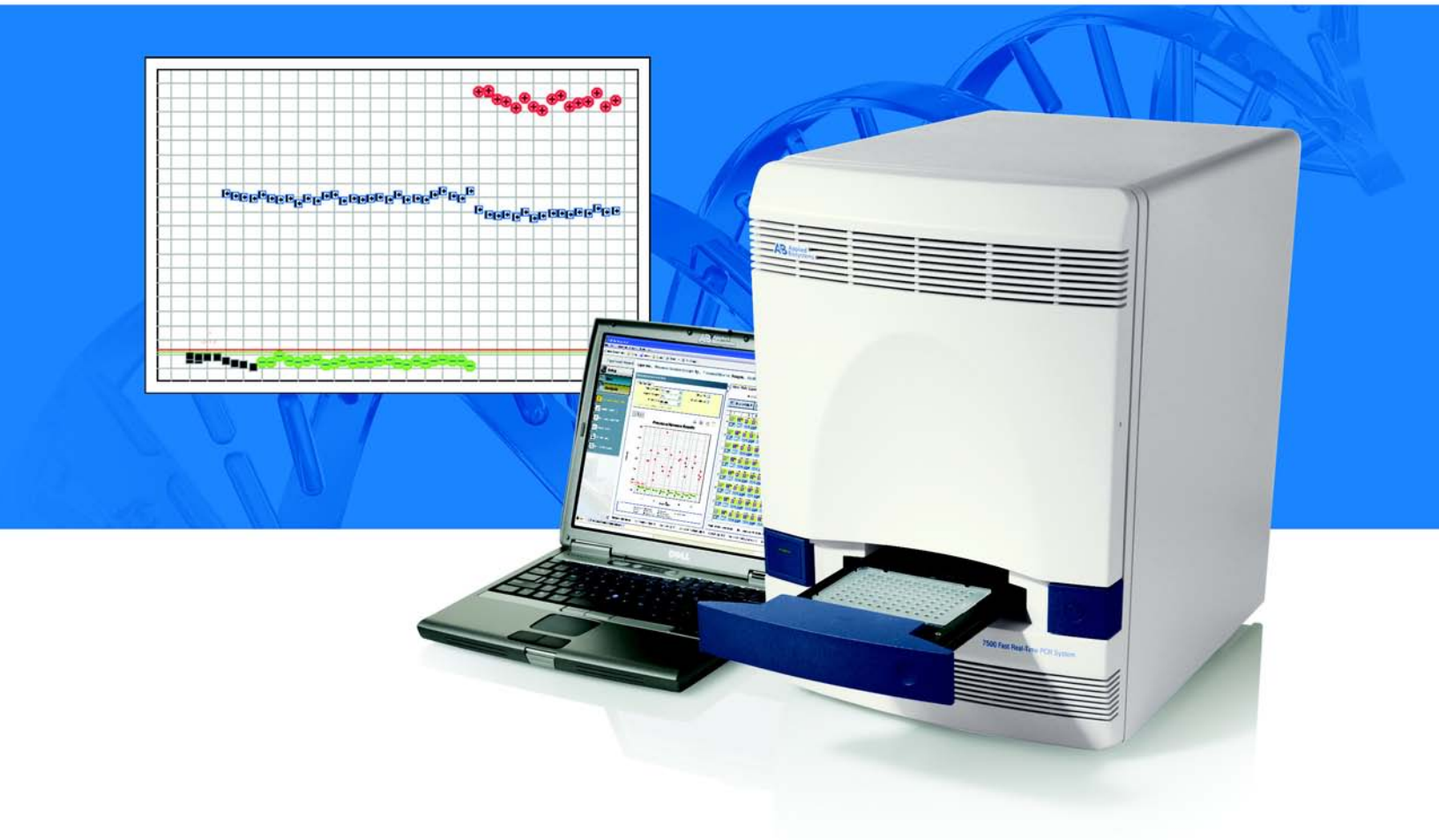
Applied Biosystems

Applied Biosystems 7500/7500 Fast

Real-Time PCR System

Presence/Absence Experiments



Applied Biosystems

Applied Biosystems 7500/7500 Fast

Real-Time PCR System

Presence/Absence Experiments

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Preface

How to Use This Guide

About the System Documentation

The guides listed below are shipped with the Applied Biosystems 7500/7500 Fast Real-Time PCR System (7500/7500 Fast system).

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments	Explains how to perform experiments on the 7500/7500 Fast system. Each Getting Started Guide functions as both a: • Tutorial, using example experiment data provided with the Applied Biosystems 7500/7500 Fast Real-Time PCR Software (7500 software). • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system.	4387784
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Presence/Absence Experiments		4387785
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments		4387783
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments		4387779
Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide	Explains how to maintain the 7500/7500 Fast system. Intended for laboratory staff responsible for the maintenance of the 7500/7500 Fast system.	4387777
Applied Biosystems 7500/7500 Fast Real-Time PCR System Computer Setup Guide		4387778
Applied Biosystems Real-Time PCR Systems Reagent Guide	Provides information about the reagents you can use on Applied Biosystems Real-Time PCR 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 7500/7500 Fast system.	4387787
Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide	Explains how to prepare your site to receive and install the 7500/7500 Fast system. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the 7500/7500 Fast system.	4387776

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 Help	Explains how to use the 7500 software to: • Set up, run, and analyze experiments using the 7500/7500 Fast system. • Calibrate a 7500/7500 Fast instrument. • Verify that the performance of a 7500/7500 Fast instrument with an RNase P run. Intended for: • Laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system. • Laboratory staff responsible for the installation and maintenance of the 7500/7500 Fast system.	NA

Assumptions

This guide assumes that you:

- Are familiar with the Microsoft Windows® operating system.
- Are familiar with the Internet and Internet browsers.
- Know how to handle DNA and/or RNA samples and prepare them for PCR.
- Understand data storage, file transfer, and copying and pasting.
- Have networking experience, if you plan to integrate the 7500/7500 Fast system into your existing laboratory data flow.

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 Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

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

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate reagent 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 xii.

How to Obtain More Information

Related Documentation

Documents Related to Genotyping Experiments

Document	PN
Allelic Discrimination Pre-Developed TaqMan® Assay Reagents Quick Reference Card	4312212
Custom TaqMan® Genomic Assays Protocol Submission Guidelines	4367671
Custom TaqMan® SNP Genotyping Assays Protocol	4334431
Ordering TaqMan® SNP Genotyping Assays Quick Reference Card	4374204
Performing a Custom TaqMan® SNP Genotyping Assay for 96-Well Plates Quick Reference Card	4371394
Performing a TaqMan® Drug Metabolism Genotyping Assay for 96-Well Plates Quick Reference Card	4367636
Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol	4312214
TaqMan® Drug Metabolism Genotyping Assays Protocol	4362038
TaqMan® SNP Genotyping Assays Protocol	4332856

Documents Related to Presence/Absence Experiments

Document	PN
DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol	4343586
NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue Protocol	4333959

Documents Related to Relative Standard Curve and Comparative C_T Experiments

Document	PN
Amplification Efficiency of TaqMan® Gene Expression Assays Application Note	127AP05
Applied Biosystems High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
Custom TaqMan® Gene Expression Assays Protocol	4334429
Primer Express® Software Version 3.0 Getting Started Guide	4362460
TaqMan® Gene Expression Assays Protocol	4333458
User Bulletin #2: Relative Quantitation of Gene Expression	4303859

Documents Related to Standard Curve Experiments

Document	PN
Amplification Efficiency of TaqMan® Gene Expression Assays Application Note	127AP05
Custom TaqMan® Gene Expression Assays Protocol	4334429
Primer Express® Software Version 3.0 Getting Started Guide	4362460
TaqMan® Gene Expression Assays Protocol	4333458
User Bulletin #2: Relative Quantitation of Gene Expression	4303859

Documents Related to the Reagent Guide

Document	PN
Applied Biosystems High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
Custom TaqMan® Gene Expression Assays Protocol	4334429
Custom TaqMan® Genomic Assays Protocol: Submission Guidelines	4367671
Custom TaqMan® SNP Genotyping Assays Protocol	4334431
Power SYBR® Green PCR Master Mix and RT-PCR Protocol	4367218
Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol	4312214
Primer Express® Software Version 3.0 Getting Started Guide	4362460
SYBR® Green PCR and RT-PCR Reagents Protocol	4304965
SYBR® Green PCR Master Mix and RT-PCR Reagents Protocol	4310251
TaqMan® Drug Metabolism Genotyping Assays Protocol	4362038
TaqMan® Exogenous Internal Positive Control Reagents Protocol	4308335
TaqMan® Fast Universal PCR Master Mix (2X) Protocol	4351891
TaqMan® Gene Expression Assays Protocol	4333458
TaqMan® Gene Expression Master Mix Protocol	4371135
TaqMan® Genotyping Master Mix Protocol	4371131
TaqMan® SNP Genotyping Assays Protocol	4332856
TaqMan® Universal PCR Master Mix Protocol	4304449
User Bulletin #2: Relative Quantitation of Gene Expression	4303859
Using TaqMan® Endogenous Control Assays to Select an Endogenous Control for Experimental Studies Application Note	127AP08

Note: For more documentation, see "How to Obtain Support" on page xi.

Obtaining Information from the Software Help

The 7500 Software Help describes how to use each feature of the user interface. Access the Help from within the software by doing one of the following:

- Press **F1**.
- Click in the toolbar.
- Select **Help ▸ 7500 Software Help**.

To find topics of interest in the Help:

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

Send Us Your Comments

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

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" on page xi).

How to Obtain Support

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

At the Support page, you can:

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

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

IMPORTANT! When directed to do so by this guide, or when you need to schedule maintenance for your 7500/7500 Fast instrument (such as annual planned maintenance or temperature verification/calibration), contact the Applied Biosystems Care Center. To obtain a phone number for or to send an e-mail to the center, go to **http://www.appliedbiosystems.com/support/contact**.

Safety Conventions Used in This Document

Safety Alert Words

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

Definitions

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

CAUTION – 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.

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

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

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

Examples

The following examples show the use of safety alert words:

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

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

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

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

Symbols on Instruments

Electrical Symbols on Instruments

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

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

Safety Symbols

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

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

Environmental Symbols on Instruments

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

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

Safety Labels on Instruments

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

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

Locations of Warnings

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

General Instrument Safety

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

Moving and Lifting the Instrument

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

Moving and Lifting Stand-Alone Computers and Monitors

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

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

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

Operating the Instrument

Ensure that anyone who operates the instrument has:

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

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

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 Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

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

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

About MSDSs

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

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

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

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

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

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

Chemical Waste Safety

Chemical Waste Hazard

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

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

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

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

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

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

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

Electrical Safety

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

Fuses

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 Applied Biosystems 7500/7500 Fast Real-Time PCR System has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Moving Parts

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

Biological Hazard Safety

General Biohazard

WARNING **BIOHAZARD.** Biological samples such as tissues, body fluids, 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.

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

To minimize musculoskeletal and repetitive motion risks:

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

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standard
- European Safety and EMC Standards
- Australian EMC Standards

U.S. and Canadian Safety Standards

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

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

Canadian EMC Standard

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

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials," and with standard EN 61010-2-081:2002+A1:2003 "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

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

1 Get Started

This chapter covers:

- About the 7500/7500 Fast System 2
- Supported Consumables 4
- About Presence/Absence Experiments 7
- How to Use This Guide. 11
- About the Example Presence/Absence Experiment 12
- Example Experiment Workflow 14

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

About the 7500/7500 Fast System

The Applied Biosystems 7500/7500 Fast Real-Time PCR System is a 96-well, five-color platform that uses fluorescence-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).

About Data Collection

The 7500/7500 Fast system collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs:

Run Type		Data Collection Point
Real-time runs	Standard curve	The instrument collects data after 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 7500/7500 Fast 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 7500 software stores the raw fluorescence image for analysis.

After a run, the 7500 software uses region of interest (ROI), optical, dye, and background calibrations to determine the location and intensity of the fluorescence in each read, the dye associated with each fluorescence signal, and the significance of the signals.

Notes

About the Filters

The 7500/7500 Fast system uses the following filters:

Filter	1	2	3	4	5
Dye	• FAM™ dye • SYBR® Green dye	• JOE™ dye • VIC® dye	• TAMRA™ dye • NED™ dye • Cy3® dye	• ROX™ dye • Texas Red® dye	Cy5® dye

For More Information

For information on:

- The 7500/7500 Fast system – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 Help*.

  Note: To access the Help, select **Help ▸ 7500 Software Help** in the 7500 software.

- Genotyping experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments*.
- Relative standard curve and/or comparative C_T ($\Delta\Delta C_T$) experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments*.
- Standard curve experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

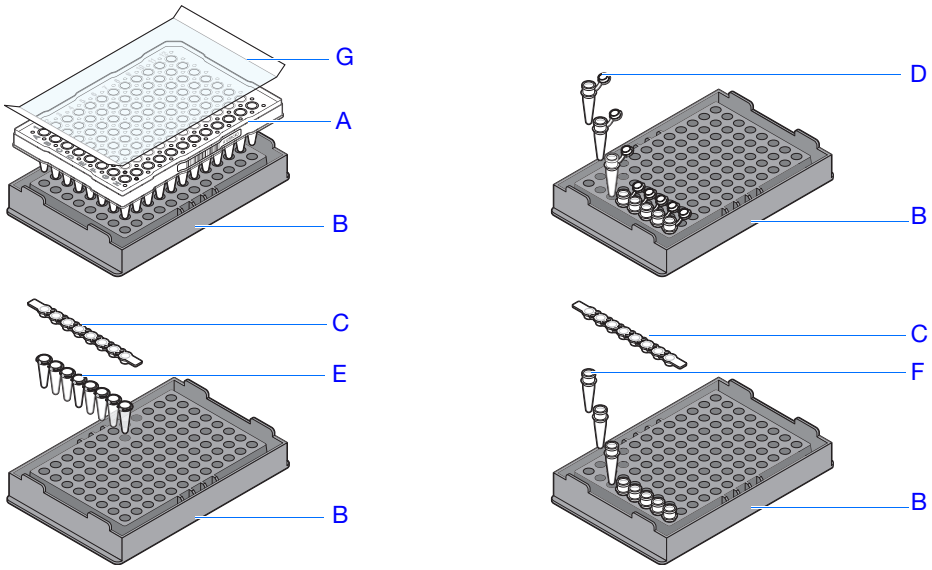
Notes

Supported Consumables

7500 System

The 7500 system supports the consumables listed below.

Consumable	Part Number
• MicroAmp® Optical 96-Well Reaction Plate with Barcode, 0.2-mL • MicroAmp™ Optical Adhesive Film	• 4306737 • 4311971
• MicroAmp™ Optical 8-Tube Strip, 0.2-mL • MicroAmp™ Optical 8-Cap Strip	• 4316567 • 4323032
• MicroAmp® Optical Tube without Cap, 0.2-mL • MicroAmp® Reaction Tube with Cap, 0.2-mL	• N8010933 • N8010540
• Microamp® Splash Free Support Base	• N8010531
• MicroAmp™ Adhesive Film Applicator • MicroAmp® Cap Installing Tool (Handle) • MicroAmp™ Multi-Removal Tool	• 4333183 • 4330015 • 4313950



#	Consumable
A	MicroAmp® Optical 96-Well Reaction Plate, 0.2-mL
B	Microamp® Splash Free Support Base
C	MicroAmp™ Optical 8-Cap Strip, 0.2-mL
D	MicroAmp® Reaction Tube with Cap, 0.2-mL

#	Consumable
E	MicroAmp™ Optical 8-Tube Strip
F	MicroAmp® Optical Tube without Cap
G	MicroAmp™ Optical Adhesive Film

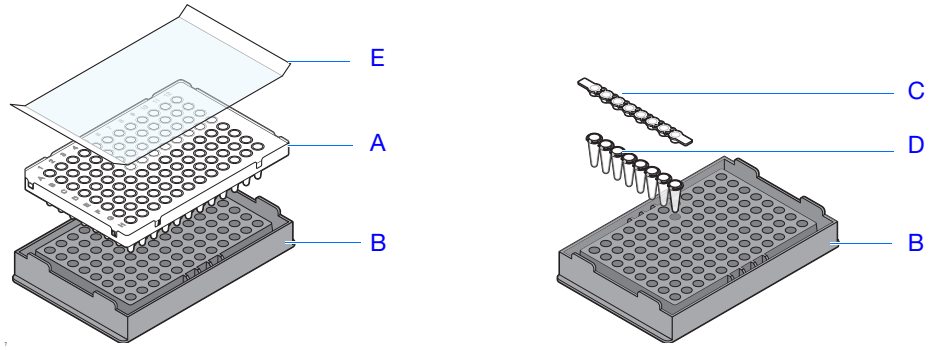
Notes

7500 Fast System

The 7500 Fast system supports the consumables listed below.

IMPORTANT! Use only Fast consumables (reaction plates, tube strips, and tubes) with the 7500 Fast system, even when performing an experiment with standard reagents.

Consumable	Part Number
• MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL • MicroAmp™ Optical Adhesive Film	• 4346906 • 4311971
• MicroAmp™ Fast 8-Tube Strip, 0.1-mL • MicroAmp™ Optical 8-Cap Strip	• 4358293 • 4323032
• Microamp® Splash Free Support Base	• N8010531
• MicroAmp™ Adhesive Film Applicator • MicroAmp® Cap Installing Tool (Handle) • MicroAmp™ Multi-Removal Tool	• 4333183 • 4330015 • 4313950



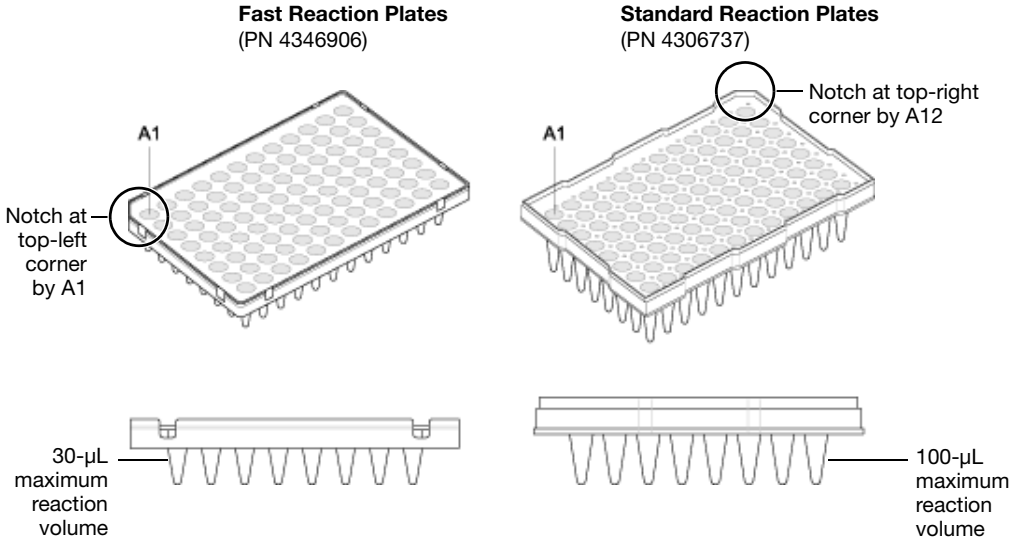
#	Consumable
A	MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1-mL
B	Microamp® Splash Free Support Base
C	MicroAmp™ Optical 8-Cap Strip
D	MicroAmp™ Fast 8-Tube Strip
E	MicroAmp™ Optical Adhesive Film

Notes

Standard vs. Fast Reaction Plates and Tubes

Make sure that you use the correct reaction plate for your system:

System	Reaction Plate
7500 system	• MicroAmp® Optical 96-Well Reaction Plate (PN 4306737, also called *standard reaction plates*) • MicroAmp® Optical Tubes without Caps, 0.2-mL (PN N8010933, also called *standard reaction tubes*) • MicroAmp® Reaction Tubes with Caps, 0.2-mL (PN N2070540) • MicroAmp™ Optical 8-Tube Strip, 0.2-mL (PN 4316567, also called *standard reaction tube strips*) **IMPORTANT!** Fast reaction plates and tubes do not fit into the standard sample block correctly and will result in loss of data.
7500 Fast system	• MicroAmp™ Fast Optical 96-Well Reaction Plate, (PN 4346906, also called *Fast reaction plates*) • MicroAmp™ Fast 8-Tube Strip, 0.1-mL (PN 4358293, also called *Fast reaction tube strips*) **IMPORTANT!** Standard reaction plates and tube strips will not properly function and might be crushed when using the Fast sample block.



Notes

About Presence/Absence Experiments

Endpoint Experiments

Presence/absence experiments are endpoint experiments. In endpoint experiments:

- Data are collected at the end of the PCR process.
- Reactions are characterized by the quantity of target accumulated at the end of PCR.
- The datapoint is the normalized intensity of the reporter dye, or Rn.

Note: Some endpoint experiments also include pre-PCR datapoints. If so, the system calculates the delta Rn (ΔRn) value per the following formula:

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

Note: In this guide, the term *experiment* refers to the entire process of performing a run using the 7500/7500 Fast system, including setup, run, and analysis.

About Presence/Absence Experiments

Perform a presence/absence experiment to detect a specific target in a sample.

Supported Reagents

The 7500/7500 Fast system supports the following reagents for presence/absence experiments:

- TaqMan® reagents (page 8)
- Other fluorescent-based reagents (page 8)

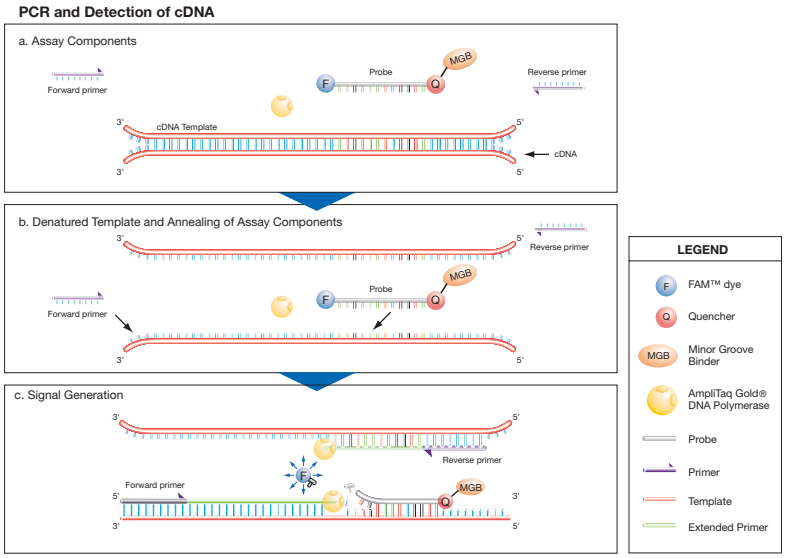
Note: Presence/absence experiments are not supported for Fast master mix or Fast protocols.

Notes

TaqMan Reagents

TaqMan reagents use a fluorogenic probe to enable detection of a target sequence (unknown target) and IPC sequence (IPC target). During amplification, the unknown target and the IPC target generate reporter fluorescence such that positive or negative calls can be made on samples.

Note: If you use TaqMan® reagents, the 7500 software automatically calculates reaction volumes in the Reaction Setup screen.

TaqMan® Reagents or Kits	Process
Advantages • Increased specificity with the addition of a fluorogenic probe. • Provides multiplex capability. • Preformulated assays, optimized to run under universal thermal cycling conditions, are available. **Limitations** Requires synthesis of a unique fluorogenic probe.	

Other Reagents

If you use fluorescence-based reagents other than TaqMan® reagents, you must design your experiment using Advanced Setup instead of the Design Wizard (see "Advanced Setup Workflow" on page 92).

Notes

About the PCR Reactions

With presence/absence experiments, you prepare PCR reactions that contain primers and probes to amplify the target and a reagent to detect amplification of the target. You can set up the PCR reactions for the presence/absence experiments three different ways:

IPC setup – Use an internal positive control (IPC) to monitor the PCR progress and ensure that a negative result is not caused by failed PCR in the sample. PCR reactions contain two primer/probe sets: One to detect the unknown target (unknown target primer set and TaqMan probe to detect the unknown target) and one to detect the IPC (IPC primer set and a VIC dye-labeled TaqMan probe to detect the IPC template). With this setup, there are three well types:

- **Unknown-IPC wells** – Wells contain sample template and IPC template; the presence of the target is not known.
- **Negative control-IPC wells** – Wells contain IPC template and water or buffer instead of sample template in the PCR reaction. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample template. Also called *IPC+*.
- **Negative control-blocked IPC wells** – Wells contain IPC blocking agent instead of sample template in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample template and amplification of the IPC is blocked. Also called *no amplification control (NAC)*.

No IPC, singleplex setup – Use Advanced Setup to omit the IPC from your presence/absence experiment. PCR reactions contain one primer/probe set. PCR reactions do not contain the IPC. With this setup, there are two well types:

- **Unknown wells** – Wells contain sample template; the presence of the target is not known.
- **Negative controls** –Wells contain water or buffer instead of sample template.

