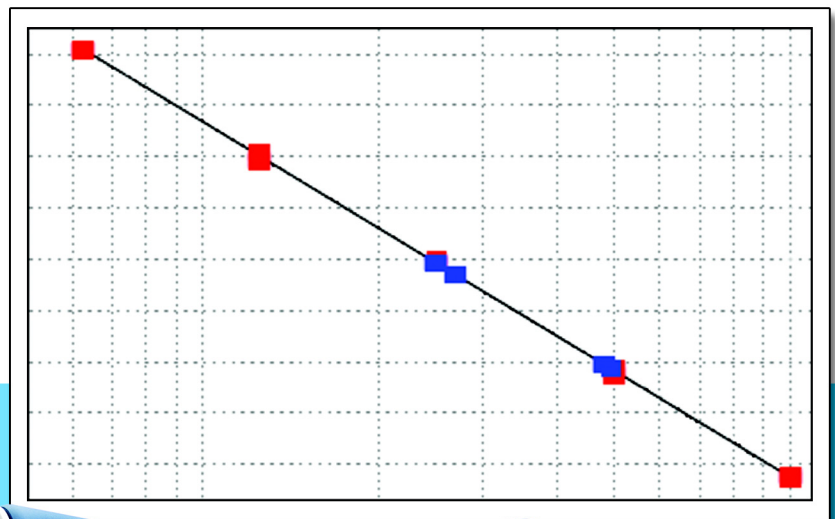


Applied Biosystems StepOne™ and StepOnePlus™

Real-Time PCR Systems

Standard Curve Experiments



Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems

Standard Curve Experiments

Get Started

1

Design the
Experiment

2

Prepare the
Reactions

3

Run the
Experiment

4

Analyze the
Experiment

5

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Contents

	Preface	vii
	How to Use This Guide	vii
	How to Obtain More Information	ix
	How to Obtain Support	xii
	Safety Conventions Used in This Document	xiii
	Symbols on Instruments	xiv
	Safety Labels on Instruments	xvi
	General Instrument Safety	xvii
	Chemical Safety	xviii
	Chemical Waste Safety	xix
	Electrical Safety	xx
	LED Safety	xxi
	Biological Hazard Safety	xxi
	Workstation Safety	xxii
	Safety and Electromagnetic Compatibility (EMC) Standards	xxii
Chapter 1	Get Started	1
	About the StepOne™ and StepOnePlus™ Systems	2
	Supported Consumables	4
	About Standard Curve Experiments	6
	How to Use This Guide	9
	About the Example Experiment	10
	Example Experiment Workflow	12
Chapter 2	Design the Experiment	15
	Chapter Overview	16
	Create a New Experiment	17
	Define the Experiment Properties	20
	Define the Methods and Materials	22
	Set Up the Targets	25
	Set Up the Standards	27
	Set Up the Samples	29
	Set Up the Run Method	31
	Review the Reaction Setup	33
	Order Materials for the Experiment	38
	Finish the Design Wizard	42

Chapter 3	Prepare the Reactions	45
	Chapter Overview	46
	Prepare the Sample Dilutions	47
	Prepare the Standard Dilution Series	49
	Prepare the Reaction Mix	51
	Prepare the Reaction Plate	53
Chapter 4	Run the Experiment	59
	Chapter Overview	60
	Prepare for the Run	61
	(Optional) Enable the Notification Settings	63
	Start the Run	65
	Monitor the Run	69
	Unload the Instrument and Transfer the Data	76
Chapter 5	Analyze the Experiment	81
	Chapter Overview	82
	Section 5.1: Review Results	83
	Analyze the Experiment	84
	View the Standard Curve	90
	View the Amplification Plot	92
	View the Well Table	99
	Publish the Data	102
	Section 5.2: Troubleshoot (If Needed)	103
	View the Analysis Settings	104
	View the QC Summary	106
	Omit Wells from the Analysis	108
	View the Multicomponent Plot	109
	View the Raw Data Plot	112
Appendix A	Alternate Experiment Workflows	115
	Advanced Setup Workflow	116
	QuickStart Workflow	117
	Template Workflow	119
	Export/Import Workflow	121

