOne™ software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample DNA is added to the reaction mix at a 10× concentration. For example, if the reaction volume is 20 µL, the calculated volume of sample for 1 reaction is 2 µL.
Sample Library	In the StepOne™ software, a collection of samples. The Sample Library contains the sample name and the sample color.
Sample or Standard (10×)	In the StepOne™ software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample or standard is added to the reaction mix at a 10× concentration. For example, if the reaction volume is 20 µL, the calculated volume of sample or standard for 1 reaction is 2 µL.
sample/SNP assay reaction	In genotyping experiments, the combination of which sample to test and which SNP assay to perform in one PCR reaction. Each PCR reaction can contain only one sample and one SNP assay.
sample/target reaction	In quantitation experiments, the combination of which sample to test and which target to detect and quantify in one PCR reaction. In the Design Wizard, you can detect and quantify only one target in one PCR reaction. Use Advanced Setup to detect and quantify more than one target in one PCR reaction.
serial factor	In the StepOne™ software, a numerical value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10×, the difference between any 2 adjacent points in the curve is 10-fold.
series	See standard dilution series.
slope	Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of −3.32 indicates 100% amplification efficiency. See also amplification efficiency (EFF%) and regression line.
SNP	Abbreviation for single nucleotide polymorphism. The SNP can consist of a base difference or an insertion or deletion of one base.

SNP assay Used in genotyping experiments, a PCR reaction that contains primers to amplify the SNP and two probes to detect different alleles.

SNP Assay Library In the StepOne™ software, a collection of SNP assays to add to genotyping experiments. The SNP assays in the library contain the SNP assay name, SNP assay color, and for each allele, the allele name or base(s), reporter, quencher, and allele colors. The SNP assays in the library may also contain the assay ID and comments about the SNP assay.

spatial calibration Type of StepOne™ and StepOnePlus™ system calibration in which the system maps the positions of the wells in the sample block(s). Spatial calibration data are used so that the software can associate increases in fluorescence during a run with specific wells in the reaction plate.

stage In the thermal profile, a group of one or more steps. There are three types of stages: holding stage (including pre-PCR read and post-PCR read), cycling stage (also called amplification stage), and melt curve stage.

standalone layout A system layout in which the StepOne™ or StepOnePlus™ instrument is *not* connected to a computer by the yellow cable. In this layout, you control the instrument only with the instrument touchscreen, and you use a USB drive or network connection to transfer data between the instrument and computer.

standard Sample that contains known standard quantities. Standard reactions are used in quantitation experiments to generate standard curves. See also standard curve and standard dilution series.

standard curve In standard curve and relative standard curve experiments:

- The best-fit line in a plot of the C_T values from the standard reactions plotted against standard quantities. See also regression line.
- A set of standards containing a range of known quantities. Results from the standard curve reactions are used to generate the standard curve. The standard curve is defined by the number of points in the dilution series, the number of standard replicates, the starting quantity, and the serial factor. See also standard dilution series.

standard curve method Method for determining absolute target quantity in samples. With the standard curve method, the StepOne™ software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples. See also standard and standard curve.

standard dilution series In standard curve and relative standard curve experiments, a set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards. For example, the standard stock is used to prepare the first dilution point, the first dilution point is used to prepare the second dilution point, and so on. In the StepOne™ software, the volumes needed to prepare a standard dilution series are calculated by the number of dilution points, the number of standard replicates, the starting quantity, the serial factor, and the standard concentration in the stock. See also standard curve.

standard quantity A known quantity in the PCR reaction.

- In standard curve experiments, the quantity of target in the standard. In the StepOne™ software, the units for standard quantity can be for mass, copy number, viral load, or other units for measuring the quantity of target.
- In relative standard curve experiments, a known quantity in the standard. Standard quantity can refer to the quantity of cDNA or the quantity of standard stock in the PCR reaction. The units are not relevant for relative standard curve experiments because they cancel out in the calculations.

starting quantity When defining a standard curve in the StepOne™ software, corresponds to the highest or lowest quantity.

step A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt curve steps), hold temperature, hold time (duration), and you can turn data collection on or off for the ramp or the hold parts of the step. For cycling stages, a step is also defined by the AutoDelta status. With StepOnePlus™ systems, which contain the VeriFlex™ blocks, each step contains 6 temperatures (1 for each VeriFlex block).

study name Entered during study setup, the name that is used to identify the study. Study names cannot exceed 100 characters and cannot include any of the following characters: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (|), colon (:), semicolon (;), and sign (&), percent sign (%), dollar sign ($), at sign (@), circumflex (^), left parenthesis (( ), right parenthesis ( )), or exclamation point (!).