No IPC, multiplex setup – Use Advanced Setup to omit the IPC from your presence/absence experiment and detect two targets in one reaction. PCR reactions contain two primer/probe sets. PCR reactions do not include the IPC. With this setup, there are two well types:

- **Unknown-Unknown wells** – Wells contain sample template; the presence of the target is not known.
- **Negative control-Negative control wells** – Wells contain water or buffer instead of sample template.

About the Instrument Run

With presence/absence experiments, the instrument runs can include:

- **Pre-PCR read** – Perform the pre-PCR read on the 7500/7500 Fast instrument before PCR amplification to collect baseline fluorescence data.
- **Amplification** – Perform amplification on the 7500/7500 Fast instrument. If you do not include amplification in the run method, you need to perform amplification on another instrument.
- **Post-PCR read** – The post-PCR read is required to determine the results for presence/absence experiments. Perform the post-PCR read on the instrument after PCR amplification to collect endpoint fluorescence data.

Fluorescence data collected during the instrument run are stored in an experiment data file (EDS).

Notes

About the Analysis

Data from the instrument run are used to determine presence/absence calls. Results are plotted in a presence/absence plot. If the experiment includes amplification, results are plotted in an amplification plot.

Pre-PCR read – If included, the data collected from the pre-PCR read can be used to normalize data collected from the post-PCR read.

Amplification – If included, the data collected from the amplification can be used to troubleshoot.

Post-PCR read – The data collected from the post-PCR read are used to make presence/absence calls:

- **Presence** – The target amplified above the target's threshold. The target is present in the sample.
- **Absence** – The target did not amplify above the target's threshold. The target is absent in the sample.
- **Unconfirmed** – The target exhibited amplification but was below the target threshold. The target cannot be confirmed and the sample must be retested.

With the IPC setup, the data collected form the post-PCR read are used to make the following calls:

- **IPC Failed** – The IPC target did not amplify in the IPC wells and/or the IPC target amplified in the blocked IPC wells.
- **IPC Succeeded** – The IPC target amplified in the IPC wells and the IPC target did not amplify in the blocked IPC wells.

Notes

How to Use This Guide

This guide functions as both a tutorial and as a guide for performing your own experiments.

Using This Guide as a Tutorial

Using example experiment data provided with the 7500 software, you can use this guide as a tutorial for performing a presence/absence experiment on a 7500/7500 Fast system. Follow the procedures in chapters 2 through 5:

Chapter	Procedure
2	Design the experiment using the Design Wizard in the 7500 software.
3	Prepare the experiment, using the reagents and volumes calculated by the Design Wizard in Chapter 2.
4	Run the experiment on a 7500/7500 Fast instrument.
5	Analyze the results.

For more information, see "About the Example Presence/Absence Experiment" on page 12.

Using This Guide with Your Own Experiments

After completing the tutorial exercises in Chapter 2 through 5, use this guide to lead you through your own presence/absence experiments. Each procedure in Chapters 2 through 5 includes a set of guidelines that you can use to perform your own experiments.

Additionally, you can use one of the other workflows provided in the 7500 software to perform your experiments. The table below provides a summary of all the workflows available in the 7500 software.

Workflow	Description	See...
Design Wizard	Set up a new experiment with guidance from the software. The Design Wizard guides you through best practices as you create your own experiment. The Design Wizard is recommended for new users. **Note:** Design options are more limited in the Design Wizard than in Advanced Setup.	Chapter 2
Advanced Setup	Set up a new experiment using advanced options. Advanced Setup allows design flexibility as you create your own experiment. Advanced Setup is recommended for experienced users.	page 92
QuickStart	Run a new experiment with no plate setup information. If desired, you can add all design parameters after the run.	page 93
Template	Set up a new experiment using setup information from a template.	page 95
Export/Import	Import experiment designs from ASCII text files that contain experiment setup information.	page 96

Notes

About the Example Presence/Absence Experiment

To illustrate how to perform presence/absence experiments, this guide leads you through the process of designing, preparing, running and analyzing an example experiment. The example experiment represents a typical setup that you can use to quickly familiarize yourself with a 7500/7500 Fast system.

Description

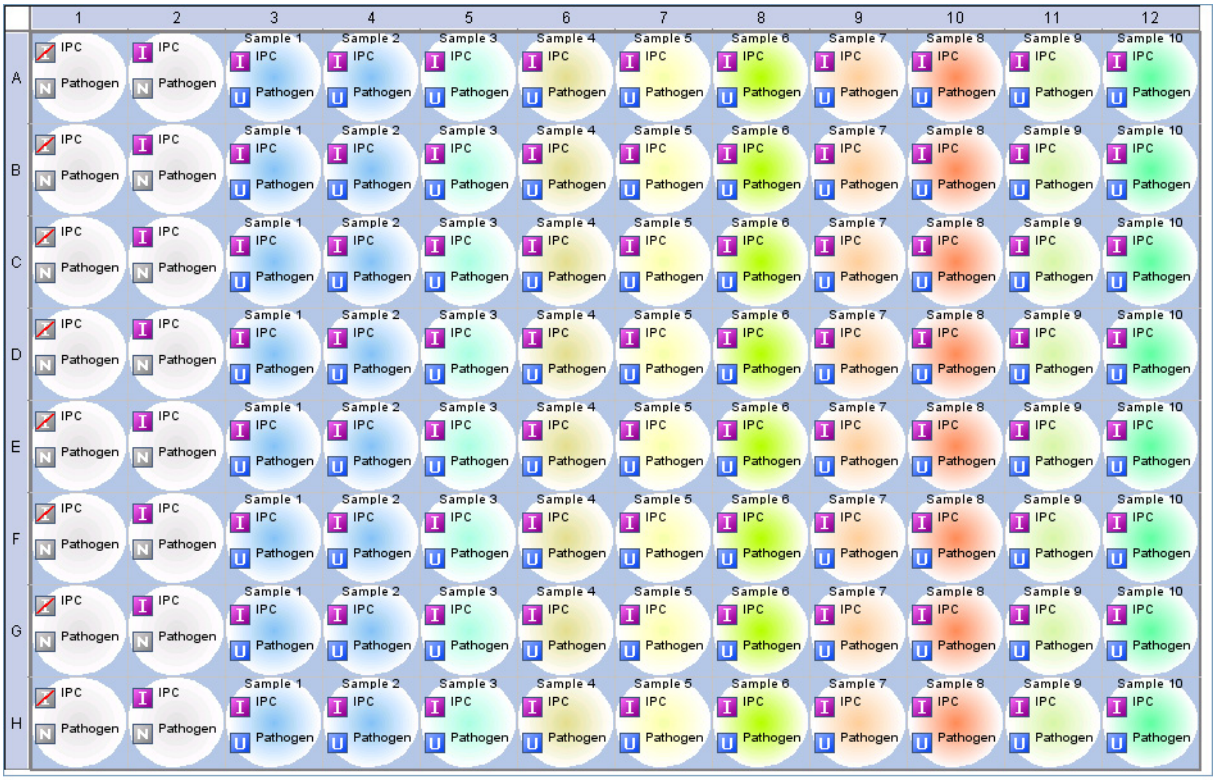
The objective of the presence/absence example experiment is to determine if a pathogen is present in each batch of ground beef.

In the presence/absence example experiment:

- DNA is extracted from samples using the PrepMan® Ultra Sample Preparation Reagent. The DNA is extracted from bacteria found in five samples of ground beef.
- The target is a pathogen.
- The experiment is designed for duplex PCR, where each reaction contains two primer/probe sets. One set detects the unknown target (primer set and FAM™ dye-labeled probe to amplify and detect the target sequence). The other primer/probe set detects the IPC primer set and VIC® dye-labeled TaqMan probe amplifies and detects the IPC template.

Reaction Plate Layout

The 7500 software displays the 96-well reaction plate layout as shown below:



Notes

About the Example Experiment Data

In this getting started guide, you will use two files:

- In Chapter 2, you will create a presence/absence example experiment that contains setup data, then you will save the file to your computer.
- In Chapter 5, you will view results in a presence/absence example experiment file that contains run data. The data file for the example experiment installs with the 7500 software. You can find the data file for the example experiment on your computer:

  <*drive*>:\Applied Biosystems\<*software name*>\experiments\
  Presence Absence Example.eds

  where:

  – <*drive*> is the computer hard drive on which the 7500 software is installed.

  – <*software name*> is the current version of the 7500 software.

Example Files in the Experiments Folder

The experiments folder of the 7500 software contains several example files that you can reference when analyzing your own data. The following example files install with the 7500 software:

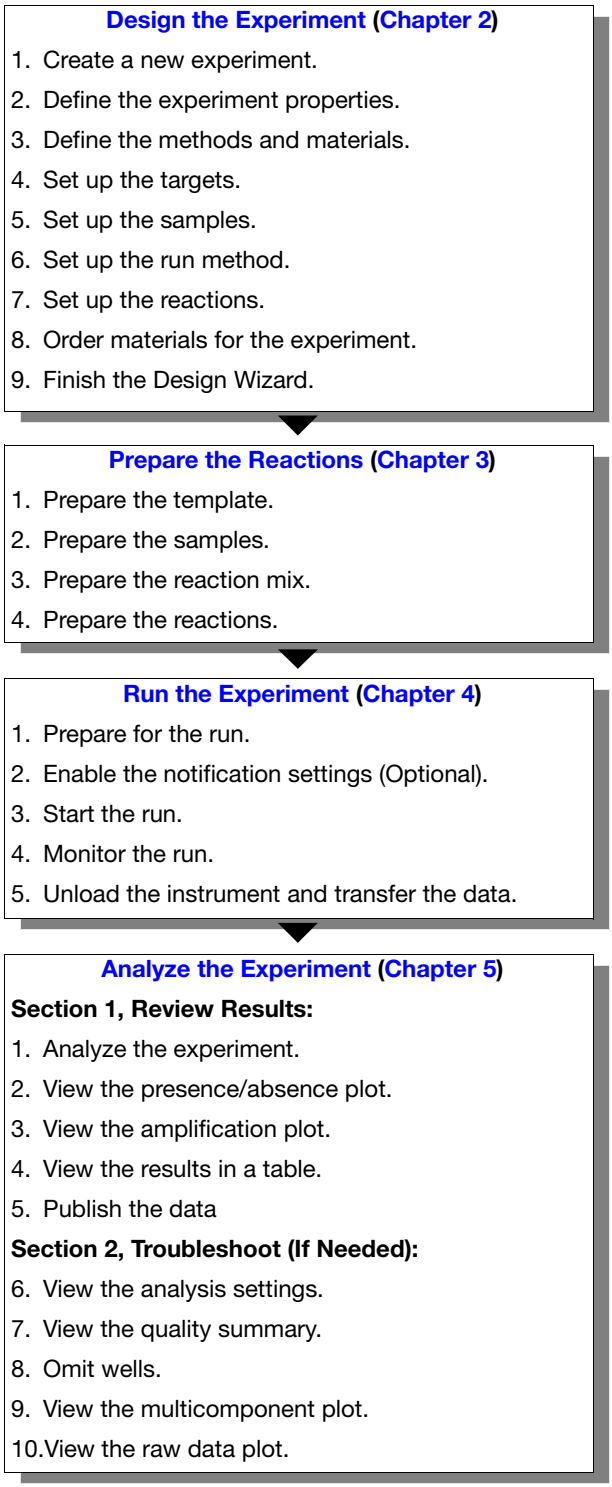
- Comparative Ct Example.eds
- Comparative Ct Study Example.edm
- Comparative Ct Study (Biological Groups).edm
- Genotyping Example.eds
- Presence Absence Example.eds
- Relative Standard Curve Example.eds
- Standard Curve Example.eds

Note: Be sure to use the *Presence Absence Example.eds* file when you perform the procedures in this guide.

Notes

Example Experiment Workflow

The figure below shows the workflow for the presence/absence example experiment.



Notes

2 Design the Experiment

This chapter covers:

- Chapter Overview 16
- Create a New Experiment 17
- Define the Experiment Properties 18
- Define the Methods and Materials 20
- Set Up the Targets 22
- Set Up the Samples and Replicates 23
- Set Up the Run Method 25
- Review the Reaction Setup 27
- Order Materials for the Experiment 31
- Finish the Design Wizard 33

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

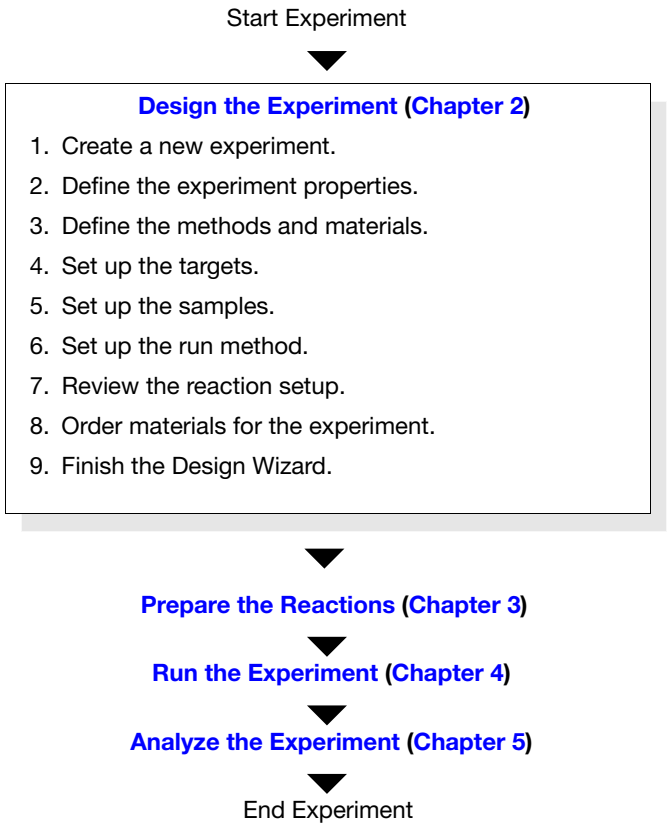
Notes

Chapter Overview

This chapter explains how to use the Design Wizard in the 7500 software to set up the presence/absence example experiment. The Design Wizard guides you through Applied Biosystems recommended best practices as you enter design parameters for the example experiment.

Note: When you design your own experiments, you can select alternate workflows (see "Using This Guide with Your Own Experiments" on page 11).

Example Experiment Workflow



Notes

Create a New Experiment

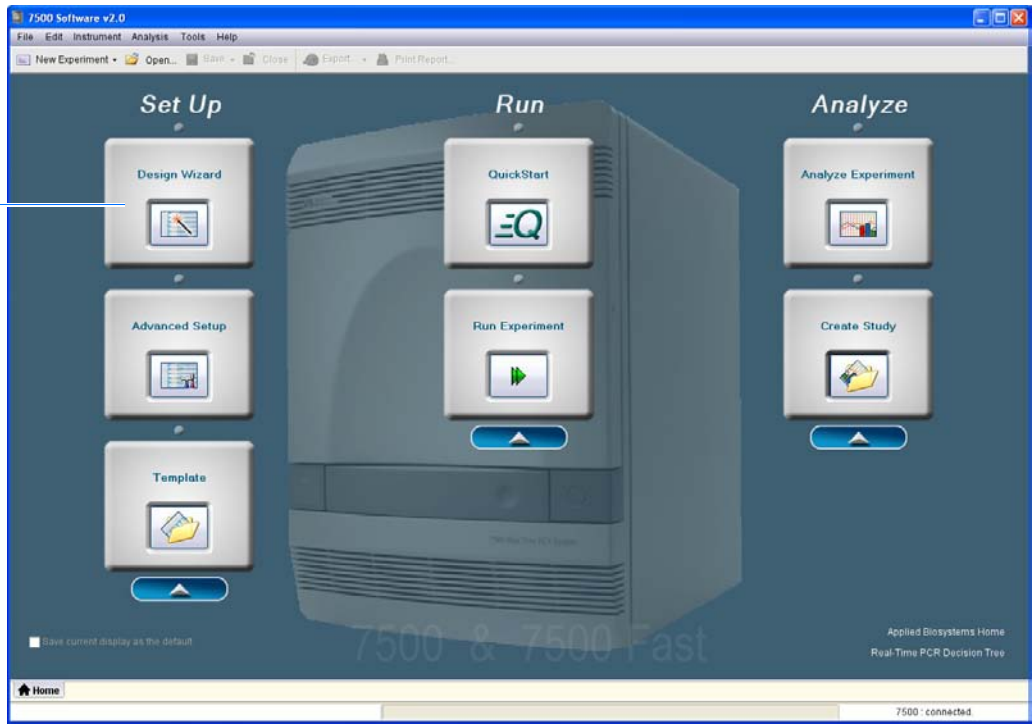
Create a new experiment using the Design Wizard in the 7500 software.

Create an Experiment

1. Double-click (7500 software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where <*software name*> is the current version of the 7500 software.

2. In the Home screen, click **Design Wizard** to open the Design Wizard.



Notes

Define the Experiment Properties

In the Experiment Properties screen, enter identifying information for the experiment, select the instrument type, then select the type of experiment to design.

About the Example Experiment

In the presence/absence example experiment:

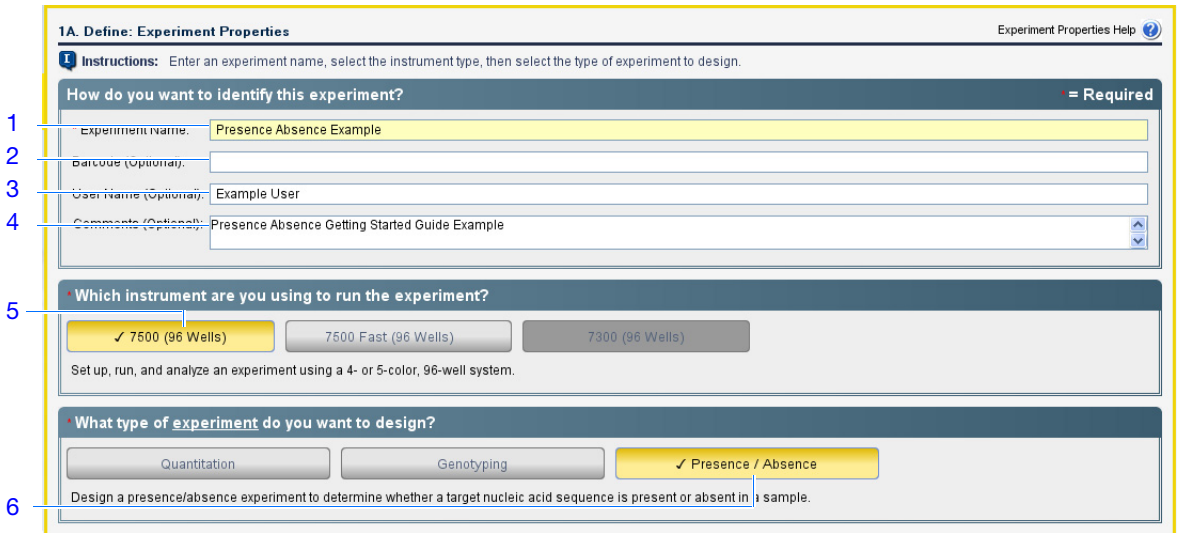
- The experiment is identified as an example.
- The instrument selected to run the experiment is the 7500 instrument.
- A MicroAmp® Optical 96-Well Reaction Plate is used.
- The experiment type is presence/absence.

Complete the Experiment Properties Screen

1. Click the **Experiment Name** field, then enter **Presence Absence Example**.

   Note: The experiment header updates with the experiment name you entered.

2. Leave the Barcode field empty.
3. Click the **User Name** field, then enter **Example User**.
4. Click the **Comments** field, then enter **Presence Absence Getting Started Guide Example**.
5. Select **7500 (96 Wells)**.
6. Select **Presence/Absence** for the experiment type.
7. Click **Next**.



Notes

Design Guidelines

When you design your own presence/absence experiment:

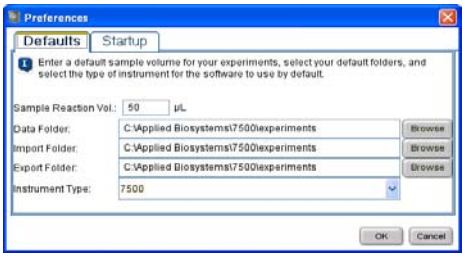
- Enter an experiment name that is descriptive and easy to remember. You can enter up to 100 characters in the Experiment Name field. You cannot use the following characters in the Experiment Name field: / \ > < * ? " | : ;

  Note: The experiment name is used as the default file name.

- *(Optional)* Enter a barcode to identify the barcode on the reaction plate. You can enter up to 100 characters in the Barcode field.
- (Optional) Enter a user name to identify the owner of the experiment. You can enter up to 100 characters in the User Name field.
- (Optional) Enter comments to describe the experiment. You can enter up to 1000 characters in the Comments field.
- Select the instrument you are using to run the experiment:
  - **7500 (96 Wells)**
  - **7500 Fast (96 Wells)**

  Note: You can use 7500 software design experiments for the 7500/7500 Fast instrument. The instrument you select in the Experiment Properties screen affects the reaction plate layout and materials list.

  Note: To set the default instrument type, select **Tools** ▸ **Preferences**, then select the **Defaults** tab (default). In the Default Instrument Type drop-down list, select the appropriate instrument.



- Select **Presence/Absence** as the experiment type.

For More Information

For more information on:

- Completing the Experiment Properties screen – Access the 7500 Software Help by clicking or pressing **F1**.
- Consumables – See "Order Materials for the Experiment" on page 31.
- Presence/absence experiments – Refer to the *Applied Biosystems Real-Time PCR Systems Reagent Guide*.

Notes

Define the Methods and Materials

In the Methods & Materials screen, view the reagents, DNA template and ramp speed to use for the experiment.

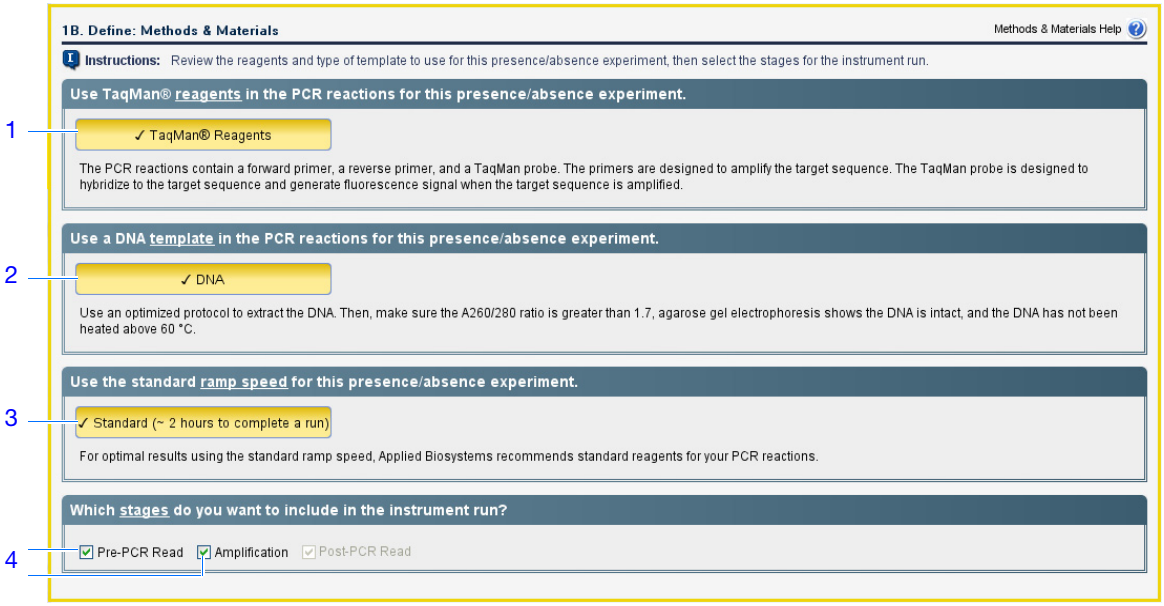
About the Example Experiment

In the presence/absence example experiment:

- TaqMan® reagents are used.
- The standard ramp speed is used in the instrument run.
- DNA template is the type of nucleic acid sample added to the PCR reactions.

Complete the Methods & Materials Screen

1. Make sure **TaqMan® Reagents** is displayed for the reagents.
2. Make sure **DNA** is displayed for the template type.
3. Make sure **Standard (~2 hours to complete a run)** is displayed for the ramp speed.
4. Make sure **Pre-PCR Read** and **Amplification** are selected for the stages.
5. Click **Next**.



Notes

Design Guidelines

When you design your own presence/absence experiment:

- TaqMan® reagents use a TaqMan® probe to detect amplification of the target. In the presence/absence experiments, TaqMan reagents consist of two primer/probe sets. The primers are designed to amplify the target and the IPC template. The TaqMan probes are designed to hybridize to the target and the IPC and generate fluorescence when the target and IPC are amplified.

  Note: Applied Biosystems recommends that you use TaqMan reagents for presence/absence experiments. If you want to use other reagents, use Advanced Setup (see "Advanced Setup Workflow" on page 92) to set up your experiment.