Bibliography	125
Glossary	127
Index	143

How to Use This Guide

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

Guide	Purpose and Audience	PN
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Genotyping Experiments</i>	<p>Explains how to perform experiments on the StepOne and StepOnePlus systems. Each Getting Started Guide functions as both:</p> <ul style="list-style-type: none"> • A tutorial, using example experiment data provided with the Applied Biosystems StepOne™ Real-Time PCR Software (StepOne™ software). • A guide for your own experiments. <p>Intended for laboratory staff and principal investigators who perform experiments using the StepOne or StepOnePlus system.</p>	4376786
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Presence/Absence Experiments</i>		4376787
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments</i>		4376785
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments</i>		4376784
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking, and Maintenance Guide</i>	<p>Explains how to install and maintain the StepOne and StepOnePlus systems.</p> <p>Intended for laboratory staff responsible for the installation and maintenance of the StepOne or StepOnePlus system</p>	4376782
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation Quick Reference Card</i>		4376783
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide</i>	<p>Provides information about the reagents you can use on the StepOne and StepOnePlus systems, including:</p> <ul style="list-style-type: none"> • An introduction to TaqMan® and SYBR® Green reagents • Descriptions and design guidelines for the following experiment types: <ul style="list-style-type: none"> – Quantitation experiments – Genotyping experiments – Presence/absence experiments <p>Intended for laboratory staff and principal investigators who perform experiments using the StepOne or StepOnePlus system.</p>	4379704

Guide	Purpose and Audience	PN
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Site Preparation Guide</i>	Explains how to prepare your site to receive and install the StepOne and StepOnePlus systems. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the StepOne or StepOnePlus system.	4376768
<i>Applied Biosystems StepOne™ Real-Time PCR Software Help</i>	Explains how to use the StepOne software to: <ul style="list-style-type: none"> • Set up, run, and analyze experiments using the StepOne and StepOnePlus systems. • Monitor networked StepOne and StepOnePlus instruments. • Calibrate StepOne and StepOnePlus instruments. • Verify the performance of StepOne and StepOnePlus instruments with an RNase P run. Intended for: <ul style="list-style-type: none"> • Laboratory staff and principal investigators who perform experiments using the StepOne or StepOnePlus system. • Laboratory staff responsible for the installation and maintenance of the StepOne or StepOnePlus system. 	NA

Assumptions This guide assumes that you:

- Are familiar with the Microsoft Windows® XP 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 StepOne or StepOnePlus 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 xiii](#).

How to Obtain More Information

Related Documentation

Other StepOne and StepOnePlus System Documents

The documents listed in the table below are not shipped with the StepOne or StepOnePlus instrument.

Document	PN
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation Performance Verification Protocol</i>	4376791
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation Qualification-Operation Qualification Protocol</i>	4376790
<i>Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Planned Maintenance Protocol</i>	4376788

Documents Related to Genotyping Experiments

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

Documents Related to Presence/Absence Experiments

Document	PN
<i>DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol</i>	4343586
<i>NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue Protocol</i>	4333959
<i>PrepMan® Ultra Sample Preparation Reagent Protocol</i>	4318925

Documents Related to Relative Standard Curve and Comparative C_T Experiments

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

Documents Related to Standard Curve Experiments

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


Documents Related to the Reagent Guide

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

Note: For more documentation, see [“How to Obtain Support”](#) on [page xii](#).

Obtaining Information from the Software Help

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

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

To find topics of interest in the Help:

- Review the table of contents.
- Search for a specific topic.
- Search an 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 xii).

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 StepOne™ or StepOnePlus™ 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 email 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 reagent 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 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 symbols that are affixed to Applied Biosystems instruments (see “[Safety Symbols](#)” on [page xv](#)).

Examples

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



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.



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











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

Symbols on Instruments






Electrical Symbols

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 symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see “[Safety Labels on Instruments](#)” on [page xvi](#)). 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	Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.		Indicates the presence of moving parts 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 an electrical shock hazard and to proceed with appropriate caution.
			Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.

Environmental Symbols

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

Symbol	Description
	<p>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).</p> <p>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.</p>

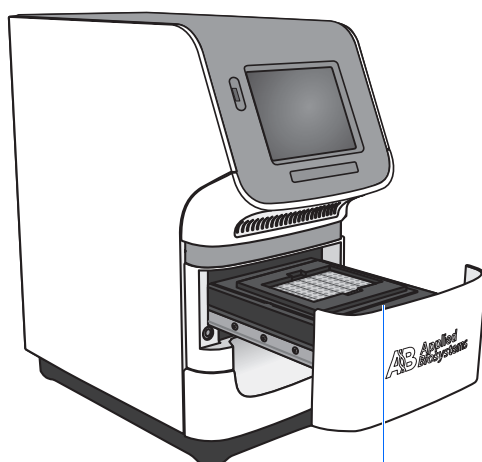
Safety Labels on Instruments

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 réglementation locale associées à la manipulation et l'élimination des déchets.
CAUTION Hot surface.	ATTENTION Surface brûlante.
DANGER High voltage.	DANGER Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to 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.
DANGER Class 3B (III) visible and/or invisible LED radiation present when open and interlocks defeated. Avoid exposure to beam.	DANGER Rayonnement visible ou invisible d'un faisceau LED de Classe 3B (III) en cas d'ouverture et de neutralisation des dispositifs de sécurité. Evitez toute exposition au faisceau.