SYBR® Green reagents PCR reaction components that consist of two primers designed to amplify the target and SYBR® Green dye to detect double-stranded DNA.

system dye

Dye supplied by Thermo Fisher Scientific and precalibrated on the StepOne™ or StepOnePlus™ system. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Maintenance Manager.

System dyes on the StepOne™ system:

- FAM™ dye
- JOE™ dye
- ROX™ dye
- SYBR® Green dye
- VIC® dye

System dyes on the StepOnePlus™ system:

- FAM™ dye
- JOE™ dye
- NED™ dye
- ROX™ dye
- SYBR® Green dye
- TAMRA™ dye
- VIC® dye

IMPORTANT! We do not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus™ system.

TaqMan® reagents

PCR reaction components that consist of primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.

target

The nucleic acid sequence that you want to amplify and detect.

target color

In the StepOne™ software, a color assigned to a target to identify the target in the plate layout and analysis plots.

Target Library

In the StepOne™ software, a collection of targets to add to experiments. The targets in the library contain the target name, reporter, quencher, and target color. The target in the library may also contain comments about the target.

task

In the StepOne™ software, the type of reaction performed in the well for the target or SNP assay. Available tasks:

- Unknown
- Negative Control
- Standard (standard curve and relative standard curve experiments)
- Positive control (genotyping experiments)
- IPC (presence/absence experiments)
- Blocked IPC (presence/absence experiments)

technical replicate Reactions that contain identical components and volumes, and that evaluate the same sample.

temperature plot In the StepOne™ software, a display of temperatures for the sample, instrument cover, and instrument block during the StepOne™ or StepOnePlus™ instrument run.

template In the Design Wizard of the StepOne™ software (and in QuickStart for quantitation experiments), the type of nucleic acid to add to the PCR reaction. The recommended template varies according to experiment type:

- Quantitation experiments (standard curve, relative standard curve, and comparative C_T) – cDNA (complementary cDNA), RNA, or gDNA (genomic DNA)

  For quantitation experiments, the template type selection affects the run method, reaction setup, and materials list.
- Genotyping experiments – Wet DNA (gDNA or cDNA) or dry DNA (gDNA or cDNA)

  For genotyping experiments, the template type selection affects the reaction setup.
- Presence/absence experiments - DNA

  For presence/absence experiments, we recommend adding DNA templates to the PCR reactions.

thermal profile Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the StepOne™ or StepOnePlus™ instrument run.

threshold

1. In amplification plots, the level of fluorescence above the baseline and within the exponential growth region The threshold can be determined automatically (see automatic CT) or can be set manually (see manual CT).
2. In presence/absence experiments, the level of fluorescence above which the StepOne™ software assigns a presence call.

threshold cycle (C_T) The PCR cycle number at which the fluorescence meets the threshold in the amplification plot.

Tm See melting temperature (Tm).

touchscreen Instrument display that you touch to control the StepOne™ or StepOnePlus™ instrument.

unknown In the StepOne™ software, the task for the target or SNP assay in wells that contain the sample you are testing:

- In quantitation experiments, the task for the target in wells that contain a sample with unknown target quantities.
- In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype.
- In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known.

unknown-IPC wells In presence/absence experiments, wells that contain a sample and internal positive control (IPC).

VeriFlex™ Technology

The StepOnePlus™ instrument contains six independently thermally regulated VeriFlex™ blocks, creating up to six different zones for the 96 sample wells. After you enable the VeriFlex blocks in the StepOne™ software, you can set a different temperature for one or more of the VeriFlex blocks.

y-intercept

In the standard curve, the value of y where the regression line crosses the y-axis. The y-intercept indicates the expected threshold cycle (C_T) for a sample with quantity equal to 1.

zone

One of up to six sample temperatures among the 96 wells formed by independently thermally regulated VeriFlex™ blocks during the StepOnePlus™ instrument run. You can set a different temperature for one or more of the VeriFlex blocks, or you can set the same temperature for each of the VeriFlex blocks.

Note: For melt curve steps, you need to set the same temperature for each of the VeriFlex blocks.

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zone boundary

The edge of a zone for samples formed by the six independently thermally regulated VeriFlex™ blocks. In the StepOne™ software, the zone boundaries are displayed in the plate layout as thick red lines.

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