- DNA template is used in the presence/absence experiment.
- Select the **Standard** ramp speed for the instrument run.

  IMPORTANT! The Design Wizard does not support Fast ramp speeds for presence/absence experiments. If you want to use Fast ramp speeds, use Advanced Setup to set up your experiment.

- Select the appropriate stages for the instrument run:
  - **Pre-PCR Read** – If you do not want to collect data before amplification, deselect the **Pre-PCR Read** check box.

    Note: Applied Biosystems recommends that you include the pre-PCR read in the 7500/7500 Fast instrument run.

  - **Amplification** – If you want to perform amplification on a thermal cycler, deselect the **Amplification** check box.

    Note: Applied Biosystems recommends that you include the amplification stage in the 7500/7500 Fast instrument run. You can use the data collected during amplification to troubleshoot.

  - **Post-PCR Read** – This required stage is preselected for you.

For More Information

For more information on:

- Completing the Methods & Materials screen – Access the 7500 Software Help by clicking or pressing **F1**.
- TaqMan reagents – Refer to the *Applied Biosystems Real-Time PCR Systems Reagent Guide*.
- Using Advanced Setup – See "Advanced Setup Workflow" on page 92.
- PCR, including singleplex vs. multiplex PCR – Refer to the *Applied Biosystems Real-Time PCR Systems Reagent Guide*.

Notes

Set Up the Targets

In the Targets screen, enter the name of the target that you want to detect in the PCR reaction plate (Unknown), then enter the name of the internal positive control (IPC) target.

About the Example Experiment

In the presence/absence example experiment:

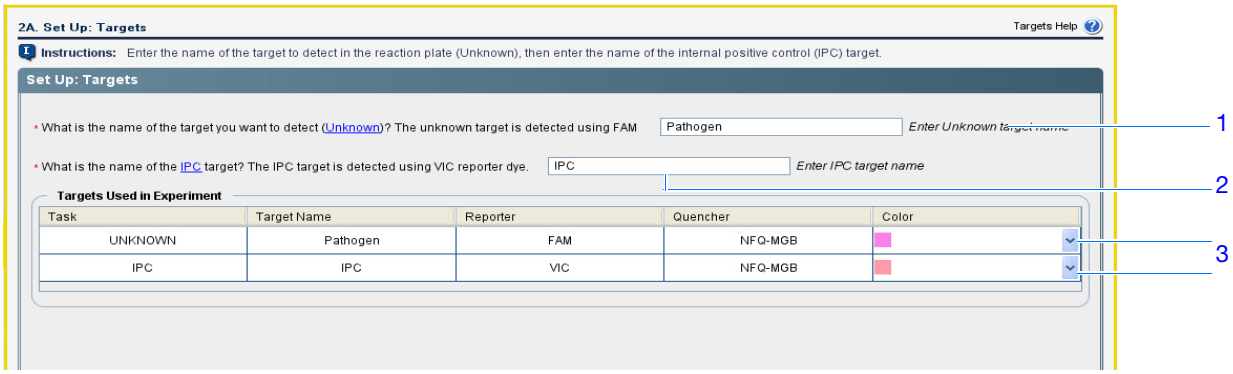
- A pathogen is used as the unknown target and an IPC is used as the IPC target. The IPC target ensures that a negative result is not the result of a failed PCR reaction.
- Each well in the reaction plate contains an IPC.

Complete the Targets Screen

1. Click the **What is the name of the target you want to detect (Unknown)?** field, then enter **Pathogen**.
2. Click the **What is the name of the IPC target?** field, then enter **IPC**.

Note: The Targets Used in Experiment table updates with the target names.

3. Leave the default colors in the Color drop-down lists.
4. Click **Next**.



Design Guidelines

When you design your own presence/absence experiment, identify the target and the IPC with a unique name and color. You can enter up to 100 characters each for the unknown target name and the IPC target name.

Note: In the Design Wizard, the unknown target is detected using a FAM™ dye-labeled TaqMan probe and the IPC target is detected using a VIC® dye-labeled TaqMan probe.

Note: If you want to set up your presence/absence experiments without the IPC, perform advanced setup. See "Advanced Setup Workflow" on page 92.

For More information

For more information on completing the Targets screen, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Set Up the Samples and Replicates

In the Samples & Replicates screen, enter the number of samples, replicates, and negative controls to include in the reaction plate, enter the sample names, then select which sample/target reactions to set up.

About the Example Experiment

In the presence/absence example experiment:

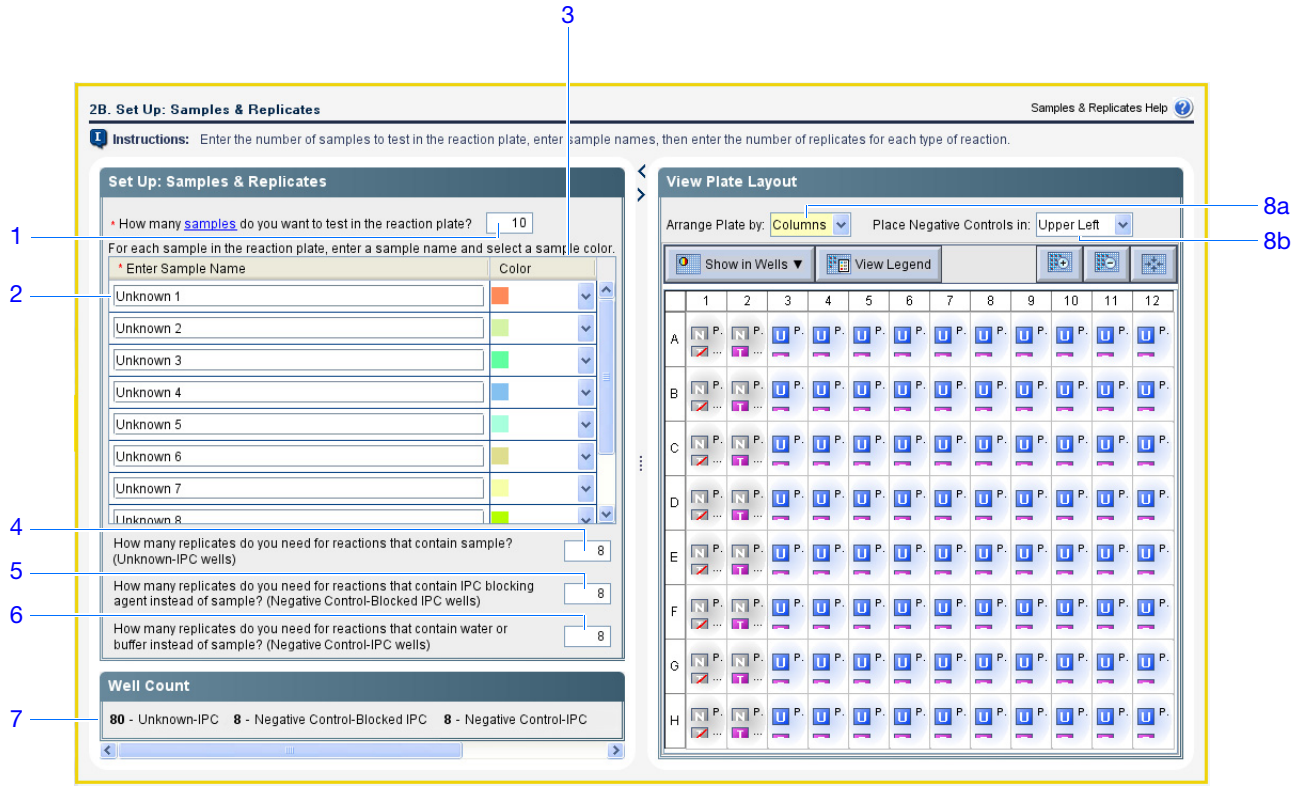
- Ten samples are used, where the presence/absence of the target (a pathogen) is unknown.
- Eight Unknown-IPC replicates are used. Replicates are identical to the sample reactions, containing identical components and volumes.
- Eight Negative Control-Blocked IPC replicates are used. Replicates contain IPC template and IPC blocking agent instead of sample template.
- Eight Negative Control-IPC replicates are used. Replicates contain IPC template and buffer or water instead of sample template.

Complete the Samples & Replicates Screen

1. Click the **How many samples do you want to test in the reaction plate?** field, then enter **10**.
2. In the Enter Sample Name fields, enter:
   - **Unknown 1**
   - **Unknown 2**
   - **Unknown 3**
   - **Unknown 4**
   - **Unknown 5**
   - **Unknown 6**
   - **Unknown 7**
   - **Unknown 8**
   - **Unknown 9**
   - **Unknown 10**
3. Leave the default colors in the Color drop-down lists.
4. Click the **How many replicates do you need for reactions that contain sample? (Unknown-IPC wells)** field, then enter **8**.
5. Click the **How many replicates do you need for reactions that contain IPC blocking agent instead of sample? (Negative Control-Blocked IPC wells)** field, then enter **8**.
6. Click the **How many replicates do you need for reactions that contain water or buffer instead of sample? (Negative Control-IPC wells)** field, then enter **8**.
7. In the Well Count pane, confirm that there are:
   - 80 Unknown-IPC
   - 8 Negative Control-Blocked IPC
   - 8 Negative Control-IPC

Notes

8. In the View Plate Layout pane:
   a. In the Arrange Plate by drop-down list, select **Columns**.
   b. In the Place Negative Controls in drop-down list, select **Upper Left**.
9. Click **Next**.



Design Guidelines

When you design your own presence/absence experiment:

- Identify each sample with a unique name and color. You can enter up to 100 characters in the Sample Name field.
- Enter the number of Unknown-IPC replicates to set up. Applied Biosystems recommends 3 Unknown-IPC replicates.
- Enter the number of Negative Control-Blocked IPC replicates to set up. Applied Biosystems recommends 3 Negative Control-Blocked IPC replicates.
- Enter the number of Negative Control-IPC replicates to set up. Applied Biosystems recommends 3 Negative Control-IPC replicates.
- If the number of reactions required exceeds the number of wells in the reaction plate, reduce the number of samples, Unknown-IPC replicates, Negative Control-Blocked IPC replicates, and/or Negative Control-IPC replicates.

For More Information

For more information on completing the Samples & Replicates screen, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Set Up the Run Method

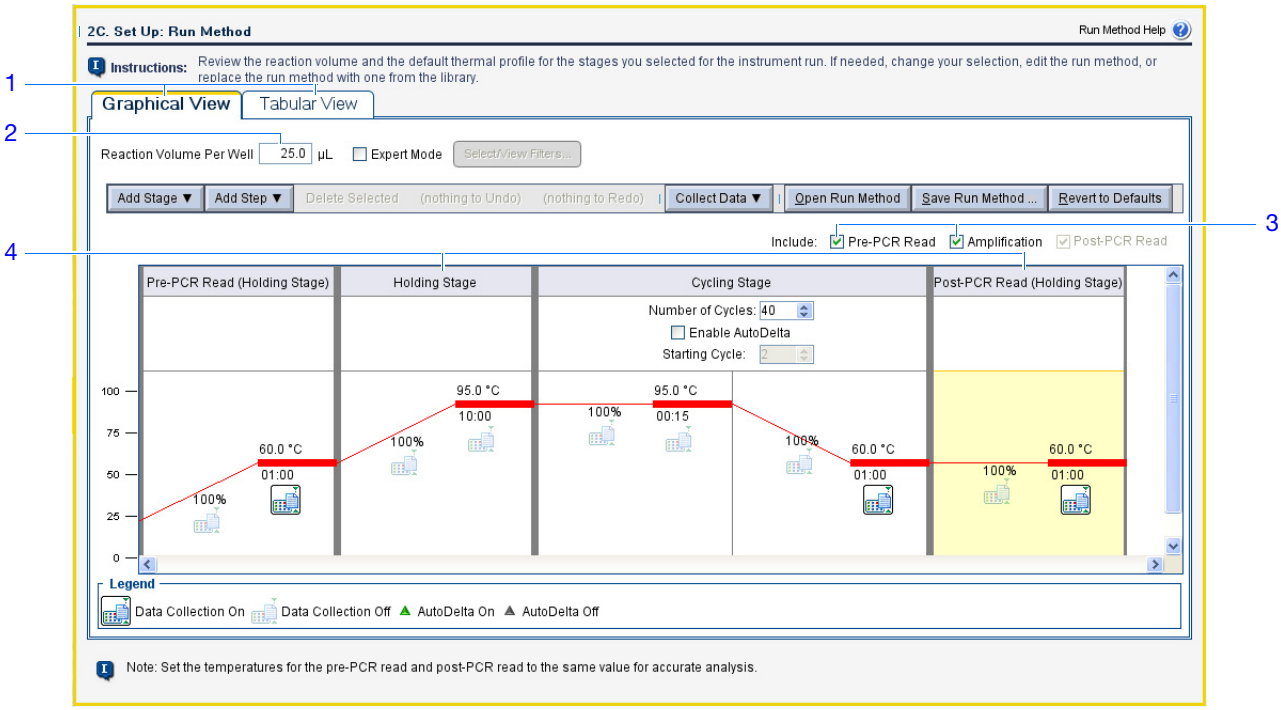
In the Run Method screen, review the reaction volume and the thermal profile for the default run method. If needed, you can edit the default run method, or replace it with one from the library.

About the Example Experiment

In the presence/absence example experiment, the default run method is used without edits.

Review the Run Method Screen

1. Click either the **Graphical View** tab (default) or **Tabular View** tab.
2. Click the **Reaction Volume Per Well** field, then enter **25 µL**.
3. Make sure the Pre-PCR Read and Amplification check boxes are selected.
4. Make sure the thermal profile displays the holding and cycling stages shown below.
5. Click **Next**.



Notes

Design Guidelines

When you design your own presence/absence experiment:

- Enter a reaction volume/well. Applied Biosystems recommends a reaction volume of 25 µL for presence/absence experiments. The 7500 system supports reaction volumes from 20 to 100 µL. The 7500 Fast system supports reaction volumes from 10 to 30 µL.
- Review the thermal profile to make sure that it is appropriate for your reagents.

  If your experiment requires a different thermal profile, edit the thermal profile or replace the run method with one from the Run Method library. The Run Method library is included in the 7500 software.

For More Information

For more information on:

- The run method library or completing the Run Method screen – Access the 7500 Software Help by clicking or pressing **F1.**
- Using Advanced Setup – See "Advanced Setup Workflow" on page 92.

Notes

Review the Reaction Setup

In the Reaction Setup screen, select the assay type, then review the calculated volumes for preparing the samples, controls, and PCR reactions.

IMPORTANT! Perform these steps for each target in the reaction plate.

About the Example Experiment

In the presence/absence example experiment:

- The assay type is Inventoried/Made to Order
- The reaction volume per well is 25 µL with a 10% excess.
- The reaction components are:
  - TaqMan® Universal PCR Master Mix (2.0×)
  - IPC Mix (10×)
  - IPC DNA (50×)
  - Primer/Probe Mix (20.0×)
  - Water
- For all samples:
  - The stock concentration of sample DNA is 100.0 ng/µL.
  - The sample DNA is diluted to a concentration of 50.0 ng/µL.
  - The diluted sample DNA (50.0 ng/µL) is at a 10× concentration: 2.5 µL is used to prepare a 25-µL reaction.
  - The final concentration of sample DNA in the reactions is 5.0 ng/µL.

Complete the Reaction Setup Screen

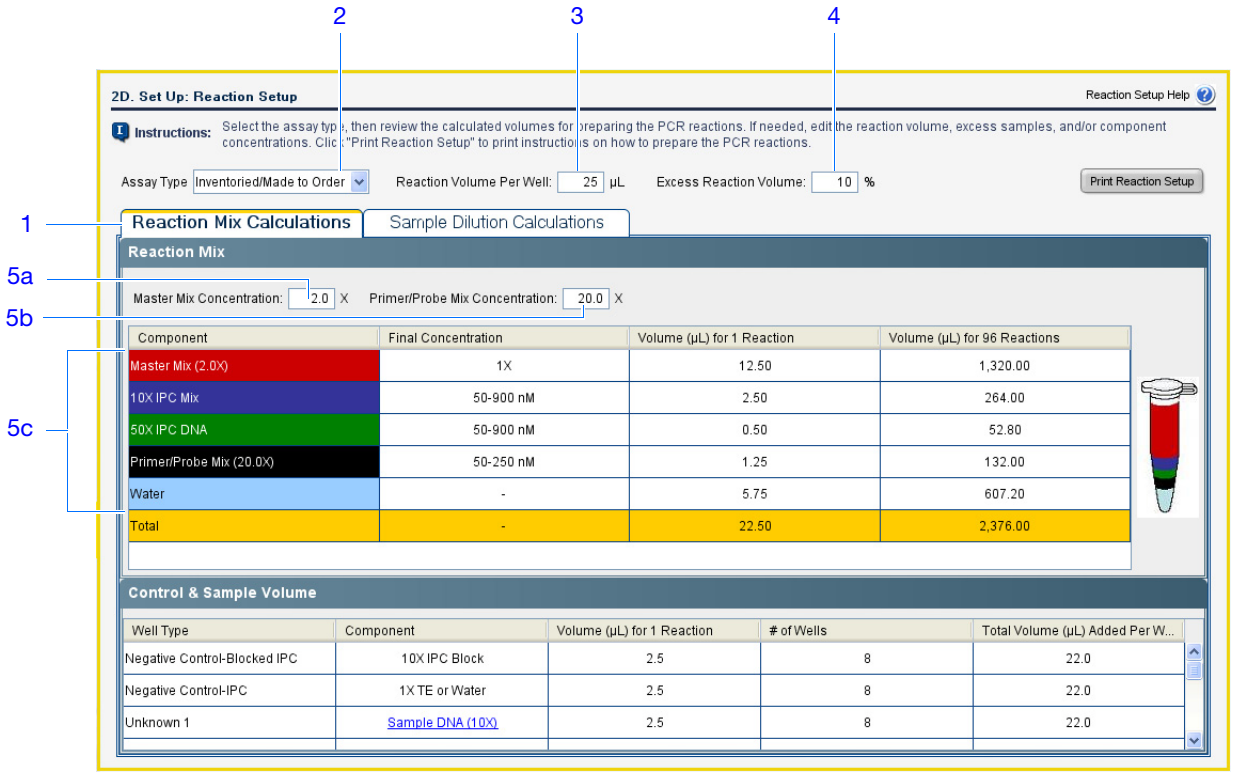
Complete the Reaction Mix Calculations Tab

1. Select the **Reaction Mix Calculations** tab (default).
2. Make sure the Assay Type field displays **Inventoried/Made to Order**.
3. Make sure the Reaction Volume Per Well field displays **25 µL**.
4. Make sure the Excess Reaction Volume field displays **10%**.
5. In the Reaction Mix pane:
   a. Make sure the Master Mix Concentration field displays **2.0×**.
   b. Make sure the Primer/Probe Mix Concentration field displays **20.0×**.

Notes

c. Review the components and calculated volumes for the PCR reactions:

Component	Volume (µL) for 1 Reaction	Volume (µL) for 96 Reactions (Including Excess)
Master Mix (2.0X)	12.50	1320.00
10X IPC Mix	2.50	264.00
50X IPC DNA	0.50	52.80
Primer/Probe Mix (20.0X)	1.25	132.00
Water	5.75	607.20
Total Volume	22.50	2376.00



6. In the Control & Sample Volume pane, review the calculated volumes for preparing the reactions:

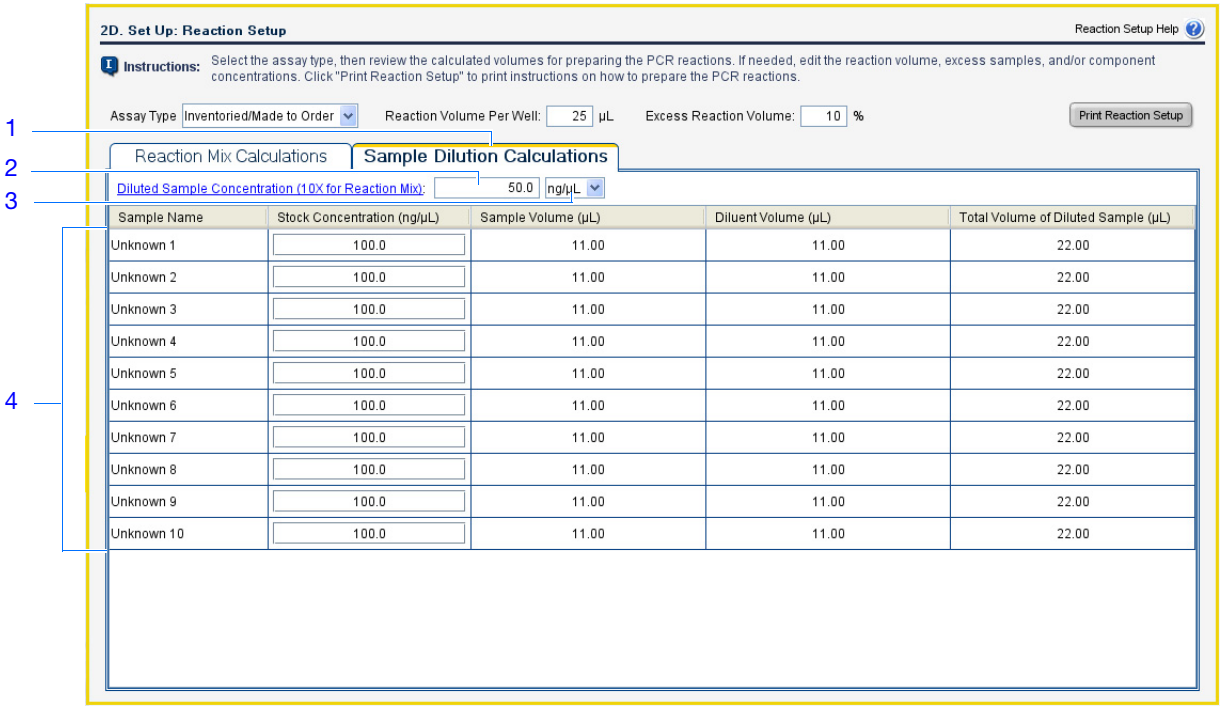
Well Type	Component	Volume (µL) for 1 Reaction	# of Wells	Total Volume (µL) Added per Well Type
Negative Control-Blocked IPC	10X IPC Block	2.5	8	22.0
Negative Control-IPC	1X TE or Water	2.5	8	22.0
Unknown 1	Sample DNA (10X)	2.5	8	22.0
Unknown…10	Sample DNA (10X)	2.5	8	22.0

Notes

Complete the Sample Dilution Calculations Tab

1. Select the **Sample Dilution Calculations** tab.
2. Click the **Diluted Sample Concentration (10× for Reaction Mix)** field, then enter **50.0**.
3. In the unit drop-down list, select **ng/µL** (default).
4. Review the calculated volumes for the sample dilutions:

Sample Name	Stock Concentration (ng/µL)	Sample Volume (µL)	Diluent Volume (µL)	Total Volume of Diluted Sample (µL)
Unknown 1	100.0	11.00	11.00	22.00
Unknown 2	100.0	11.00	11.00	22.00
Unknown 3	100.0	11.00	11.00	22.00
Unknown 4	100.0	11.00	11.00	22.00
Unknown 5	100.0	11.00	11.00	22.00
Unknown 6	100.0	11.00	11.00	22.00
Unknown 7	100.0	11.00	11.00	22.00
Unknown 8	100.0	11.00	11.00	22.00
Unknown 9	100.0	11.00	11.00	22.00
Unknown 10	100.0	11.00	11.00	22.00

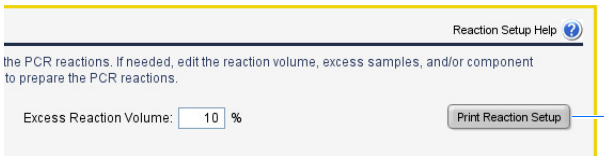


Notes

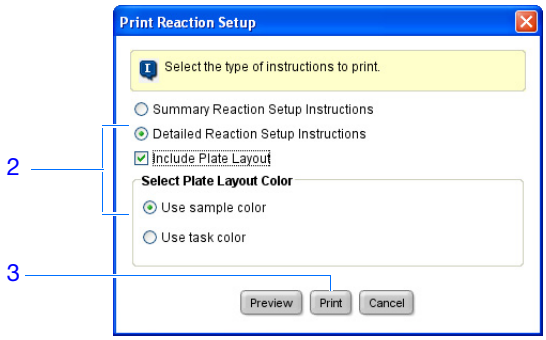
Print Reaction Setup Instructions

Print detailed reaction setup instructions, then save the instructions for Chapter 3.

1. Click **Print Reaction Setup**.



2. In the dialog box, select:
   - **Detailed Reaction Setup Instructions**
   - **Include Plate Layout**
   - **Use sample color**
3. Click **Print** to print the reaction setup instructions.