Locations of Warnings


The StepOne and StepOnePlus instruments contain a warning at the location shown below:




General Instrument Safety

 **WARNING** **PHYSICAL INJURY HAZARD.** Using the 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 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 xviii](#).

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

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the 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 xviii.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For more 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 more 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 in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

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 <https://docs.appliedbiosystems.com/msdssearch.html>
2. In the Search field of the MSDS Search page:
 - a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
 - b. Select the language of your choice.

- c. Click **Search**.
3. To view, download, or print the document of interest:
 - a. Right-click the document title.
 - b. Select:
 - **Open** – To view the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose
 - **Print Target** – To print the document
 4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
 - a. Select **Fax** or **Email** below the document title.
 - b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
 - c. Enter the required information.
 - d. Click **View/Deliver Selected Documents Now**.


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

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 more 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 more safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

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

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

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

Electrical Safety



DANGER ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the StepOne or StepOnePlus instrument 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 instrument into a properly grounded receptacle with adequate current capacity.


Overvoltage Rating The StepOne and StepOnePlus instruments have an installation (overvoltage) category of II, and they are classified as portable equipment.

LED Safety

To ensure safe LED operation:

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

Biological Hazard Safety

General Biohazard  **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 performed 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; <http://bmbi.od.nih.gov>)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

- Your company’s/institution’s Biosafety Program protocols for working with/handling potentially infectious materials.

More information about biohazard guidelines is available at:

<http://www.cdc.gov>

Workstation Safety

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



CAUTION MUSCULOSKELETAL AND REPETITIVE MOTION

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

To minimize musculoskeletal and repetitive motion risks:

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

Safety and Electromagnetic Compatibility (EMC) Standards

U.S. and Canadian Safety Standards



The StepOne and StepOnePlus instruments have been tested to and comply with standard:

UL 61010A-1/CAN/CSA C22.2 No. 1010.1-92, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”

UL 61010A-2-010/CAN/CSA 1010.2.010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”

FDA “Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11,” as applicable.

Canadian EMC Standard

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

European Safety and EMC Standards



Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements.”

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

EN 61010-2-081, “Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes.”

EN 60825-1, “Radiation Safety of Laser Products, Equipment Classification, Requirements, and User’s Guide.

EMC

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


Australian EMC Standards



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

This chapter covers:

■ About the StepOne™ and StepOnePlus™ Systems	2
■ Supported Consumables	4
■ About Standard Curve Experiments	6
■ How to Use This Guide	9
■ About the Example Experiment	10
■ Example Experiment Workflow	12

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

About the StepOne™ and StepOnePlus™ Systems

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

System	Features
Applied Biosystems StepOne™ Real-Time PCR System (StepOne™ system)	<ul style="list-style-type: none"> • 48-well platform • Three-color system
Applied Biosystems StepOnePlus™ Real-Time PCR System (StepOnePlus™ system)	<ul style="list-style-type: none"> • 96-well platform • Four-color system • VeriFlex™ sample blocks

The StepOne and StepOnePlus systems use fluorescent-based polymerase chain reaction (PCR) reagents to provide:

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

About Data Collection

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

Run Type		Data Collection Point
Real-time runs	Standard curve	The instrument collects data following each extension step of the PCR.
	Relative standard curve	
	Comparative C _T ($\Delta\Delta C_T$)	
Post-PCR (endpoint) runs	Genotyping	The instrument collects data: <ul style="list-style-type: none"> • Before the PCR (For presence/absence experiments, data collection before the PCR is optional, but recommended.) • (Optional) During the PCR. The instrument can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting. • After the PCR
	Presence/absence	

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

- 1. Excitation** – The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.

Notes _____

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

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

About the Filters

The StepOne and StepOnePlus systems use the following filters:

StepOne system		StepOnePlus system	
Filter	Dye	Filter	Dye
1	FAM™ dye	1	FAM™ dye
	SYBR® Green dye		SYBR® Green dye
2	JOE™ dye	2	JOE™ dye
	VIC® dye		VIC® dye
3	ROX™ dye	3	TAMRA™ dye
			NED™ dye
		4	ROX™ dye

About the VeriFlex™ Technology

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

For More Information

For information on:

- The StepOne and StepOnePlus systems, refer to *Applied Biosystems StepOne™ Real-Time PCR Software Help*.