4. Click **Next**.

Design Guidelines

When you design your own presence/absence experiment:

- Select the type of assay you are using:
  - **Inventoried/Made to Order** – The primers and TaqMan probe are provided together in one ready-to-use assay mix.
  - **Custom** – The primers and TaqMan probe are designed and provided separately.
- Enter a reaction volume/well. Applied Biosystems recommends a reaction volume of 25 µL for presence/absence experiments. The 7500 system supports reaction volumes from 20 to 100 µL. The 7500 Fast system supports reaction volumes from 10 to 30 µL.
- Include excess reaction volume to account for the loss that occurs during pipetting. Applied Biosystems recommends an excess reaction volume of at least 10%.
- Review the components and calculated volumes for the PCR reactions. If needed, edit the master mix and primer/probe mix concentrations.

For More Information

For more information on completing the Reaction Setup screen, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Order Materials for the Experiment

In the Materials List screen, review the list of materials recommended to prepare the PCR reaction plate. (Optional) Print the materials list, create a shopping list, then order the recommended materials from the Applied Biosystems Store.

Note: To access the Applied Biosystems Store, you need to have an Internet connection. Product availability and pricing may vary according to your region or country. Online ordering through the Applied Biosystems Store is not available in all countries. Contact your local Applied Biosystems representative for help.

Note: The 7500 software recommends the materials to order based on your experiment design. It is assumed that you will design your experiment, order your materials, then prepare (Chapter 3) and run (Chapter 4) the reaction plate when your materials arrive.

About the Example Experiment

In the presence/absence example experiment, the recommended materials are:

- MicroAmp® Optical 96-Well Reaction Plate
- MicroAmp™ Optical Adhesive Film
- MicroAmp® Splash Free Support Base
- TaqMan® Universal PCR Master Mix (2×)

Complete the Experiment Materials List Screen

1. In the Display drop-down list, select **All Items** (default), then review the recommended materials. If needed, use the scroll bar at right to see all items.

   Note: For more information on a specific item, click the part number link. You will be connected to the product information page in the Applied Biosystems Store. To access the Applied Biosystems Store, you need to have an Internet connection.

2. (Optional) Click **Print Materials List** to print the materials list.
3. (Optional) Create a shopping list:
   a. Select the check box next to each of the following items:
      - MicroAmp® Optical 96-Well Reaction Plate
      - MicroAmp™ Optical Adhesive Film
      - MicroAmp® Splash Free Support Base
      - TaqMan® Universal PCR Master Mix (2×)

   b. Click **Add Selected Items to Shopping List**.

Notes

4. (Optional) Create a shopping basket in the Applied Biosystems Store:

   Note: To access the Applied Biosystems Store, you need to have an Internet connection. Product availability and pricing may vary according to your region or country. Online ordering through the Applied Biosystems Store is not available in all countries. Contact your local Applied Biosystems representative for help.

   a. Check that the Experiment Shopping List contains the desired materials and that the quantities are correct, then click **Order Materials in List**.

   b. In the Order Materials - Log In dialog box, enter your user name and password for the Applied Biosystems Store, then click **Log In and Submit**.

      Note: If you do not have an account with the Applied Biosystems Store, click **Register Now** to create an account.

   c. When you are connected to the Applied Biosystems Store, follow the prompts to complete your order.

5. Go to "Finish the Design Wizard" on page 33.

Design Guidelines

When you design your own presence/absence experiment:

- Select all the materials you require for your experiment and add them to your shopping list.
- To access and use the Applied Biosystems Store:
  - Confirm that your computer has an Internet connection.
  - Use the following Applied Biosystems-recommended browsers and versions of Adobe® Acrobat® Reader:

Desktop Operating System	Netscape® Navigator	Microsoft® Internet Explorer	Macintosh® Safari	Adobe® Acrobat® Reader
Windows® 2000/NT/XP/Vista	v6.x or later	v6.x or later	Not applicable	v4.0 or later
Macintosh® OS 9+ or later	Not supported	Not supported	v2.0.4 or later	v4.0 or later

IMPORTANT! Make sure that cookies and Java Script are turned on.

For More Information

For more information on completing the Experiment Materials List screen, access the 7500 Software Help by clicking ? or pressing **F1**.

Notes

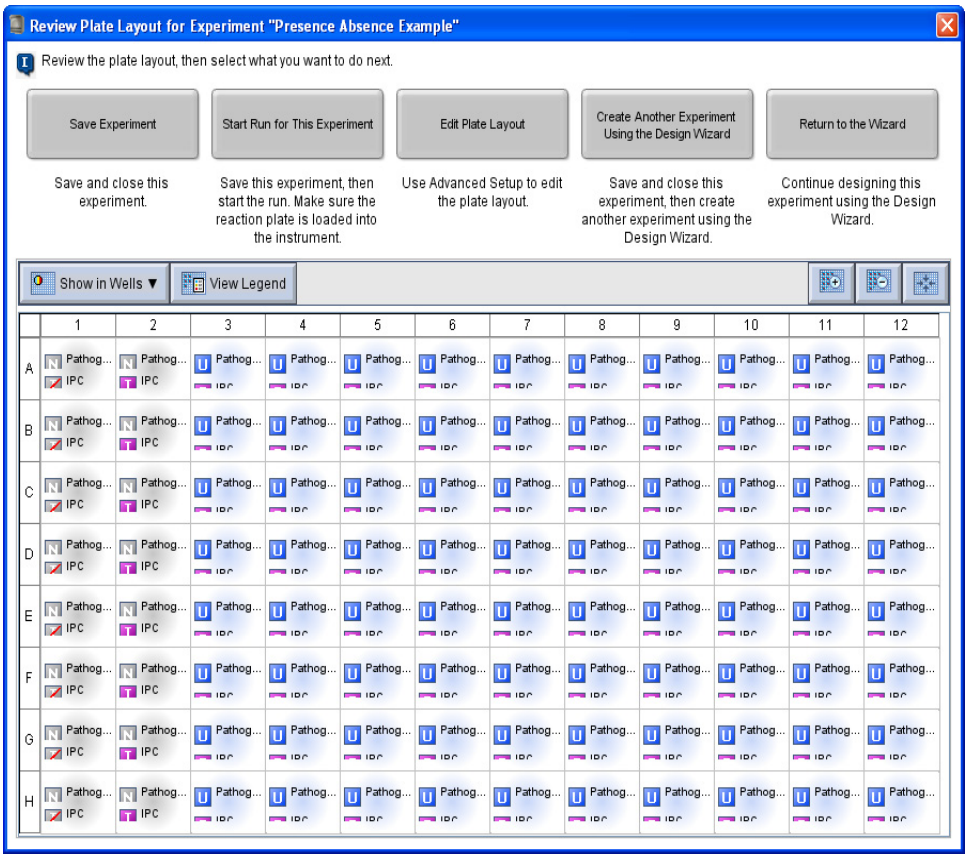
Finish the Design Wizard

To finish the Design Wizard, review the plate layout, then select an exit option.

About the Example Experiment

The 7500 software automatically selects locations for the wells in the reaction plate. In the presence/absence experiment:

- The wells are arranged as shown below.



- The experiment is saved as is and closed.

Note: For the example experiment, do not perform the run at this time.

Notes

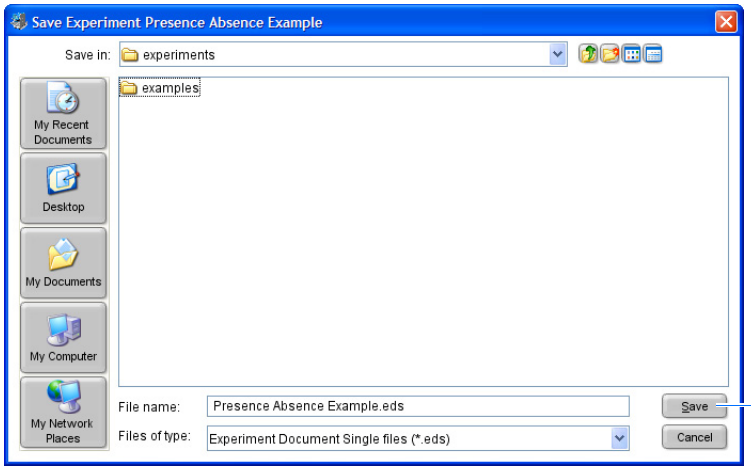
Finish the Design Wizard

1. At the bottom of the 7500 software screen, click **Finish Designing Experiment**.
2. In the Review Plate Layout for Experiment window, review the plate layout. Make sure there are:
   - 80 Unknown-IPC
   - 8 Negative Control-Blocked IPC
   - 8 Negative Control-IPC

   Note: If the plate layout is incorrect, click **Return to the Wizard** and check your entered values.
3. Click **Save Experiment**.
4. In the Save Experiment dialog box, enter **Presence Absence Example Setup.eds** in the File name drop-down list, then click **Save**. The example experiment is saved and closed, and you are returned to the Home screen.

   IMPORTANT! Do not save the experiment using the default file name. Doing so will overwrite the example experiment already present in the experiments folder.

   Note: By default, the example experiment is saved to the <*drive*>:\Applied Biosystems\<*software name*>\experiments folder.



Notes

Design Guidelines

When you design your own presence/absence experiment:

- In the Review Plate Layout for Experiment window, select the appropriate exit option:

Click	To...
Save Experiment	Save and close the experiment without making any further changes or starting the run.
Start Run for This Experiment	Save the experiment and start the run. Make sure the reaction plate is loaded in the instrument.
Edit Plate Layout	Use Advanced Setup to edit the plate layout.
Create Another Experiment Using the Design Wizard	Save and close the current experiment, then create another experiment using the Design Wizard.
Return to the Wizard	Return to the experiment to make changes using the Design Wizard.

- By default, experiments are saved to:

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

  To change the:

  – Save location for a specific experiment – Navigate to the desired location using the Save Experiment dialog box.

  – Default save location – Select **Tools** ▸ **Preferences**, then select the **Defaults** tab. In the Data Folder field, browse to then select the desired location.

For More Information

For more information on using Advanced Setup, see "Advanced Setup Workflow" on page 92.

Notes

Notes

3 Prepare the Reactions

This chapter covers:

- Chapter Overview 38
- Prepare the Template. 39
- Prepare the Samples 40
- Prepare the Reaction Mix 42
- Prepare the Reactions 44

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

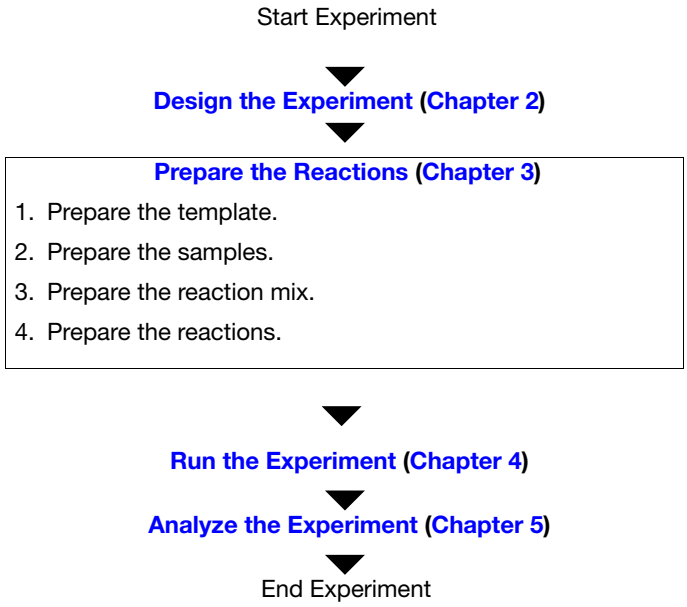
Notes

Chapter Overview

This chapter explains how to prepare the PCR reactions for the presence/absence example experiment and provides guidelines for how to prepare the PCR reactions for your own presence/absence experiment.

Example Experiment Workflow

The workflow for preparing the PCR reactions for the example experiment provided with this getting started guide is shown below.



Notes

Prepare the Template

In this section, prepare the DNA template for the PCR reactions using the PrepMan® Ultra Sample Preparation Reagent kit.

About the Example Experiment

For the presence/absence example experiment, the template for the PCR reactions is DNA extracted from bacteria found in ground beef. There are 10 samples of ground beef to analyze for the presence or absence of a pathogen.

Required Materials

- DNA isolated from blood, tissue culture cells, and buccal swabs.
- DNA isolated from animal or plant tissue.
- Purified plasmid DNA.
- One of the products listed below for DNA isolation:

Kit‡	Part Number
PrepMan® Ultra Sample Preparation Reagent Kit	4322547
NucPrep® DNA Chemistry for Tissues	4340274
BloodPrep® Chemistry for Blood, Tissue Culture Cells, and Buccal Swabs	4346860
Plasmid Miniprep Kit	402790

‡ Applied Biosystems recommends using the kits with the ABI Prism® 6100 Nucleic Acid PrepStation or the ABI Prism® 6700 Nucleic Acid Automated Workstation.

Prepare the Template

Use the DNA isolation kits recommended in the table above, or follow your own protocol to isolate DNA.

Preparation Guidelines

When you prepare your own presence/absence experiment, extract DNA from the tissue or sample. Ensure that the DNA you use:

- Has a $A_{260/280}$ ratio of greater than 1.7.
- Has a stock concentration of 100 ng/µL.
- Is extracted from the raw material you are testing using an optimized protocol.
- Does not contain PCR inhibitors.
- Is intact as visualized by gel electrophoresis.
- Has not been heated above 60 °C.

For More Information

Applied Biosystems supplies several chemistries for isolating DNA from blood, tissue, cell cultures, plant material, and food. For more information refer to:

- *DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol*
- *NucPrep® Chemistry Isolation of Genomic DNA from Animal and Plant Tissue Protocol*
- *PrepMan® Ultra Sample Preparation Reagent Protocol*

Notes

Prepare the Samples

In this section, dilute the DNA samples using the volumes that were calculated by the 7500 software.

About the Example Experiment

For the presence/absence example experiment, 2.5 µL of 50 ng/µL sample DNA is added to 22.5 µL of Reaction Mix. The final reaction volume per well is 25 µL. For all wells containing sample DNA, the final concentration of DNA per well is 5 ng/µL, and the total amount of DNA per well is 100 ng.

For the presence/absence example experiment:

- The stock concentration of sample (Unknown) DNA, after preparing the template, is 100 ng/µL.
- The desired concentration of sample (Unknown) DNA to add to the reaction is 50 ng/µL.
- The reaction volume per well is 25 µL.
- The excess reaction volume is 10%.
- The volumes calculated in the software are:

Sample Name	Stock Concentration (ng/µL)	Sample Volume (µL)	Diluent Volume (µL)	Total Volume of Diluted Sample (µL)
Unknown 1	100	11.0	11.0	22.0
Unknown 2	100	11.0	11.0	22.0
…	…	…	…	…
Unknown 10	100	11.0	11.0	22.0

Required Materials

- Buffer or water to dilute the sample
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Sample stock
- Vortexer
- Centrifuge

Notes

Prepare the Sample Dilutions

1. Label a separate microcentrifuge tube for each unknown sample:
   - Unknown 1
   - Unknown 2
   - Unknown 3
   - Unknown 4
   - Unknown 5
   - Unknown 6
   - Unknown 7
   - Unknown 8
   - Unknown 9
   - Unknown 10

2. Add the required volume of water to each empty tube:

Tube	Sample Name	Diluent Volume (µL)
1	Unknown 1	11.0
2	Unknown 2	11.0
…	…	…
10	Unknown 10	11.0

3. Add the required volume of sample stock to each empty tube:

Tube	Sample Name	Sample Volume (µL)
1	Unknown 1	11.0
2	Unknown 2	11.0
…	…	…
10	Unknown 10	11.0

4. Vortex each sample for 3 to 5 seconds, then centrifuge the tubes briefly.

5. Place the diluted samples on ice until you prepare the reactions.

Notes

Prepare the Reaction Mix

Prepare the reaction mix using the components and volumes that were calculated by the 7500 software ("Complete the Reaction Mix Calculations Tab" on page 27).

Note: The reaction mix includes all components *except* sample and negative control (buffer or IPC blocking agent).

About the Example Experiment

All samples contain a primer and probe set for the IPC and a primer and probe set for the pathogen, therefore, only one reaction mix is required.

The reaction mix components and volumes calculated in the software are:

Component	Volume (µL) for 1 Reaction
TaqMan® Universal PCR Master Mix (2.0×) or TaqMan® Universal PCR Master Mix, No Amperase® UNG	12.50
10× IPC Mix	2.50
50× IPC DNA	0.50
20× Primer/Probe Mix	1.25
Water/Buffer	5.75
Total Reaction Mix Volume	22.50

Note: The sample is not added at this time.

Required Materials

- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Reaction mix components (listed above)
- Centrifuge

Prepare the Reaction Mix

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

CAUTION **CHEMICAL HAZARD. TaqMan® 2× Universal PCR Master Mix, No AmpErase UNG** may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Notes

1. Label an appropriately sized tube for the reaction mix: **Pathogen Reaction Mix**.
2. Add the required volumes of each component to the tube:

Component	Volume (µL) for 1 Reaction	Volume (µL) for 96 Reactions (Including Excess)
TaqMan® Universal PCR Master Mix (2.0×)	12.50	1320.0
10× IPC Mix	2.50	264.0
50× IPC DNA	0.50	52.80
20× Primer/Probe Mix	1.25	132.00
Water/Buffer	5.75	607.20
Total Reaction Mix Volume	22.50	2376.00

3. Mix the reaction mix by gently pipetting up and down, then cap the tube.
4. Centrifuge the tube briefly.
5. Place the reaction mix on ice until you prepare the reactions.

Preparation Guidelines

When you prepare your own presence/absence reactions:

- Prepare the reaction mix for each target separately.
- Include excess volume in your calculations to provide excess volume for the loss that occurs during reagent transfers.
- Include all required components.
- Prepare the reagents according to the manufacturer's instructions.
- Keep the TaqMan probe protected from light, in the freezer, until you are ready to use it. Excessive exposure to light may affect the fluorescent probes.
- Prior to use:
  - Mix the master mix thoroughly by swirling the bottle.
  - Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
  - Thaw any frozen samples by placing them on ice. When thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.

Notes

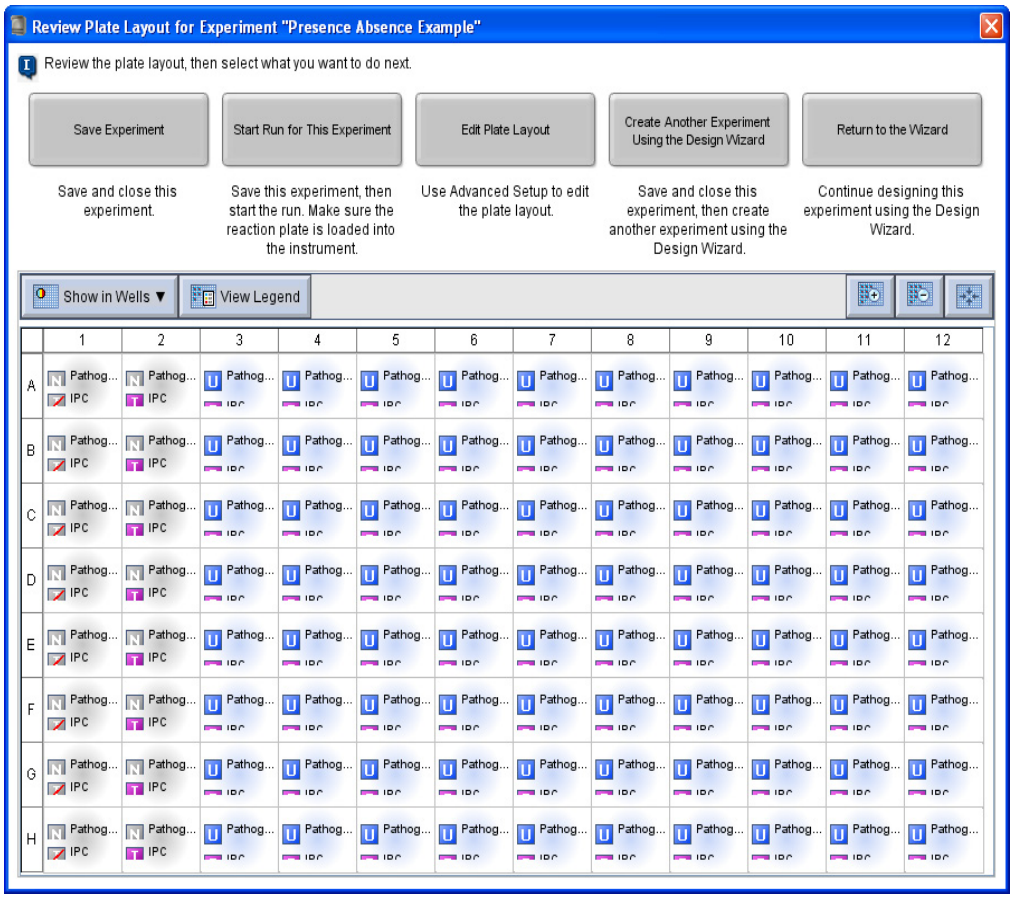
Prepare the Reactions

Prepare the reactions using the volumes that were calculated by the 7500 software. See "Review the Reaction Setup" on page 27.

About the Example Experiment

For the presence/absence experiment:

- A MicroAmp® Optical 96-Well Reaction Plate is used.
- The final concentration of DNA for all of the samples is 5 ng/µL.
- The reaction volume is 25 µL/well.
- The reaction plate contains:
  - 8 Negative Control-Blocked IPC
  - 8 Negative Control-IPC
  - 80 Unknown-IPC
- The plate layout that the 7500 software automatically generates is used:



Notes

Required Materials

- Pipettors
- Pipette tips
- Pathogen reaction mix (from page 42)
- Water or buffer
- Samples (from page 41)
- MicroAmp® Optical 96-Well Reaction Plate
- MicroAmp™ Optical Adhesive Film
- Centrifuge

Prepare the Reactions

Use the plate layout and instructions to prepare the PCR reactions. Make sure the arrangement of PCR reactions matches the plate layout in the software:

1. Add 22.5 µL of reaction mix to the appropriate wells.

Reaction Mix	Add 22.5 µL Reaction Mix to Wells
Pathogen	A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12 B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12 C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12 D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12 E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12 F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12 G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12 H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12

2. Prepare Negative Control-IPC reactions (Task = NC-IPC): Add 2.5 µL of buffer or water to the appropriate wells.

Add 2.5 µL Buffer or Water to Wells
A2, B2, C2, D2, E2, F2, G2, H2

3. Prepare Negative Control-Blocked IPC reactions (Task = NC-Blocked IPC): Add 2.5 µL of IPC blocking agent to the appropriate wells.

Add 2.5 µL Blocking Agent to Wells
A1, B1, C1, D1, E1, F1, G1, H1

Notes

4. Prepare unknown reactions (Task = Unknown): Add 2.5 µL of sample to the appropriate wells.

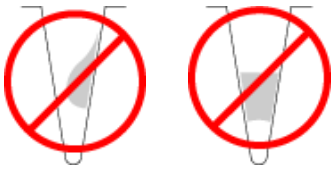
Sample	Add 2 µL Sample to Wells
Unknown 1	A3, B3, C3, D3, E3, F3, G3, H3
Unknown 2	A4, B4, C4, D4, E4, F4, G4, H4
Unknown 3	A5, B5, C5, D5, E5, F5, G5, H5
Unknown 4	A6, B6, C6, D6, E6, F6, G6, H6
Unknown 5	A7, B7, C7, D7, E7, F7, G7, H7
Unknown 6	A8, B8, C8, D8, E8, F8, G8, H8
Unknown 7	A9, B9, C9, D9, E9, F9, G9, H9
Unknown 8	A10, B10, C10, D10, E10, F10, G10, H10
Unknown 9	A11, B11, C11, D11, E11, F11, G11, H11
Unknown 10	A12, B12, C12, D12, E12, F12, G12, H12

5. Cover the reactions with the appropriate consumable:
   - Optical reaction plate – Seal the reaction plate with optical adhesive film.
   - Tube strips – Cover the tubes with caps.

6. Centrifuge the tubes briefly to remove air bubbles.

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

IMPORTANT! Do not allow the bottom of the reaction plate to become dirty. Fluids and other contaminants that adhere to the bottom of the reaction plate can contaminate the sample block 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. Until you are ready to perform the run, place the reaction plate on ice in the dark.

Notes

Preparation Guidelines

When you prepare your own presence/absence experiment:

- Wear appropriate protective eyewear, clothing, and gloves.
- Make sure you use the appropriate consumables.
- Make sure the arrangement of the PCR reactions matches the plate layout in the 7500 software. You can either:
  - Accept the plate layout automatically generated by the 7500 software.

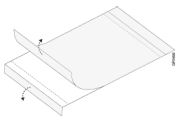
    or
  - Use Advanced Setup to change the plate layout in the software (page 92).
- If you use optical adhesive film, seal each reaction plate as follows:

  a. Place the reaction plate onto the center of the 96-well base.

  b. Load the reaction plate as desired.

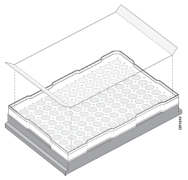
  c. Remove a single optical adhesive film (film) from the box. Fold back one of the end-tabs. Hold the film with its backing side up.

  d. In one continuous movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.

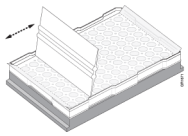


  IMPORTANT! Improper peeling of the optical adhesive film may result in haziness, but does not affect results. Haziness disappears when the film comes into contact with the heated cover in the instrument.

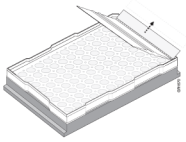
  e. While holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure the film completely covers all wells of the reaction plate.



  f. While applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.



  g. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat for the other end-tab.



  h. Repeat step f to ensure a tight, evaporation-free seal. While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film.

  Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight seal.

Notes

i. Inspect the reaction plate to be sure all wells are sealed. You should see an imprint of all wells on the surface of the film.

For More Information

For more information on:

- Consumables – See "Supported Consumables" on page 4.
- Using Advanced Setup to change the plate layout – See "Advanced Setup Workflow" on page 92.

Notes

4 Run the Experiment

This chapter covers:

- Chapter Overview 50
- Prepare for the Run 51
- Enable the Notification Settings (Optional) 53
- Start the Run 55
- Monitor the Run 55
- Unload the Instrument 57

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

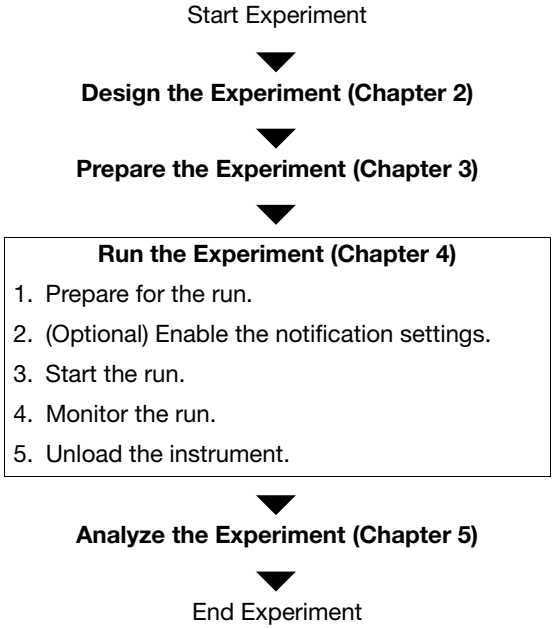
Notes

Chapter Overview

This chapter explains how to perform a run on the Applied Biosystems 7500/7500 Fast Real-Time PCR System.

Example Experiment Workflow

The workflow for running the example experiment provided with this getting started guide is shown below.



Notes

Prepare for the Run

Prepare for the run by opening the example experiment file you created in Chapter 2, then loading the sealed reaction plate into the 7500/7500 Fast instrument.

Open the Example Experiment

1. Double-click (7500 software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***

   where *<software name>* is the current version of the 7500 software.

2. In the Home screen, click **Open**.

3. In the Open dialog box, navigate to the **experiments** folder (default):

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

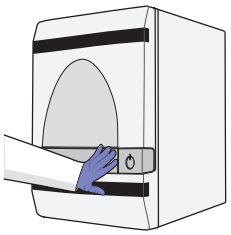
4. Double-click **Presence Absence Example Setup.eds** to open the example experiment file you created in Chapter 2.

Load the Reaction Plate Into the Instrument

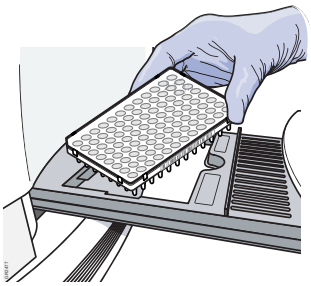
WARNING **PHYSICAL INJURY HAZARD.** During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

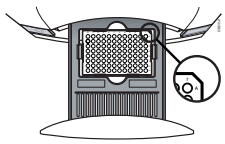
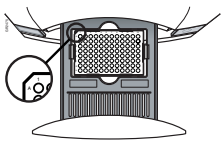
IMPORTANT! Wear powder-free gloves when you handle the reaction plate.

1. Push the tray door to open it.



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



7500 system	7500 Fast system
Load standard plates with the notched A12 position at the top-right of the tray.	Load Fast plates with the notched A1 position at the top-left of the tray.
	

Notes

3. Place the reactions in the precision plate holder (PPH). If you use:
   - **A reaction plate** – Place the reaction plate in the PPH with well A1 at the back-left corner.

     Note: Fast Reaction 8-tube strips can be used only on the 7500 Fast system. For the 7500 system, use MicroAmp® Optical 8-Tube Strips.
   - **Reaction tube strips** – Place the tube strips in the PPH for tube strips.

     Note: Fast Reaction tubes cannot be used in the 7500 Fast system.
   - **Reaction tubes** – Place the tubes in the PPH.

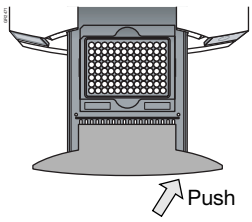
     Note: MicroAmp® Fast Reaction Tubes (PN 4358297) cannot be used in the 7500 Fast system.

     IMPORTANT! For optimal performance with partial loads:

     For a 7500 Instrument

     Load at least 16 tubes, arranging them first in the center columns of the instrument system (columns 6 and 7). Move outward towards columns 1 and 12 as you add more tubes.

     For a 7500 Fast Instrument
     - Place empty tube strips in columns 1 and 12 to prevent crushing of tubes containing samples.
     - Place tube strips with samples in the PPH vertically, starting in columns 6 and 7 and moving outward.
     - A maximum of 6 tube strips can be used in the 7500 Fast instrument. Leave columns 2, 3, 10, and 11 empty.
4. Close the tray. Apply pressure to the right side of the tray and at an angle.



Notes

Enable the Notification Settings (Optional)

Enable the notification settings so that the 7500 software alerts you via e-mail when the 7500/7500 Fast instrument begins and completes the run, or if an error occurs during the run. Enabling the notifications settings feature is optional and does not affect the performance of the 7500/7500 Fast system or the duration of the run.

IMPORTANT! The notification settings feature is available only if the computer that you are using is running the 7500/7500 Fast instrument *and* is connected to an Ethernet network.

About the Example Experiment

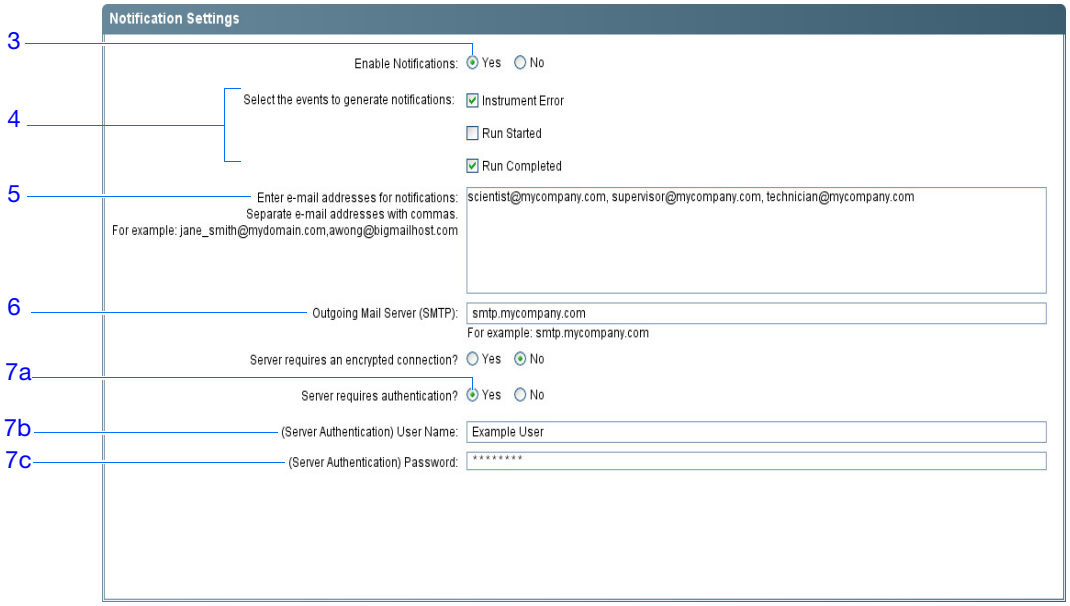
In the example experiment:

- The 7500 software is set up to send notifications to three users (scientist, supervisor, and technician at mycompany.com) when the 7500/7500 Fast system ends the run and if it encounters any errors during operation.
- The example SMTP server (www.mycompany.com) is set up for secure sockets layer (SSL) encryption and requires authentication for use.

Set Up the Notification Settings

1. In the 7500 software, click **Run** in the navigation pane.
2. Click **Notification Settings**.
3. Select **Yes** for Enable Notifications.
4. Select the events that will trigger notifications:
   a. Select **Instrument Error**.
   b. Select **Run Completed**.
5. In the Enter e-mail addresses for notifications field, enter: **scientist@mycompany.com, supervisor@mycompany.com, technician@mycompany.com**.
6. In the Outgoing Mail Server (SMTP) field, enter **smtp.mycompany.com**.
7. Set the authentication settings:
   a. Select **Yes** for Server requires authentication.
   b. In the User Name field, enter **Example User**.
   c. In the Password field, enter **password**.

Notes



Run Guidelines

When you set up the 7500/7500 Fast system for automatic notification:

- Your system must be set up for network use. Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- Select the events for which you want to receive e-mail notifications. Select:
  - **Instrument Error** – To notify recipients by email of all errors encountered by the instrument during each run.
  - **Run Started** – To notify recipients by email when the instrument starts a run.
  - **Run Completed** – To notify recipients by email when the instrument completes a run.
- Obtain e-mail addresses to receive notifications.

  IMPORTANT! Separate addresses with a comma (,).

- Contact your systems administrator or information technology department if you need:
  - E-mail addresses for users who will receive notifications
  - A network address for a simple mail transfer protocol (SMTP) server on the LAN
  - A user name and password for the server, if required for access
  - The Secure Sockets Layer (SSL) setting of the server (on or off)

Notes

Start the Run

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation mode. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

To start your 7500/7500 Fast instrument:

1. In the 7500 software, click **Run** in the navigation pane.
2. Click **START RUN** .

Monitor the Run

You can view the progress of the run in real time as described below. During the run, periodically view all three available plots from the 7500 software for potential problems.

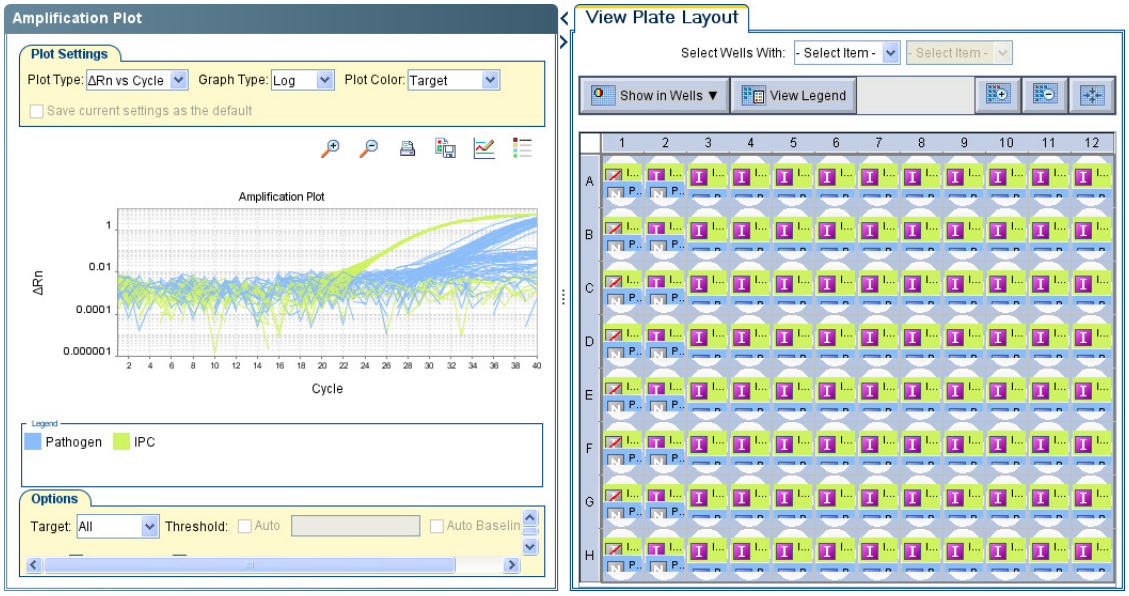
To...	Action
Stop the run	1. In the 7500 software, click **STOP RUN**. 2. In the Stop Run dialog box, click one of the following: – **Stop Immediately** to stop the run immediately. – **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold. – **Cancel** to continue the run.
View amplification data in real time	Select **Amplification Plot**. See "About the Amplification Plot Screen" on page 55.
View progress of the run in the Run Method screen	Select **Run Method**. See "About the Run Method Screen" on page 56.
Enable/disable the Notification Settings	Select or deselect **Enable Notifications**. See "Enable the Notification Settings (Optional)" on page 53.

About the Amplification Plot Screen

The screen displays sample amplification as your instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the Amplification Plot screen displays the data for the wells selected in the View Plate Layout tab. The plot contrasts normalized dye fluorescence (ΔRn) and cycle number. The figure below shows the Amplification Plot screen as it appears during the example experiment.

To view data in the Amplification Plot screen, select the wells that you want to view in the View Plate Layout tab.

Notes



The Amplification Plot screen is useful for identifying and examining abnormal amplification. Abnormal amplification can include the following:

- Increased fluorescence in negative control wells.
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

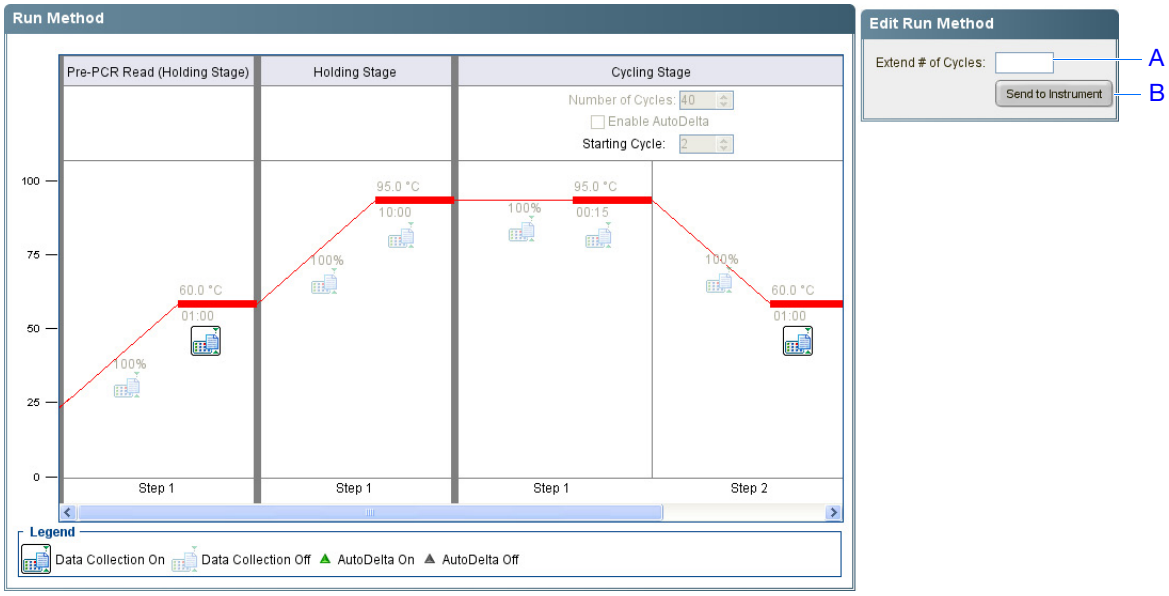
If you notice abnormal amplification or a complete absence of fluorescence, troubleshoot the error as explained in the 7500 Software Help (click or press **F1**).

About the Run Method Screen

The screen displays the run method selected for the run in progress. The software updates the Run Status field throughout the run. The figure below shows the Run Method screen as it appears in the example experiment.

	To...	Action
A	Change the number of cycles	In the Extend # of Cycles field, enter the number of cycles to apply to the Cycling Stage.
B	Apply your changes	Click **Send to Instrument**.

Notes



If an alert appears, click the error for more information and troubleshoot the problem as explained in the 7500 Software Help (click or press **F1**).

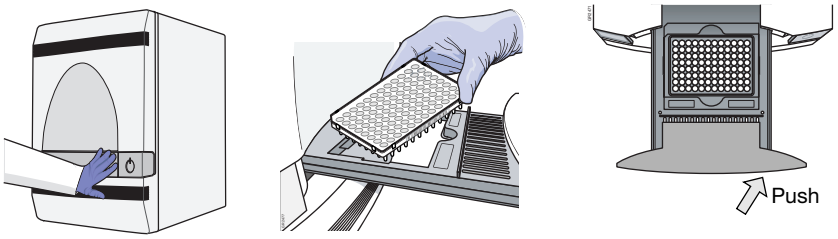
Unload the Instrument

When your 7500/7500 Fast system displays the Run Complete message, unload the reaction plate from the instrument.

Unload the Reaction Plate

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

1. Push the tray door to open it.
2. Remove the reaction plate.
3. Push the tray door to close it.



Notes

Notes

5 Analyze the Experiment

This chapter covers:

- Chapter Overview 60

Section 5.1 Review Results 61

- Analyze the Experiment 62
- View the Presence/Absence Plot 65
- View the Amplification Plot 67
- View the Well Table 74
- Publish the Data 77

Section 5.2 Troubleshoot (If Needed) 79

- View the Analysis Settings 80
- View the QC Summary 82
- Omit Wells from the Analysis 84
- View the Multicomponent Plot 86
- View the Raw Data Plot 88

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

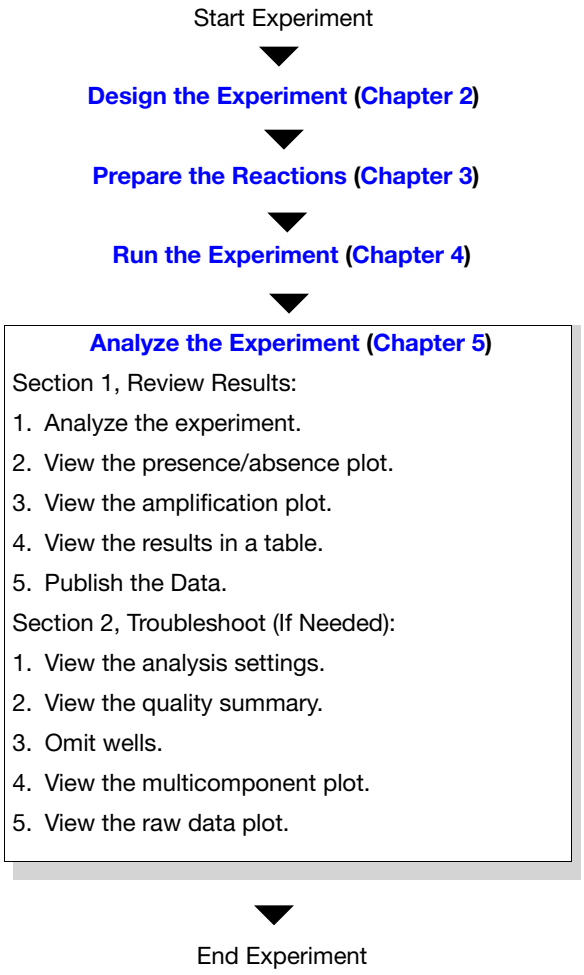
Notes

Chapter Overview

The 7500 software analyzes your data using the endpoint method. Section 1 of this chapter explains how to review the analyzed data using several of the analysis screens and how to publish data. If you receive questionable results, Section 2 of this chapter explains how to perform some troubleshooting steps.

Example Experiment Workflow

The workflow for analyzing the example experiment provided with this getting started guide is shown below.



Notes

Section 5.1 Review Results

This section covers:

- Analyze the Experiment 62
- View the Presence/Absence Plot. 65
- View the Amplification Plot 67
- View the Well Table 74
- Publish the Data 77

Notes

Analyze the Experiment

The 7500 software analyzes the experiment and displays results in the analysis screens (for example, the Amplification Plot screen or the QC Summary screen).

About the Example Experiment

For the presence/absence experiment, use the data file that installs with the 7500 software. The data file was created with the same design parameters that are provided in Chapter 2, then run and analyzed on a 7500/7500 Fast system.

You can find the data file for the example experiment on your computer:

<drive>:\Applied Biosystems*<software name>*\experiments\
Presence Absence Example.eds

where:

- *<drive>* is the computer hard drive on which the 7500 software is installed.
- *<software name>* is the current version of the 7500 software.

Analyze the Example Experiment

1. Double-click (7500 Software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where *<software name>* is the current version of the 7500 software.

2. From the Home screen, click **Open**.

3. In the Open dialog box, double-click the **experiments** folder:

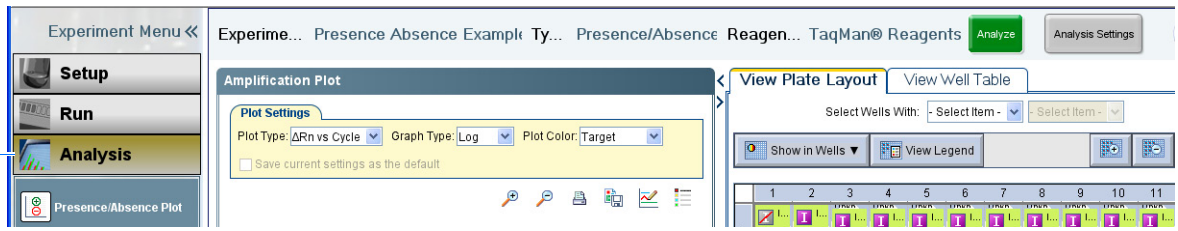
   <drive>:\Applied Biosystems*<software name>*\experiments

4. Double-click **Presence/Absence Example.eds** to open the example experiment data file.

   Note: The experiments folder contains several data files; be sure to select **Presence Absence Example.eds**.

5. In the navigation pane, click **Analysis**. The 7500 software analyzes the data using the default analysis settings.

See "Navigation Tips" on page 63 for information on navigating within the analysis screens.



Notes

Guidelines

When you analyze your own presence/absence experiment:

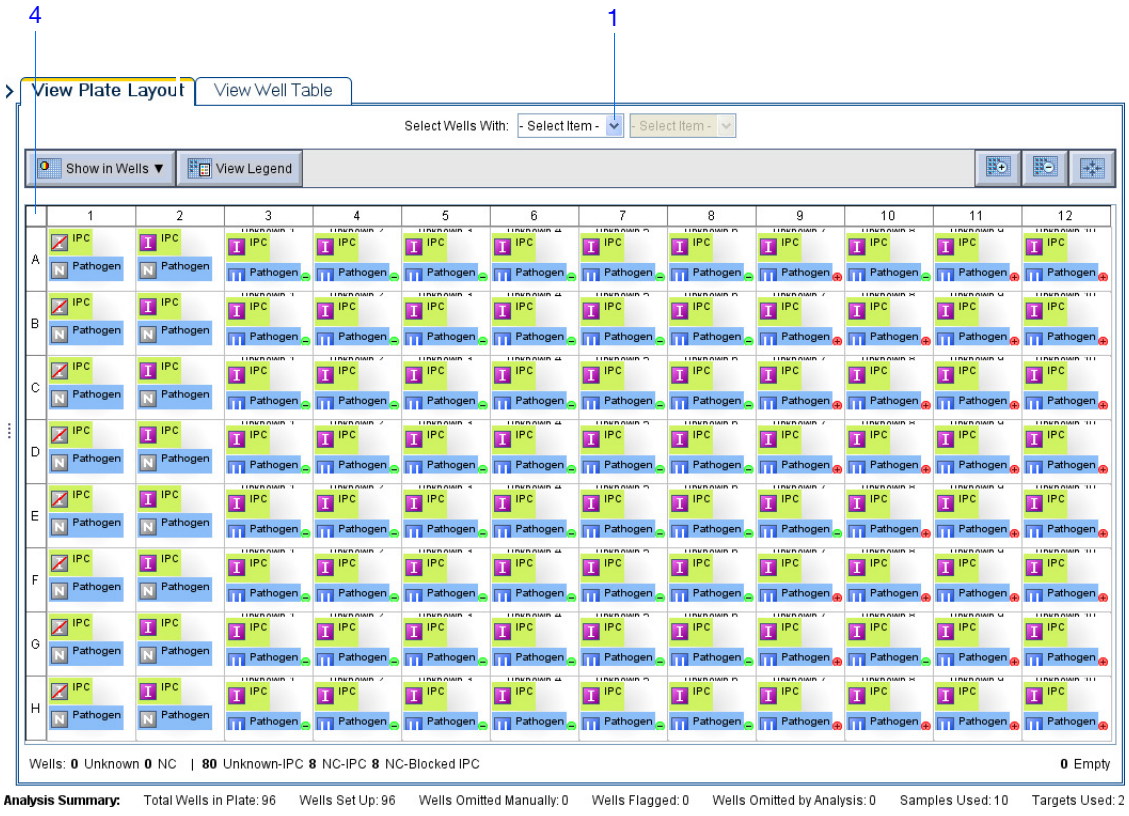
- Immediately after a run, the 7500 software automatically analyzes the data using the default analysis settings, then displays the Presence/Absence Plot on your computer.
- To reanalyze the data, select all the wells in the plate layout, then click **Analyze**.