Note: To access the Help, select **Help ▶ StepOne Software Help** from within the StepOne software.

- Genotyping experiments, refer to *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Genotyping Experiments*.
- Presence/absence experiments, refer to *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Presence/Absence Experiments*.
- Relative standard curve and/or comparative C_T (ΔΔC_T) experiments, refer to *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments*.

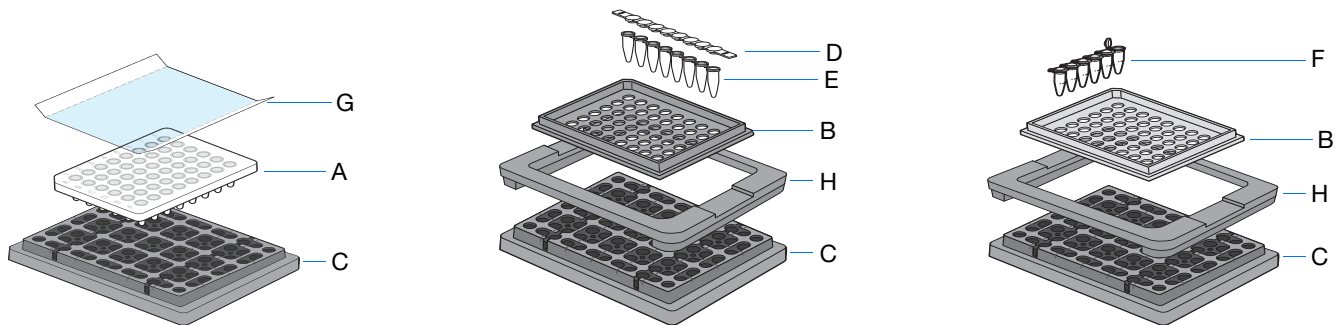
Notes

Supported Consumables

StepOne System The StepOne system supports the consumables listed below. These consumables are for use with both standard and Fast reagents/protocols.

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

Consumable	Part Number
<ul style="list-style-type: none"> • MicroAmp™ Fast Optical 48-Well Reaction Plate • MicroAmp™ 48-Well Optical Adhesive Film 	<ul style="list-style-type: none"> • 4375816 • 4375323 and 4375928
<ul style="list-style-type: none"> • MicroAmp™ Fast 8-Tube Strip • MicroAmp™ Optical 8-Cap Strip 	<ul style="list-style-type: none"> • 4358293 • 4323032
<ul style="list-style-type: none"> • MicroAmp® Fast Reaction Tube with Cap 	<ul style="list-style-type: none"> • 4358297
<ul style="list-style-type: none"> • MicroAmp™ Fast 48-Well Tray • MicroAmp™ 48-Well Base Adaptor • MicroAmp™ 96-Well Support Base 	<ul style="list-style-type: none"> • 4375282 • 4375284 • 4379590



#	Consumable
A	MicroAmp™ Fast Optical 48-Well Reaction Plate
B	MicroAmp™ Fast 48-Well Tray
C	MicroAmp™ 96-Well Support Base
D	MicroAmp™ Optical 8-Cap Strip
E	MicroAmp™ Fast 8-Tube Strip
F	MicroAmp® Fast Reaction Tube with Cap
G	MicroAmp™ 48-Well Optical Adhesive Film
H	MicroAmp™ 48-Well Base Adaptor