Navigation Tips

How to Select Wells

To display specific wells in the analysis screens, select the wells in the View Plate Layout tab as follows:

1. To select wells of a specific type, use the Select Wells With drop-down lists: Select **Sample**, **Target**, or **Task**, then select the sample, target, or task name.
2. To select one well, click the well in the plate layout.
3. To select multiple wells, click and drag over the desired wells, or **CTRL-click**, or press **Shift-click** in the plate layout.
4. To select all 96 wells, click the top left corner of the plate layout.



Notes

How To Display Multiple Plots

Use the Multiple Plots view to display up to four plots simultaneously. To navigate within the Multiple Plots view:

1. From the navigation pane, select **Analysis** ▸ **Multiple Plots View**.
2. To display four plots, click **Show plots in a 2 × 2 matrix**.
3. To display two plots in rows, click **Show plots in two rows**.
4. To display two plots in columns, click **Show plots in two columns**.
5. To display a specific plot, select the plot from the drop-down list above each plot display.

Notes

View the Presence/Absence Plot

The Presence/Absence plot displays the intensity of the fluorescence for each well position. There are four Presence/Absence plot views available:

- All Calls
- Presence calls only
- Absence calls only
- Unconfirmed calls

For each view, you can choose to:

- Show IPC
- Show Controls

About the Example Experiment

In the presence/absence experiment:

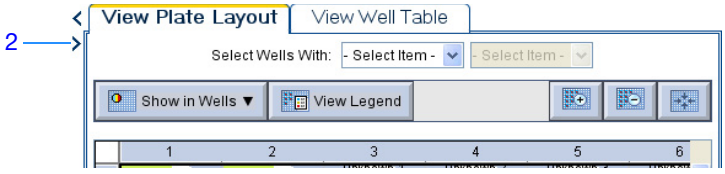
- The target is absent in samples Unknown 1, Unknown 2, Unknown 3, Unknown 4, Unknown 5, and Unknown 6.
- The target is present in samples Unknown 7, Unknown 8, Unknown 9, and Unknown 10.
- There are no unconfirmed wells.
- The IPC succeeded in all wells.

View the Presence/Absence Plot

1. From the navigation pane, select **Analysis** ▸ **Presence/Absence Plot**.

   Note: If no data are displayed, click **Analyze**.

2. For an expanded display of the 96 wells of the Presence/Absence Plot, click › in the upper left corner of the plate layout in the View Plate Layout tab.



3. From the Target Reporter drop-down list, select **Pathogen**.
4. From the Control Reporter drop-down list, select **IPC**.
5. From the Show Calls drop-down list, select **All Calls**.
6. Click the (Show a legend for the plot).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

Notes

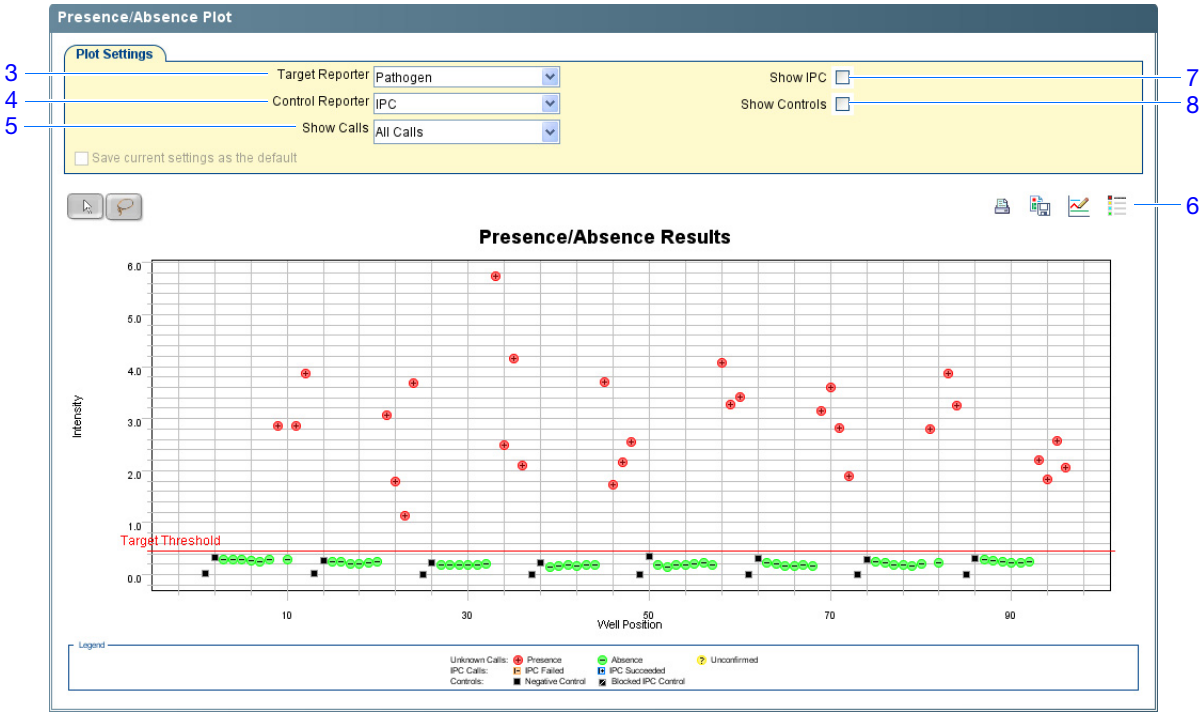
7. Click the **Show IPC** check box to view the fluorescence intensity of the IPC target in the Unknown-IPC wells.

8. Click the **Show Controls** check box to view the fluorescence intensity of the IPC target in the Negative Control-IPC wells and the Negative Control-Blocked IPC wells.

9. To view the fluorescence intensity of only the presence calls, select **Presence** from the Show Calls drop-down list.

10. To view the fluorescence intensity of only the absence calls, select **Absence** from the Show Calls drop-down list.

11. To view the fluorescence intensity of only the unconfirmed calls, select **Unconfirmed** from the Show Calls drop-down list. The presence/absence example experiment does not contain unconfirmed calls.



Analysis Guidelines

When you analyze your own experiment, review the presence/absence plot for:

- **The IPC threshold** – this value is calculated from the Negative Control-Blocked IPC reactions.
- **The Target Threshold** – this value is calculated from the Negative Control-IPC reactions. If the target's intensity is:
  - Above the target threshold, the call is present (regardless of the intensity of the IPC).
  - Below the target threshold, and the IPC's intensity is above the IPC threshold, the call is absent.

Notes

  - Below the target threshold, and the IPC's intensity is below the IPC threshold, the call is unconfirmed.
- **Target Calls**:
  - Presence
  - Absence
  - Unconfirmed
- **IPC Calls**:
  - IPC Succeeded
  - IPC Failed
- **Control Well Calls**:
  - Negative Control - IPC
  - Negative Control - Blocked IPC

For More Information For more information on the Presence/Absence Plot screen, access the 7500 Software Help by clicking or pressing **F1**.

View the Amplification Plot

Note: The amplification plot is available only if the Run Method included an amplification stage that included data collection. For more information, see "About Data Collection" on page 2, "Set Up the Run Method" on page 25, and "About the Amplification Plot Screen" on page 55.

The amplification plot displays amplification of all samples in the selected wells. There are three amplification plot views available:

- **ΔRn vs Cycle** – ΔRn is the difference in normalized fluorescence generated by the reporter between the pre-PCR read and the post-PCR read. This plot displays ΔRn as a function of cycle number. You can use this plot to identify and examine irregular amplification and to view threshold and baseline values for the run.
- **Rn vs Cycle** – Rn is the fluorescence from the reporter dye normalized to the fluorescence from the passive reference. This plot displays Rn as a function of cycle number. You can use this plot to identify and examine irregular amplification.
- **C_T vs Well** – C_T is the PCR cycle number at which the fluorescence equals the threshold in the amplification plot. This plot displays C_T as a function of well position. You can use this plot to locate outlying amplification (outliers).

IMPORTANT! Amplification plots are not used to make presence/absence calls. Examine the plots to help with troubleshooting and quality control.

Notes

About the Example Experiment

In the presence/absence example experiment, you review the amplification plot for:

- Correct baseline and threshold values
- Irregular amplification
- Outliers

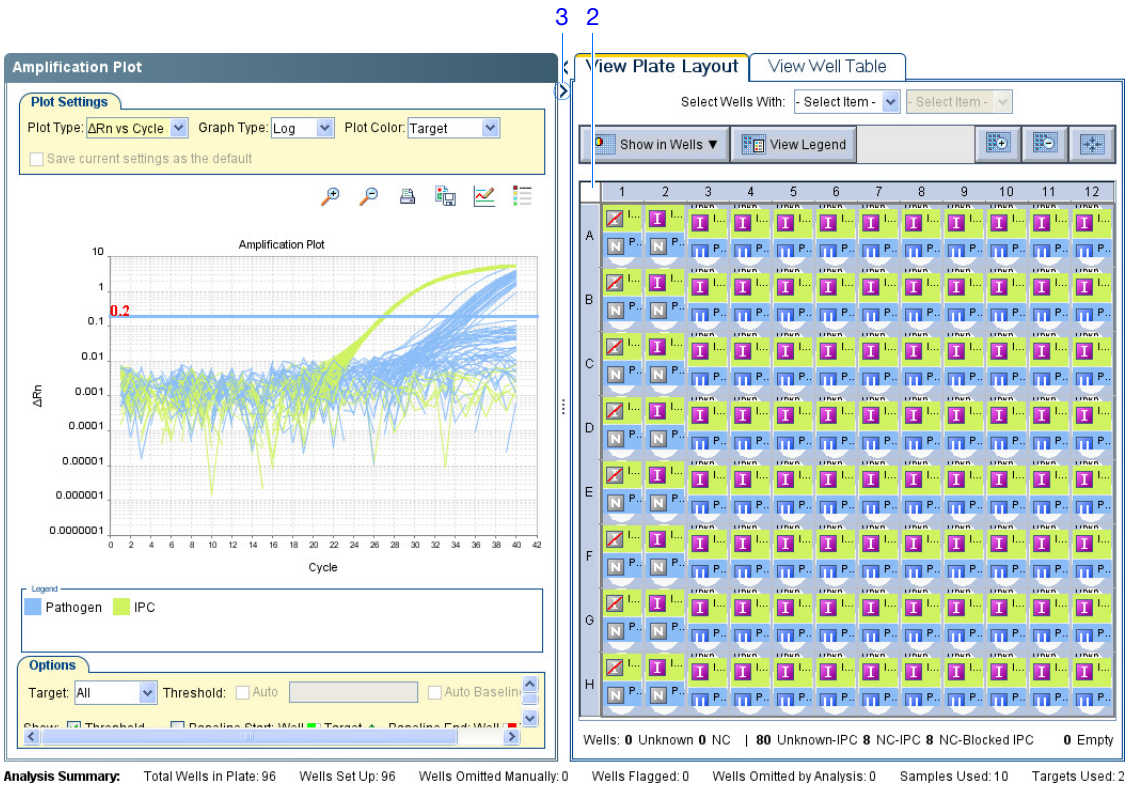
View the Amplification Plots

1. From the navigation pane, select **Analysis** ▸ **Amplification Plot**.

   Note: If no data are displayed, click **Analyze**.

2. Display all 96 wells in the amplification plot by clicking the upper left corner of the plate layout in the View Plate Layout tab.

3. For an expanded display of the Amplification Plot, click ❯ in the upper left corner of the plate layout in the View Plate Layout tab.



4. In the Amplification Plot screen:

   a. From the Plot Type drop-down list, select **ΔRn vs Cycle**.

   b. From the Plot Color drop-down list, select **Well**.

   c. Click the (Show a legend for the plot).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

Notes

d. From the Target dropdown menu, select **All**.

5. View the baseline values:

   a. From the Graph Type drop-down list, select **Linear**.

   b. Select the **Baseline** check box to show the start cycle and end cycle.

   c. Verify that the baseline is set correctly: The end cycle should be set a few cycles before the cycle number where significant fluorescence is detected. In the example experiment, the baseline is set correctly.

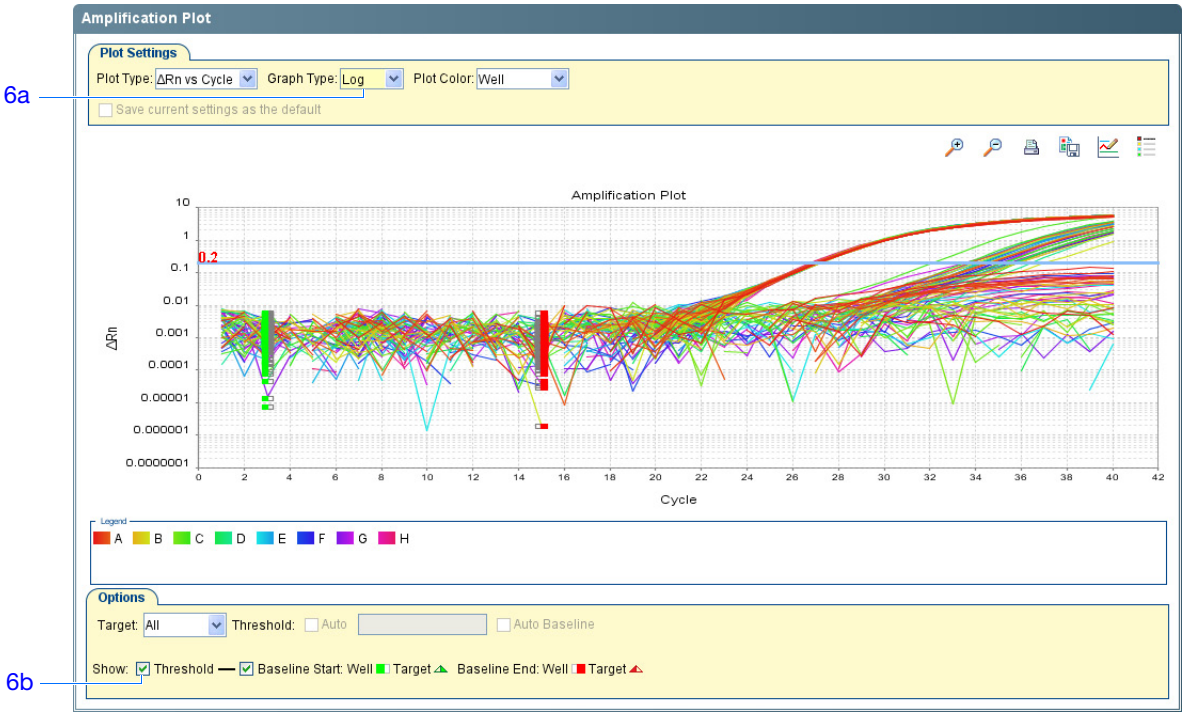
6. View the threshold values:

   a. From the Graph Type drop-down list, select **Log**.

   b. Select the Threshold check box to show the threshold.

   c. Verify that the threshold is set correctly.

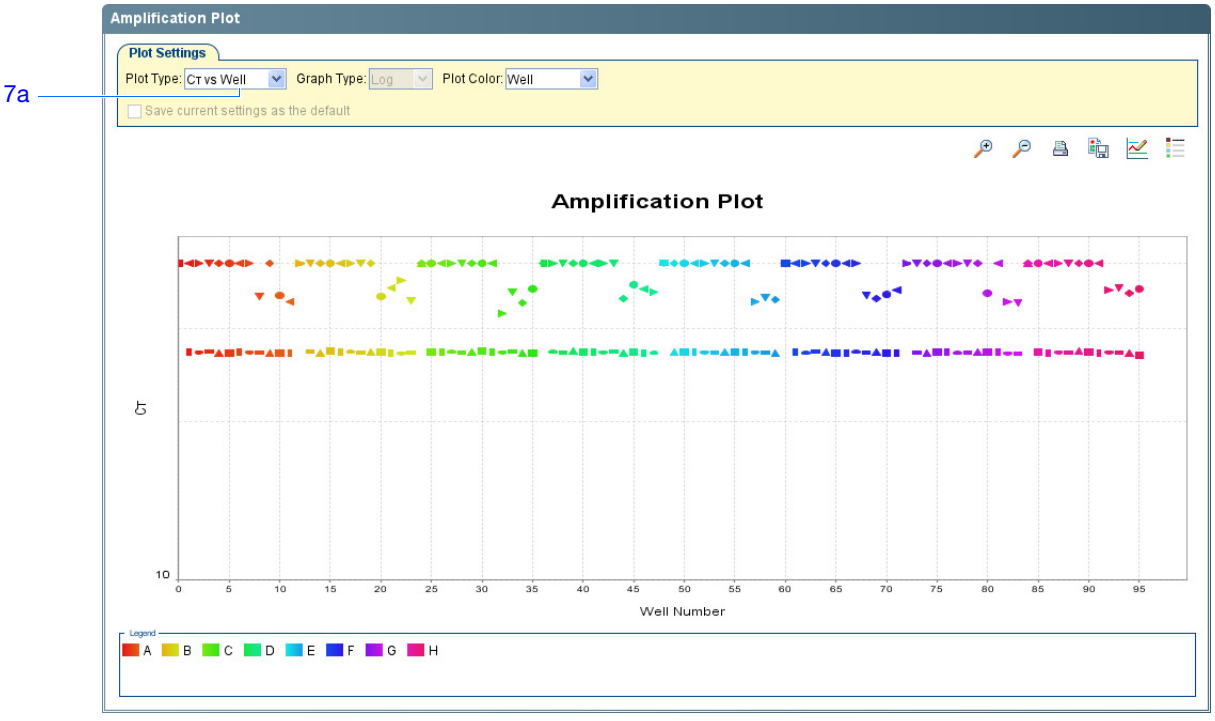
Notes



7. Locate any outliers:

   a. From the Plot Type drop-down list, select **C_T vs Well**.

   b. Look for outliers from the amplification plot.



Notes

Analysis Guidelines

When you analyze your own presence/absence experiment, look for:

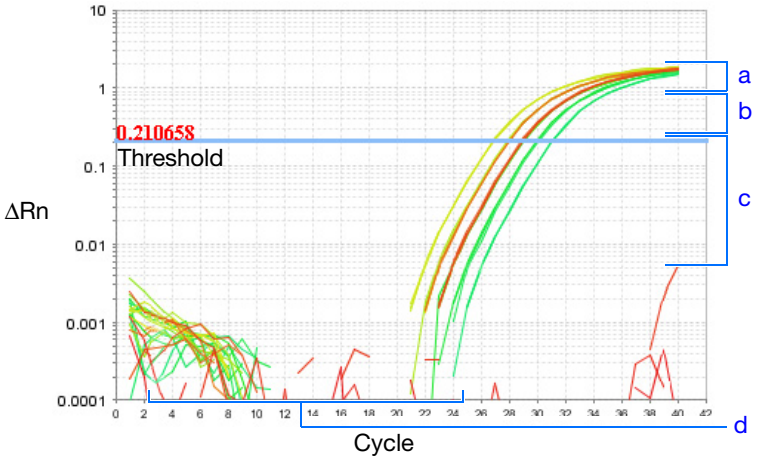
- Outliers
- A typical amplification plot – The 7500 software calculates baseline and threshold values based on the assumption that the data exhibit a *typical* amplification curve. A typical amplification plot has four distinct sections:

  a. Plateau phase

  b. Linear phase

  c. Exponential (geometric phase)

  d. Baseline

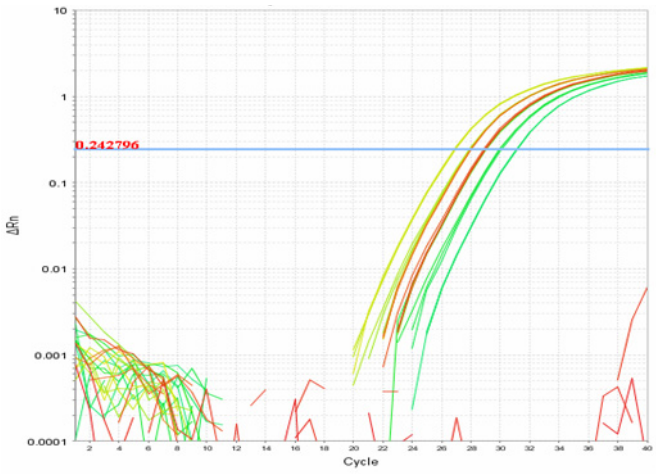
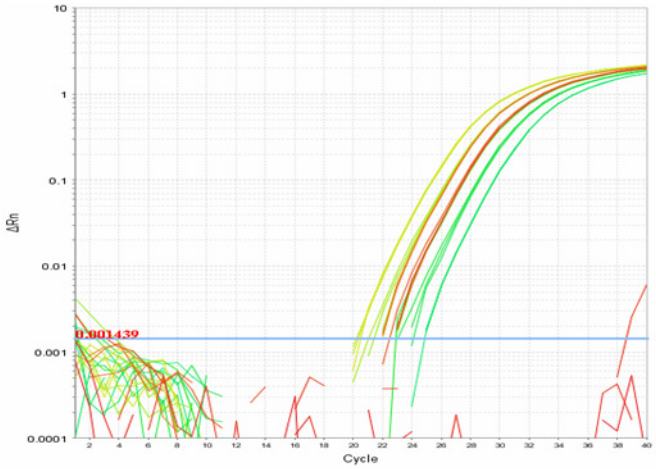
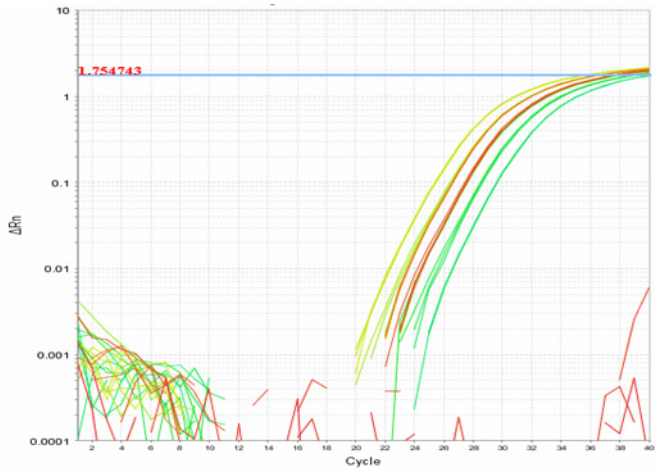


IMPORTANT! Experimental error (such as contamination or pipetting errors) can produce atypical amplification curves that can result in incorrect baseline and threshold value calculations by the 7500 software. Therefore, Applied Biosystems recommends that you examine the Amplification Plot and review the assigned baseline and threshold values for each well after analysis completes.

- Correct baseline and threshold values – See the threshold examples on page 72 and the baseline examples on page 73.

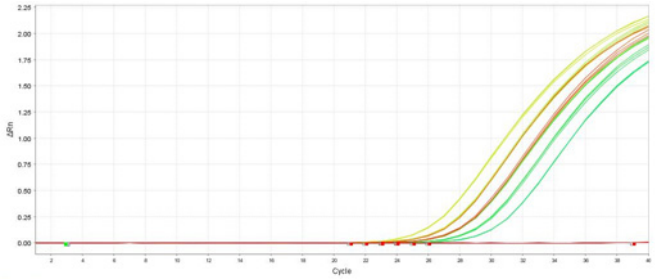
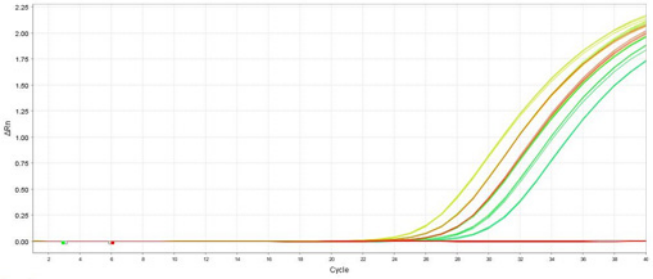
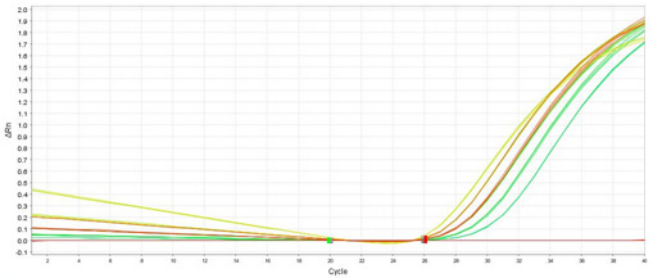
Notes

Threshold Examples

Threshold Set Correctly The threshold is set in the exponential phase of the amplification curve. Threshold settings above or below the optimum increase the standard deviation of the replicate groups.	
Threshold Set Too Low The threshold is set below the exponential phase of the amplification curve. The standard deviation is significantly higher than that for a plot where the threshold is set correctly. Drag the threshold bar up into the exponential phase of the curve.	
Threshold Set Too High The threshold is set above the exponential phase of the amplification curve. The standard deviation is significantly higher than that for a plot where the threshold is set correctly. Drag the threshold bar down into the exponential phase of the curve.	

Notes

Baseline Examples

Baseline Set Correctly The amplification curve begins after the maximum baseline.	
Baseline Set Too Low The amplification curve begins too far to the right of the maximum baseline. Increase the End Cycle value.	
Baseline Set Too High The amplification curve begins before the maximum baseline. Decrease the End Cycle value.	

If your experiment does not meet the guidelines above:

- Manually adjust the baseline and/or threshold (see "View the Analysis Settings" on page 80).

  or

- Omit wells, (see "Omit Wells from the Analysis" on page 84).

For More Information

For more information on the Amplification Plot screen, access the 7500 Software Help by clicking or pressing **F1**.

View the Well Table

The Well Table displays results data for each well in the reaction plate, including:

- The well number, sample name, target name, task, and dyes
- The calculated values: ΔRn, ΔRn mean, and ΔRn SD

Note: ΔRn, ΔRn mean, and ΔRn SD are calculated only when the analysis call settings specify to analyze data from the pre-PCR read and the post-PCR read.

- Target and IPC thresholds, Call, Comments
- Flags

About the Example Experiment

In the presence/absence example experiment, you review the Well Table for:

- Call
- ΔRn
- Flag

View the Well Table

1. From the navigation pane, select **Analysis**, then select the **View Well Table** tab.

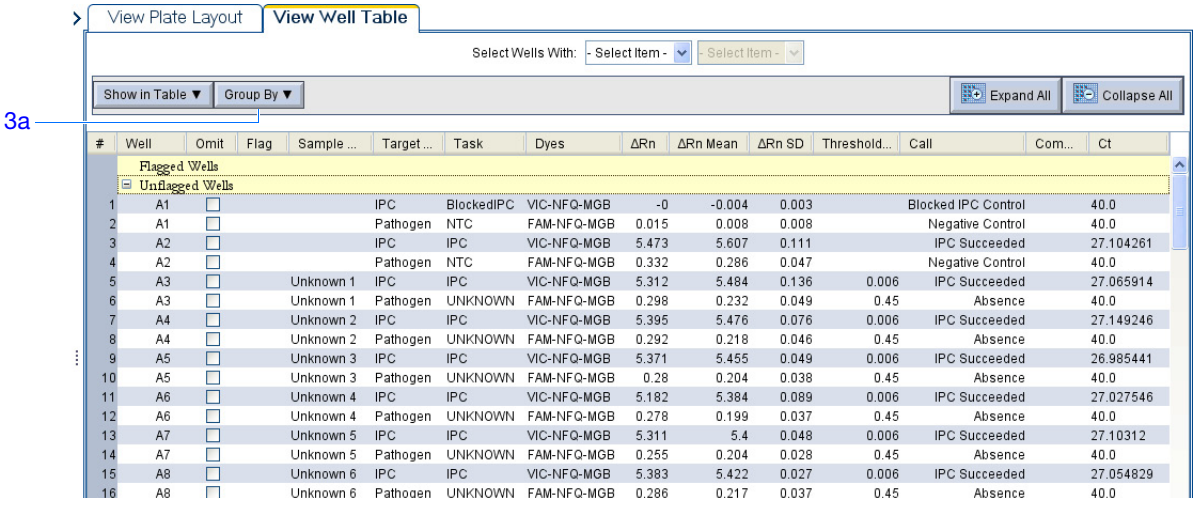
   Note: If no data are displayed, click **Analyze**.

2. For an expanded display of the Well Table, click ‹ in the upper left corner of the plate layout in the View Plate Layout tab.

3. Use the Group By drop-down list to group wells by a specific category. For the example experiment, group the wells by flag, call, and ΔRn value.

   Note: You can select only one category at a time.

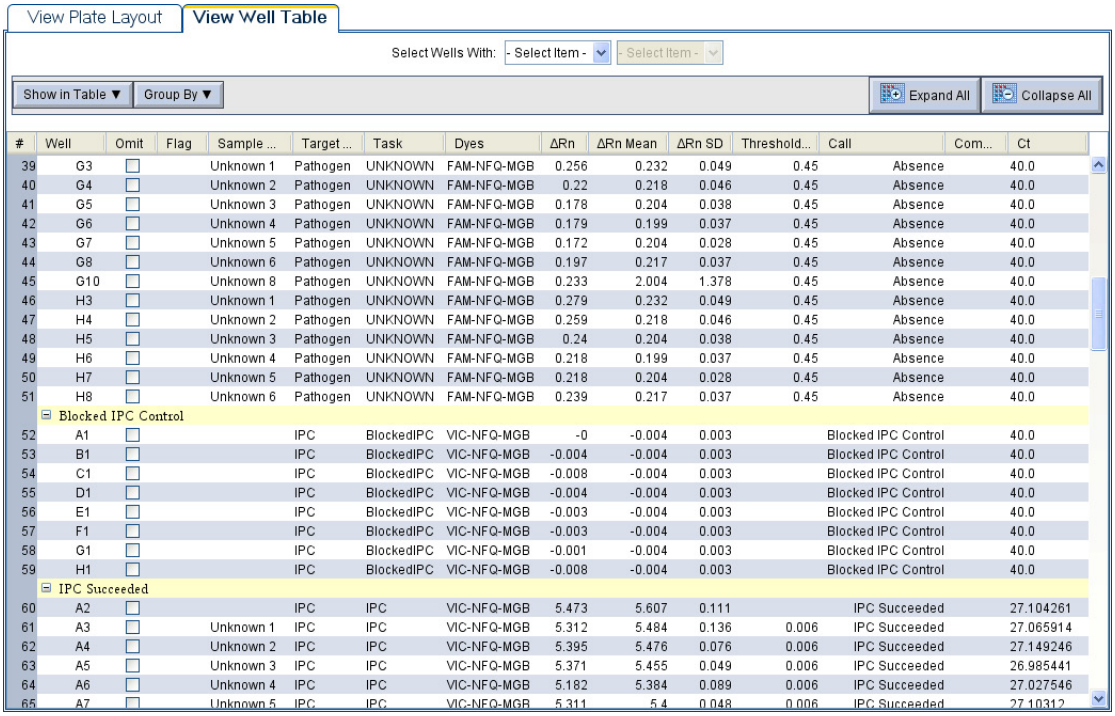
   a. From the Group By drop-down list, select **Flag**. In the example experiment, 96 wells are listed under Unflagged.



Notes

b. From the Group By drop-down list, select **Call**. Wells are listed in the order:

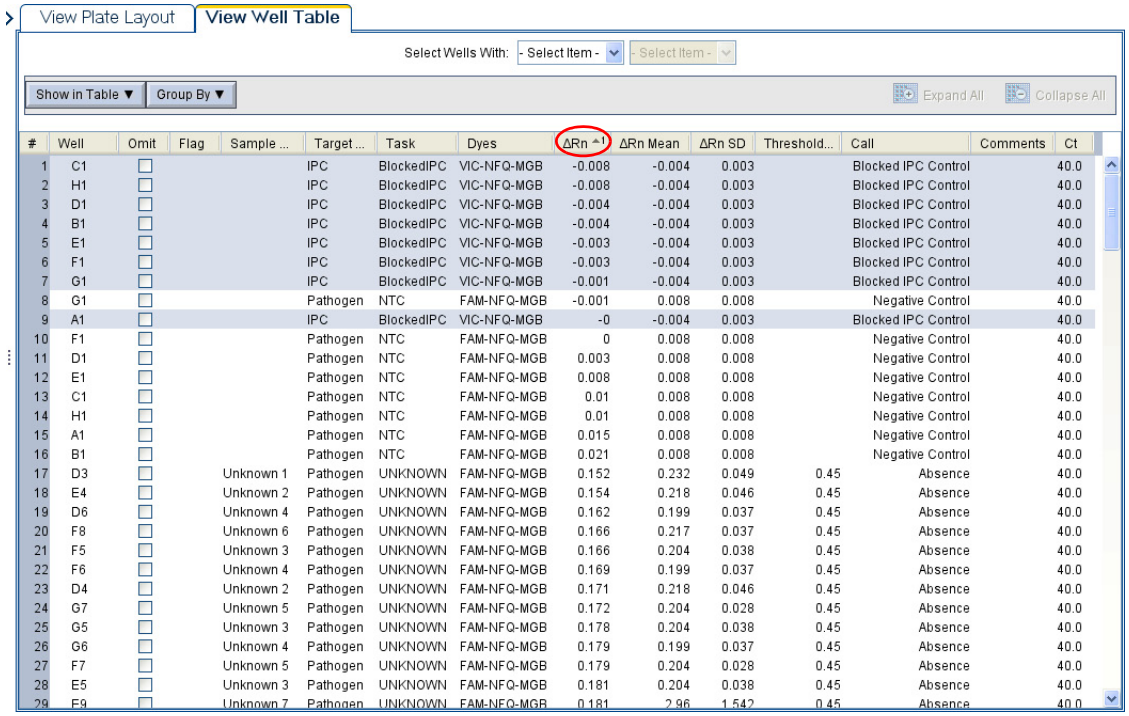
- Absence
- Blocked IPC Control
- IPC Failed
- IPC Succeeded
- Negative Control
- Presence
- Unconfirmed
- No Call



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	ΔRn	ΔRn Mean	ΔRn SD	Threshold...	Call	Com...	Ct
39	G3	☐		Unknown 1	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.256	0.232	0.049	0.45	Absence		40.0
40	G4	☐		Unknown 2	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.22	0.218	0.046	0.45	Absence		40.0
41	G5	☐		Unknown 3	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.178	0.204	0.038	0.45	Absence		40.0
42	G6	☐		Unknown 4	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.179	0.199	0.037	0.45	Absence		40.0
43	G7	☐		Unknown 5	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.172	0.204	0.028	0.45	Absence		40.0
44	G8	☐		Unknown 6	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.197	0.217	0.037	0.45	Absence		40.0
45	G10	☐		Unknown 8	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.233	2.004	1.378	0.45	Absence		40.0
46	H3	☐		Unknown 1	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.279	0.232	0.049	0.45	Absence		40.0
47	H4	☐		Unknown 2	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.259	0.218	0.046	0.45	Absence		40.0
48	H5	☐		Unknown 3	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.24	0.204	0.038	0.45	Absence		40.0
49	H6	☐		Unknown 4	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.218	0.199	0.037	0.45	Absence		40.0
50	H7	☐		Unknown 5	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.218	0.204	0.028	0.45	Absence		40.0
51	H8	☐		Unknown 6	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.239	0.217	0.037	0.45	Absence		40.0
	⊟ Blocked IPC Control													
52	A1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0	-0.004	0.003		Blocked IPC Control		40.0
53	B1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.004	-0.004	0.003		Blocked IPC Control		40.0
54	C1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.008	-0.004	0.003		Blocked IPC Control		40.0
55	D1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.004	-0.004	0.003		Blocked IPC Control		40.0
56	E1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.003	-0.004	0.003		Blocked IPC Control		40.0
57	F1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.003	-0.004	0.003		Blocked IPC Control		40.0
58	G1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.001	-0.004	0.003		Blocked IPC Control		40.0
59	H1	☐			IPC	BlockedIPC	VIC-NFQ-MGB	-0.008	-0.004	0.003		Blocked IPC Control		40.0
	⊟ IPC Succeeded													
60	A2	☐			IPC	IPC	VIC-NFQ-MGB	5.473	5.607	0.111		IPC Succeeded		27.104261
61	A3	☐		Unknown 1	IPC	IPC	VIC-NFQ-MGB	5.312	5.484	0.136	0.006	IPC Succeeded		27.065914
62	A4	☐		Unknown 2	IPC	IPC	VIC-NFQ-MGB	5.395	5.476	0.076	0.006	IPC Succeeded		27.149246
63	A5	☐		Unknown 3	IPC	IPC	VIC-NFQ-MGB	5.371	5.455	0.049	0.006	IPC Succeeded		26.985441
64	A6	☐		Unknown 4	IPC	IPC	VIC-NFQ-MGB	5.182	5.384	0.089	0.006	IPC Succeeded		27.027546
65	A7	☐		Unknown 5	IPC	IPC	VIC-NFQ-MGB	5.311	5.4	0.048	0.006	IPC Succeeded		27.10312

Notes

c. From the Group By drop-down list, select **None**. In the table, click the column heading **ΔRn**. Wells are listed in order of increasing ΔRn. Click the column heading again to reverse the sort order.



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	ΔRn	ΔRn Mean	ΔRn SD	Threshold...	Call	Comments	Ct
1	C1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.008	-0.004	0.003		Blocked IPC Control		40.0
2	H1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.008	-0.004	0.003		Blocked IPC Control		40.0
3	D1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.004	-0.004	0.003		Blocked IPC Control		40.0
4	B1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.004	-0.004	0.003		Blocked IPC Control		40.0
5	E1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.003	-0.004	0.003		Blocked IPC Control		40.0
6	F1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.003	-0.004	0.003		Blocked IPC Control		40.0
7	G1				IPC	BlockedIPC	VIC-NFQ-MGB	-0.001	-0.004	0.003		Blocked IPC Control		40.0
8	G1				Pathogen	NTC	FAM-NFQ-MGB	-0.001	0.008	0.008		Negative Control		40.0
9	A1				IPC	BlockedIPC	VIC-NFQ-MGB	-0	-0.004	0.003		Blocked IPC Control		40.0
10	F1				Pathogen	NTC	FAM-NFQ-MGB	0	0.008	0.008		Negative Control		40.0
11	D1				Pathogen	NTC	FAM-NFQ-MGB	0.003	0.008	0.008		Negative Control		40.0
12	E1				Pathogen	NTC	FAM-NFQ-MGB	0.008	0.008	0.008		Negative Control		40.0
13	C1				Pathogen	NTC	FAM-NFQ-MGB	0.01	0.008	0.008		Negative Control		40.0
14	H1				Pathogen	NTC	FAM-NFQ-MGB	0.01	0.008	0.008		Negative Control		40.0
15	A1				Pathogen	NTC	FAM-NFQ-MGB	0.015	0.008	0.008		Negative Control		40.0
16	B1				Pathogen	NTC	FAM-NFQ-MGB	0.021	0.008	0.008		Negative Control		40.0
17	D3			Unknown 1	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.152	0.232	0.049	0.45	Absence		40.0
18	E4			Unknown 2	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.154	0.218	0.046	0.45	Absence		40.0
19	D6			Unknown 4	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.162	0.199	0.037	0.45	Absence		40.0
20	F8			Unknown 6	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.166	0.217	0.037	0.45	Absence		40.0
21	F5			Unknown 3	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.166	0.204	0.038	0.45	Absence		40.0
22	F6			Unknown 4	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.169	0.199	0.037	0.45	Absence		40.0
23	D4			Unknown 2	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.171	0.218	0.046	0.45	Absence		40.0
24	G7			Unknown 5	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.172	0.204	0.028	0.45	Absence		40.0
25	G5			Unknown 3	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.178	0.204	0.038	0.45	Absence		40.0
26	G6			Unknown 4	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.179	0.199	0.037	0.45	Absence		40.0
27	F7			Unknown 5	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.179	0.204	0.028	0.45	Absence		40.0
28	E5			Unknown 3	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.181	0.204	0.038	0.45	Absence		40.0
29	F9			Unknown 7	Pathogen	UNKNOWN	FAM-NFQ-MGB	0.181	2.96	1.542	0.45	Absence		40.0

Analysis Guidelines

When you analyze your own presence/absence experiment, group the wells by:

- **Flag** –The software groups the flagged and unflagged wells. A flag indicates that the software found an error in the flagged well. For a description of the 7500 software flags, see "View the QC Summary" on page 82.
- **Call** – The software groups the wells by call: Negative Control, Blocked-IPC, Presence, Absence, Unconfirmed, IPC Succeeded, IPC Failed, and No Call.

How to Use the Plate Layout to View Calls

You can use the Plate Layout to display presence/absence calls, and to select the data displayed in the Presence Absence Plot. To:

- Change the data displayed in the Plate Layout – Click **Show in Wells**, then select (or deselect) the data that you want the Plate Layout to display.
- Select wells based on the sample, target, or task – In the Select Wells With drop-down lists, select **Sample**, **Target**, or **Task**, then select the sample, target, or task name. The 7500 software selects the associated wells in the Presence Absence Plot.

For More Information

For more information on the Well Table, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Publish the Data

You can publish the experiment data in several ways:

- Save the plot as an image file
- Print the plot
- Print the plate layout
- Create slides
- Print a report
- Export data

For More Information

For information on performing these procedures, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Notes

Section 5.2 Troubleshoot (If Needed)

This section covers:

- View the Analysis Settings 80
- View the QC Summary 82
- Omit Wells from the Analysis 84
- View the Multicomponent Plot 86
- View the Raw Data Plot 88

Notes

View the Analysis Settings

The Analysis Settings dialog box displays the analysis settings for the call settings, threshold cycle (C_T), and flags. If the default analysis settings in the 7500 software are not suitable for your experiment, you can change the settings in the Analysis Settings dialog box, then reanalyze your experiment.

About the Example Experiment

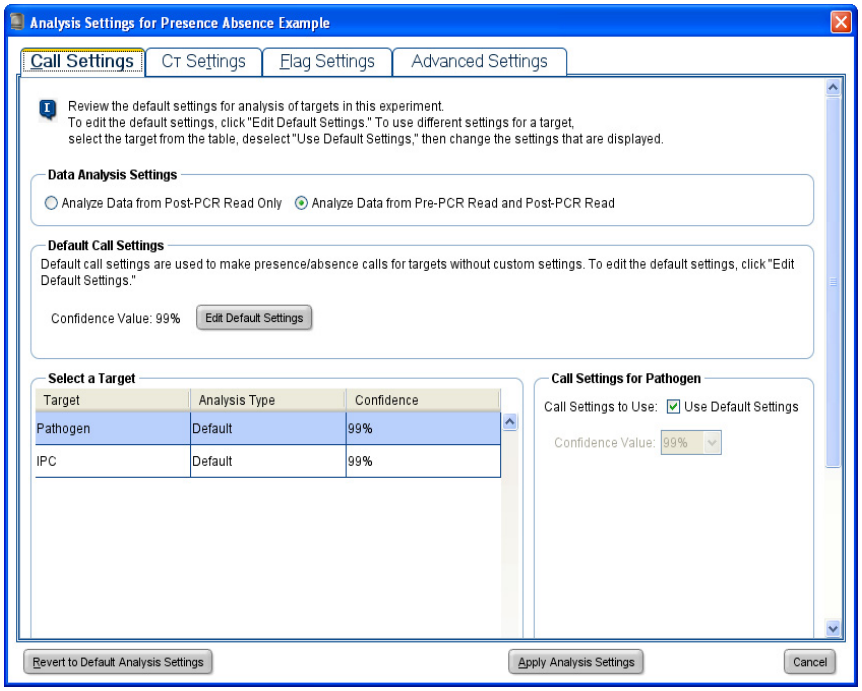
In the presence/absence example experiment, the default analysis settings are used without changes.

Review the Analysis Settings

1. From the navigation pane, select **Analysis**.
2. Click **Analysis Settings** to open the Analysis Settings dialog box.

In the example experiment, the default analysis settings are used for each tab:

- Call Settings
- C_T Settings
- Flag Settings



Notes

Analysis Guidelines

Unless you have already determined the optimal settings for your experiment, use the default analysis settings in the 7500 software. If the default settings are not suitable for your experiment, you can change the:

- **Call Settings –** Use this tab to specify whether to analyze data from the pre-PCR read or to edit the default confidence value used to make presence/absence calls for each target**.**
- **C_T Settings** (if the experiment includes amplification) – Use this tab to set the threshold and baseline either automatically or manually. When you manually set the threshold and baseline, keep the following Applied Biosystems recommendations in mind:

Setting	Recommendation
Threshold	Enter a value for the threshold so that the threshold is: • Above the background. • Below the plateau and linear regions of the amplification curve. • Within the exponential phase of the amplification curve.
Baseline	Select Start Cycle and End Cycle values so that the baseline ends before significant fluorescence is detected.

- **Flag Settings** – Use this tab to:
  - Adjust the sensitivity so that more wells or fewer wells are flagged.
  - Change the flags that are applied by the 7500 software.

For More Information

For more information on the analysis settings, access the 7500 Software Help by clicking or pressing **F1** when the Analysis Settings dialog box is open.

Notes

View the QC Summary

The QC Summary displays a list of the 7500 software flags. This summary includes the flag frequency and location for the open experiment.

About the Example Experiment

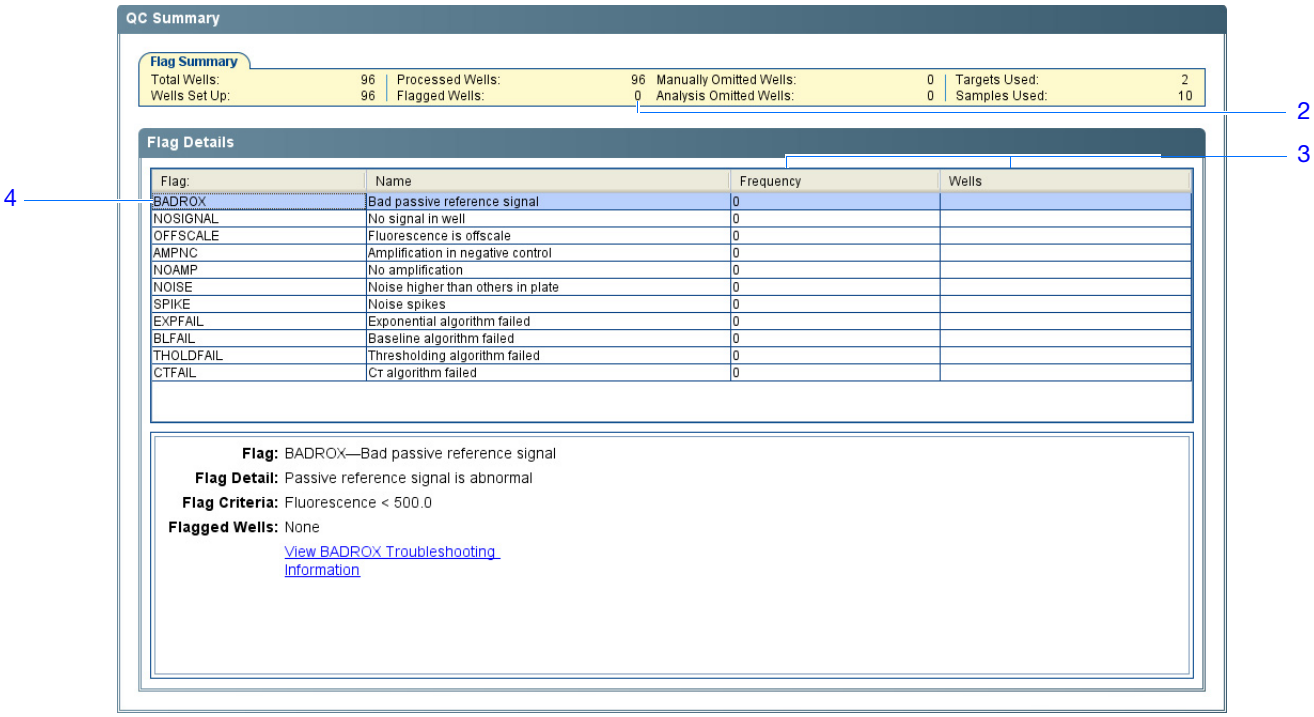
In the presence/absence example experiment, you review the QC Summary for any flags generated by the experiment data. In the example experiment, no flags have been generated.

View the QC Summary

1. From the navigation pane, select **Analysis** ▸ **QC Summary**.

   Note: If no data are displayed, click **Analyze**.

2. Review the Flag Summary.
3. In the Flag Details table, look in the Frequency and Wells columns to determine which flags appear in the experiment.
4. (Optional) Click each flag row to display detailed information about the flag.



Notes

Possible Flags

For presence/absence experiments, the flags listed below may be triggered by the experiment data.

Note: If the experiment does not include amplification, then the only flags are BADROX, NOSIGNAL, and OFFSCALE.

If a flag does not appear in the experiment, its frequency is 0. If the frequency is >0, the flag appears somewhere in the experiment; the well position is listed in the Wells column.

Flag	Description
AMPNC	Amplification in negative control
BADROX	Bad passive reference signal
BLFAIL	Baseline algorithm failed
CTFAIL	C_T algorithm failed
EXPFAIL	Exponential algorithm failed
NOAMP	No amplification
NOISE	Noise higher than others in plate
NOSIGNAL	No signal in well
OFFSCALE	Fluorescence is offscale
SPIKE	Noise spikes
THOLDFAIL	Thresholding algorithm failed

Analysis Guidelines

When you analyze your own presence/absence experiment:

- Click each flag in the Flag Details table with a frequency >0 to display detailed information about the flag. If needed, click the troubleshooting link to view information on correcting the flag.
- You can change the flag settings:
  - Adjust the sensitivity so that more wells or fewer wells are flagged.
  - Change the flags that are applied by the 7500 software.

For More Information

For more information on the QC Summary screen or on flag settings, access the 7500 Software Help by clicking or pressing **F1**.

Notes

Omit Wells from the Analysis

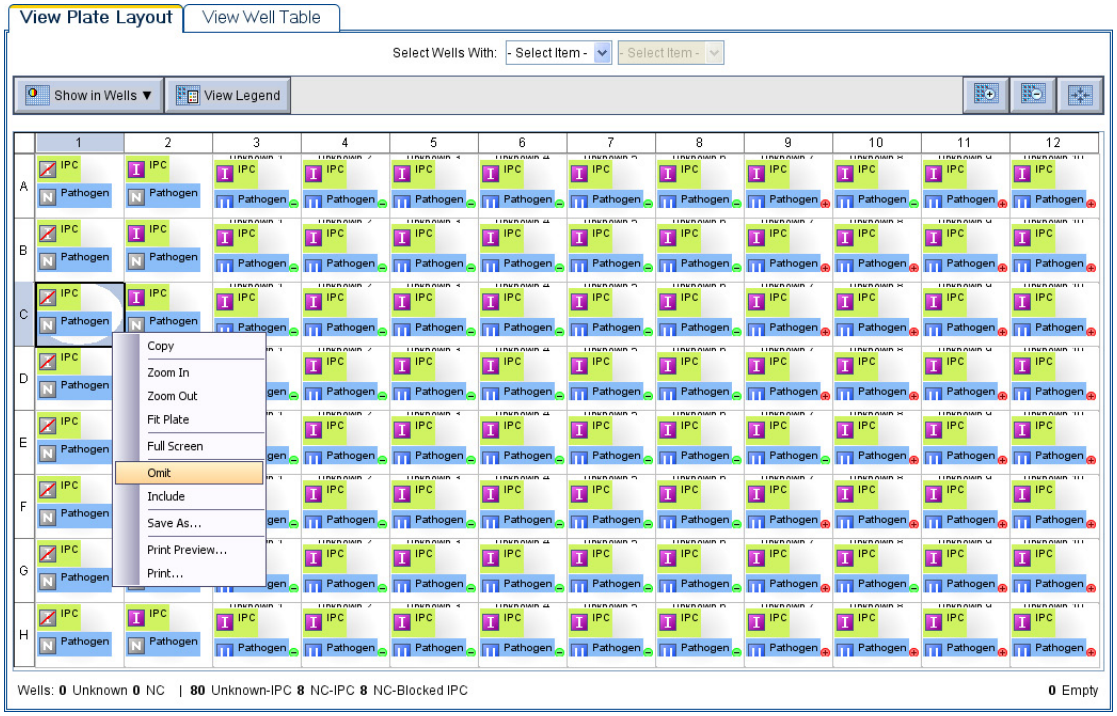
You may omit wells from analysis if you do not want to consider data generated by the well.

About the Example Experiment

In the presence/absence example experiment, there are no outliers; no wells need to be removed from analysis.

Omit Wells

1. Click the **View Plate Layout** tab.
2. Select one or more wells to omit from analysis.
3. Right-click the well(s), then select **Omit**. Results for the well are removed.



4. Click **Analyze** to reanalyze the data without the omitted wells.

Note: Alternatively, you can omit wells from the Well Table by selecting the checkbox in the Omit column for the wells you want to remove from the analysis. See "Analysis Guidelines" on page 85 for more information.

Notes

Analysis Guidelines

When you analyze your own presence/absence experiment, carefully consider which wells to omit from analysis. If needed, remove outliers manually using the Well Table.

1. From the navigation pane, select **Analysis** ▸ **Amplification Plot**.

   Note: If no data are displayed, click **Analyze**.

2. In the Amplification Plot screen, select **C_T vs Well** from the Plot Type drop-down list.
3. Select the **View Well Table** tab.
4. Review the Well Table:
   a. Look for any outliers.
   b. Select the Omit check box next to the outlying well(s).
5. Click **Analyze** to reanalyze the experiment data with the outlying well(s) removed from the analysis.

For More Information

For more information on omitting wells from the analysis, access the 7500 Software Help by clicking or pressing **F1**. Within the Help, search for the omit well topics:

1. Click the **Search** tab.
2. Enter **omit well**.
3. Click **List Topics**.
4. Double-click the topics you want to review.

Notes

View the Multicomponent Plot

The Multicomponent Plot screen displays the complete spectral contribution of each dye in a selected well over the duration of a PCR run.

About the Example Experiment

In the presence/absence example experiment, you review the Multicomponent Plot for:

- ROX™ dye
- VIC® dye
- FAM™ dye
- Spikes, dips, and/or sudden changes.
- Amplification in the negative control wells.

View the Multicomponent Plot

1. From the navigation pane, select **Analysis** ▸ **Multicomponent Plot.**

   Note: If no data are displayed, click **Analyze**.

2. Display the wells one at a time in the Multicomponent Plot screen:

   a. Click the **View Plate Layout** tab.

   b. Select one well in the plate layout; the well is shown in the Multicomponent Plot screen.

   Note: If you select multiple wells, the Multicomponent Plot screen displays the data for all selected wells simultaneously.

3. From the Plot Color drop-down list, select **Dye**.

4. Click the (Show a legend for the plot).

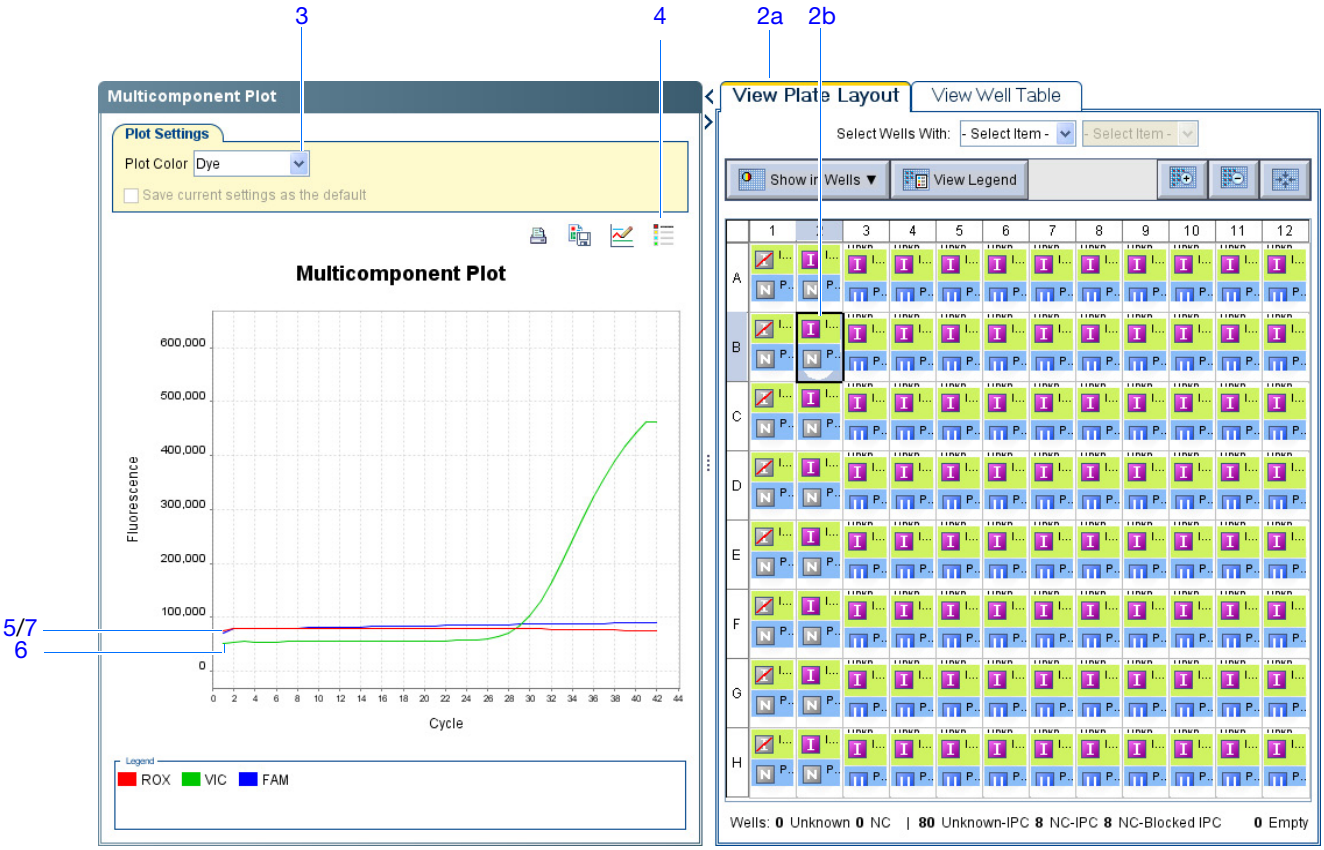
   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

5. Check the ROX dye signal. In the example experiment, the ROX dye signal remains constant throughout the run.

6. Check the VIC dye signal. In the example experiment, the VIC dye signal should not amplify for NC-Blocked IPC wells or if the IPC call for the Unknown-IPC well is IPC failed.

7. Check the FAM dye signal. In the example experiment, the FAM dye signal should amplify for Unknown-IPC wells that receive a target call of Presence.

Notes



Analysis Guidelines

When you analyze your own presence/absence experiment, look for:

- **Passive reference (ROX)**– The passive reference dye fluorescence level should remain relatively constant throughout the PCR process.
- **Reporter dye (FAM)** – The reporter dye fluorescence level should display a flat region corresponding to the baseline. If target is present in the sample (a Presence call is made), the baseline will be followed by a rapid rise in fluorescence as the amplification proceeds.
- **Any irregularities in the signal** – There should not be any spikes, dips, and/or sudden changes in the fluorescence.
- **Negative control wells** – There should not be any amplification in the negative control wells.

For More Information

For more information on the Multicomponent Plot screen, access the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Raw Data Plot

The Raw Data Plot screen displays the raw fluorescence (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

About the Example Experiment

In the presence/absence example experiment, you review the Raw Data Plot screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

View the Raw Data Plot

1. From the navigation pane, select **Analysis** ▸ **Raw Data Plot**.

   Note: If no data are displayed, click **Analyze**.

2. Click the (Show a legend for the plot).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

   Note: The legend displays the color code for each row of the reaction plate.

3. Display all 96 wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the View Plate Layout tab.

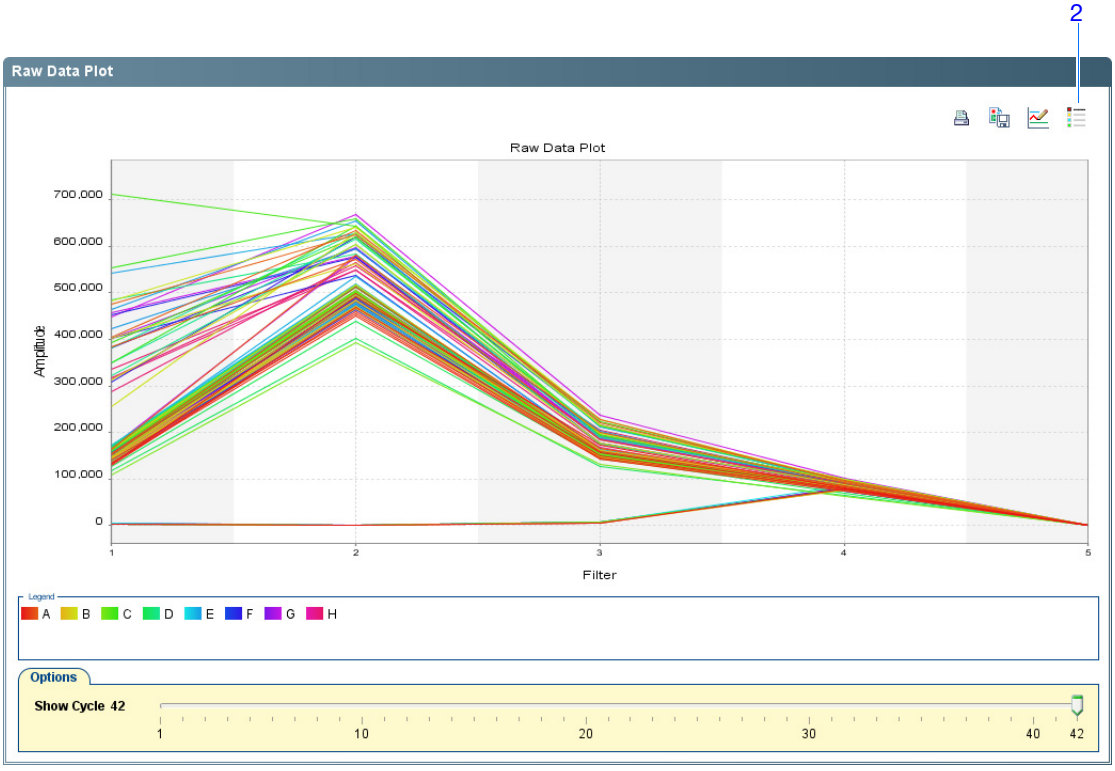
4. In the View Plate Layout tab, select wells corresponding to a replicate group. For:
   - Unknown 1 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 1**.
   - Unknown 2 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 2**.
   - Unknown 3 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 3**.
   - Unknown 4 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 4**.
   - Unknown 5 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 5**.
   - Unknown 6 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 6**.
   - Unknown 7 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 7**.
   - Unknown 8 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 8**.
   - Unknown 9 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 9**.
   - Unknown 10 wells: From the Select Wells with drop-down lists, select **Sample** and **Sample 10**.

Notes

- Negative control-IPC wells: Select wells **A2**, **B2**, **C2**, **D2**, **E2**, **F2**, **G2**, and **H2**.
- Negative control-blocked IPC wells: Select wells **A1**, **B1**, **C1**, **D1**, **E1**, **F1**, **G1**, and **H1**.

5. Click and drag the Show Cycle pointer from cycle 1 to cycle 42, and review the data from each filter:
   - The data from filter 1 correspond to the signal from FAM™ dye, the reporter for the pathogen target. Increase indicates amplification of the target.
   - The data from filter 2 correspond to the signal from VIC®dye, the reporter for the IPC target. Increase indicates amplification of the IPC.
   - The data from filter 4 correspond to the signal from ROX™ dye, which is used as the passive reference.

6. Repeat steps 4 and 5 for each replicate group.



The filters are:

Filter	1	2	3	4	5
Dye(s)	• FAM™ dye • SYBR® Green dye	• JOE™ dye • VIC® dye	• TAMRA™ dye • NED™ dye • Cy3® dye	• ROX™ dye • Texas Red® dye	Cy5® dye

Notes

Analysis Guidelines

When you analyze your own presence/absence experiment, look for the following in each filter:

- Characteristic signal growth
- No abrupt changes or dips

For More Information

For more information on the Raw Data Plot screen, access the 7500 Software Help by clicking or pressing **F1**.

Notes

A

Alternate Experiment Workflows

This appendix covers:

- Advanced Setup Workflow 92
- QuickStart Workflow 93
- Template Workflow 95
- Export/Import Workflow 96

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Advanced Setup Workflow

When you create an experiment using Advanced Setup in the 7500 software, you can set up the experiment according to your own design.

1. Double-click (7500 Software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***

   where *<software name>* is the current version of the 7500 software.

2. In the Home screen, click **Advanced Setup**.

   Note: If you do not see the Advanced Setup icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

3. Complete the setup screens to set up a new experiment:

   a. Click **Experiment Properties** (default), enter the experiment name, then select the experiment properties.

   b. Click **Plate Setup:**

Experiment Type	Action
Genotyping	Define the SNP assays, then assign them to wells in the reaction plate.
All other experiments	Define the targets, then assign them to wells in the reaction plate.

   c. Click **Run Method**, review the reaction volume and thermal profile, then edit as needed.

   d. Click **Reaction Setup**, review the components and calculated volumes for the PCR reactions, then edit as needed.

   e. (Optional) Click **Materials List**, review the list of materials, then order the materials you need to prepare the reaction plate.

4. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

Notes

5. Run the experiment:

   IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

   a. Load the reaction plate into the instrument.
   b. Start the run
   c. (Optional) Monitor the run.
   d. Unload the reaction plate from the instrument.

6. Analyze the data:

   a. Open the experiment in the 7500 software.
   b. In the navigation pane, click **Analysis**.
   c. If the data are not analyzed, click **Analyze**.
   d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

QuickStart Workflow

When you create an experiment using QuickStart, you can run the reactions on the instrument with no reaction plate setup information.

1. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

2. QuickStart the experiment:

   a. Double-click (7500 Software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***
   where *<software name>* is the current version of the 7500 software.

Notes

b. In the Home screen, click **QuickStart**.

c. Select the **Experiment Properties** tab (default), enter the experiment name, then select the experiment properties.

d. Select the **Run Method** tab, review the reaction volume and thermal profile, then edit as needed.

3. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

a. Load the reaction plate into the instrument.

b. Start the run

c. (Optional) Monitor the run.

d. Unload the reaction plate from the instrument.

4. In the 7500 software, complete the plate setup:

Experiment Type	Select and complete the…
Genotyping	a. **Define SNP Assays and Samples** tab. b. **Assign SNP Assays and Samples** tab.
All other experiments	a. **Define Targets and Samples** tab. b. **Assign Targets and Samples** tab.

5. Analyze the data:

a. Open the experiment in the 7500 software.

b. In the navigation pane, click **Analysis**.

c. If the data are not analyzed, click **Analyze**.

d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

Template Workflow

You can use a template to create a new experiment. Templates allow you to create many experiments with the same setup information.

Create a Template

1. Double-click (7500 Software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where *<software name>* is the current version of the 7500 software.

2. Open an existing experiment, or create a new experiment.

   Note: You can create a new experiment using the Design Wizard (see Chapter 2) or Advanced Setup (see page 92).

3. Select **File** ▸ **Save As Template**.
4. Enter a file name, select a location for the template, then click **Save**.
5. Click **Close**.

Create an Experiment with a Template

1. In the Home screen, click **Template**.

   Note: If you do not see the Template icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

2. Locate and select the template you created above, then click **Open**. A new experiment is created using the setup information from the template:
   - Experiment properties
   - Plate setup
   - Run method
   - Reaction setup
3. (Optional) If you want to modify the experiment, use Advanced Setup (see page 92).
4. Click **Save**, enter a file name, then click **Save** to save the experiment.

Notes

5. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

6. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

   a. Load the reaction plate into the instrument.
   b. Start the run
   c. (Optional) Monitor the run.
   d. Unload the reaction plate from the instrument.

7. Analyze the data:

   a. Open the experiment in the 7500 software.
   b. In the navigation pane, click **Analysis**.
   c. If the data are not analyzed, click **Analyze**.
   d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Export/Import Workflow

Use the Export/Import workflow to set up a new experiment using setup data exported from another experiment. Only reaction plate setup data are exported and imported.

Export Setup Data

1. Double-click (7500 Software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where *<software name>* is the current version of the 7500 software.

Notes

2. Open an existing experiment, or create a new experiment.

   Note: You can create a new experiment using the Design Wizard (see Chapter 2) or Advanced Setup (see page 92).

3. Select **File** ▸ **Export**.

4. Select the **Export Properties** tab (default), then:

   a. Select **Setup**.

   b. Select **One File** in the drop-down list.

   c. Enter a name, then select a location for the export file.

   d. Select **(*.txt)** in the File Type drop-down list.

      IMPORTANT! You cannot export *.xml files.

5. (Optional) Click the **Customize Export** tab, then select the appropriate options.

6. Click **Start Export**.

7. When prompted, click **Close Export Tool**.

Create an Experiment with an Exported Text File

You can import plate setup data from an exported text file (*.txt) to complete the reaction plate setup data for your experiment.

IMPORTANT! Be sure the exported text file you select contains only reaction plate setup data and that the experiment types match.

1. Import the reaction plate setup data from an exported text file:

   a. Using a spreadsheet application (such as Microsoft® Excel software), open an exported text file.

   b. Replace the parameters of the text file as needed. When finished, save the file as a tab-delimited text file.

   c. In the Home screen, click **Advanced Setup**.

      Note: If you do not see the Advanced Setup icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

   d. Create a new experiment or open an existing experiment.

   e. Select **File** ▸ **Import**.

   f. Click **Browse**, locate and select the text file (*.txt), then click **Select**.

Notes

g. Click **Start Import**. The setup data from the exported text file is imported into the open experiment.

Note: If your experiment already contains plate setup information, the software asks if you want to replace the plate setup with the data from the text file. Click **Yes** to replace the plate setup.

2. Use Advanced Setup to finish setting up your experiment (see page 92).

3. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

4. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

a. Load the reaction plate into the instrument.

b. Start the run

c. (Optional) Monitor the run.

d. Unload the reaction plate from the instrument.

5. Analyze the data:

a. Open the experiment in the 7500 software.

b. In the navigation pane, click **Analysis**.

c. If the data are not analyzed, click **Analyze**.

d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

Bibliography

Saiki, R.K., Scharf, S., Faloona, F.*, et al.* 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

Glossary

Advanced Setup In the 7500/7500 Fast system 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 7500/7500 Fast system, a PCR reaction mix that contains primers to amplify a target and a reagent to detect the amplified target.

Assay ID Identifier assigned by Applied Biosystems 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 Applied Biosystems 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.

baseline-corrected normalized reporter (ΔRn)

The magnitude of normalized fluorescence generated by the reporter:

1. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence 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 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 replicates

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).

See also technical replicates.

blocked IPC

In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the 7500/7500 Fast system software, the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells.

C_T

See threshold cycle (CT).

calibrator

See reference sample.

chemistry

See reagents.

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 7500/7500 Fast system 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.

custom dye

Dye that is not supplied by Applied Biosystems. Custom dyes may be adapted for use in experiments on the 7500/7500 Fast system. When using custom dyes, the custom dye should be added to the Dye Library and a custom dye calibration performed.

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 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast system 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 for the target you are quantifying. Housekeeping genes can be used as endogenous controls. See also housekeeping gene.

endpoint read

See post-PCR read.

experiment

Refers to the entire process of performing a run using the 7500/7500 Fast system, including setup, run, and analysis. The types of experiments you can perform using the 7500/7500 Fast 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 (:), or semicolon (;).

experiment type The type of experiment you are performing using the 7500/7500 Fast 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 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast system 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. 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 from the reporter dye normalized to the fluorescence 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. 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 the 7500/7500 Fast system, the grid contains 8 rows and 12 columns.

In the 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast 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 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.

QuickStart A feature in 7500/7500 Fast systems that allows you to run an experiment without entering plate setup information.

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. For the melt curve step, the ramp can also be 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, Applied Biosystems recommends using TaqMan® Fast reagents in your PCR reactions.
- For optimal results using the standard ramp speed, Applied Biosystems recommends using standard reagents in your PCR reactions.

IMPORTANT! TaqMan Fast reagents are not supported for genotyping or presence/absence experiments.

raw data plot A plot of raw fluorescence (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 7500/7500 Fast 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 Applied Biosystems Store for an Applied Biosystems 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 7500/7500 Fast system 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.

replicate group — A set of identical reactions in an experiment.

replicates — See technical replicates or biological replicates.

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 Applied Biosystems and precalibrated on the 7500/7500 Fast 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 7500/7500 Fast instrument run.

sample — The template that you are testing.

Sample DNA (10×) — In the 7500/7500 Fast system 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 7500/7500 Fast system software, a collection of samples. The Sample Library contains the sample name and the sample color.

Sample or Standard (10×) — In the 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast system 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.

region of interest (ROI) calibration — Type of 7500/7500 Fast system calibration in which the system maps the positions of the wells in the sample block. ROI 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.

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 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast system 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 7500/7500 Fast system 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.

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 Applied Biosystems and precalibrated on the 7500/7500 Fast system. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Maintenance Manager.

System dyes of the 7500/7500 Fast system include:

- FAM™ dye
- JOE™ dye
- NED™ dye
- ROX™ dye
- SYBR® Green dye
- TAMRA™ dye
- VIC® dye
- CY3 dye
- CY5 dye
- TEXAS RED® dye

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 7500/7500 Fast system software, a color assigned to a target to identify the target in the plate layout and analysis plots.

Target Library

In the 7500/7500 Fast system 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 7500/7500 Fast system 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 replicates

Identical reactions that contain identical components and volumes and evaluate the same sample.

See also biological replicates.

template

In the Design Wizard of the 7500/7500 Fast system 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, Applied Biosystems recommends 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 7500/7500 Fast 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 7500/7500 Fast system 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).

unknown

In the 7500/7500 Fast system 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).

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.

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