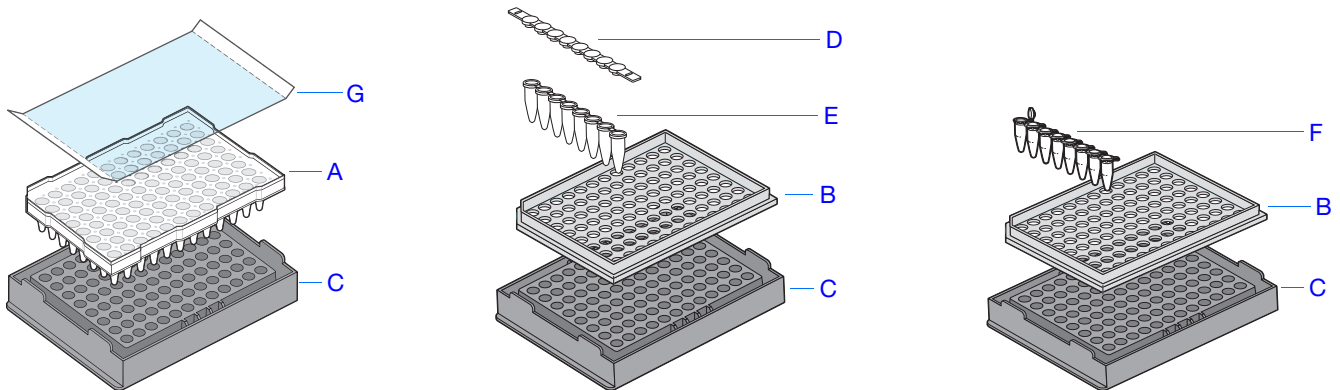
Notes _____

StepOnePlus System

The StepOnePlus system supports the consumables listed below. These consumables are for use with both standard and Fast reagents/protocols.

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

Consumable	Part Number
<ul style="list-style-type: none"> • MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode • MicroAmp™ Optical Adhesive Film 	<ul style="list-style-type: none"> • 4346906 and 4366932 • 4360954 and 4311971
<ul style="list-style-type: none"> • MicroAmp™ Fast 8-Tube Strip • MicroAmp™ Optical 8-Cap Strip 	<ul style="list-style-type: none"> • 4358293 • 4323032
<ul style="list-style-type: none"> • MicroAmp® Fast Reaction Tube with Cap 	<ul style="list-style-type: none"> • 4358297
<ul style="list-style-type: none"> • MicroAmp™ 96-Well Tray for VeriFlex™ Blocks • MicroAmp™ 96-Well Support Base 	<ul style="list-style-type: none"> • 4379983 • 4379590
<ul style="list-style-type: none"> • MicroAmp™ Adhesive Film Applicator • MicroAmp™ Cap Installing Tool (Handle) 	<ul style="list-style-type: none"> • 4333183 • 4330015



#	Consumable
A	MicroAmp™ Fast Optical 96-Well Reaction Plate
B	MicroAmp™ 96-Well Tray for VeriFlex™ Blocks
C	MicroAmp™ 96-Well Support Base
D	MicroAmp™ Optical 8-Cap Strip
E	MicroAmp™ Fast 8-Tube Strip
F	MicroAmp® Fast Reaction Tube with Cap
G	MicroAmp™ Optical Adhesive Film

Notes

About Standard Curve Experiments

Real-Time PCR Experiments

Standard curve experiments are real-time PCR experiments. In real-time PCR experiments:

- The instrument monitors the progress of the PCR as it occurs (Kwok and Higuchi, 1989).
- Data are collected throughout the PCR process.
- Reactions are characterized by the point in time during cycling when amplification of a target is first detected (Saiki *et al.*, 1985).

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

About Standard Curve Experiments

The standard curve method is used to determine the absolute target quantity in samples. With the standard curve method, the StepOne software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples.

Components

The following components are required when setting up PCR reactions for standard curve experiments:

- **Sample** – The sample in which the quantity of the target is unknown.
- **Standard** – A sample that contains known standard quantities; used in quantitation experiments to generate standard curves.
- **Standard dilution series** – A set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards.
- **Replicates** – The total number of identical reactions containing identical samples, components, and volumes.
- **Negative Controls** – Wells that contain water or buffer instead of sample template. No amplification of the target should occur in negative control wells.

PCR Options

When performing real-time PCR, choose between:

- Singleplex and multiplex PCR (below)
and
- 1-step and 2-step RT-PCR ([page 7](#))

Singleplex vs. Multiplex PCR

You can perform a PCR reaction using either:

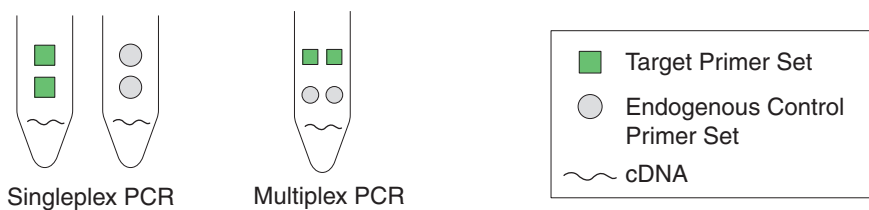
- **Singleplex PCR** – In singleplex PCR a single primer set is present in the reaction tube or well. Only one target or endogenous control can be amplified per reaction.
or

Notes _____

- **Multiplex PCR** – In multiplex PCR, two or more primer sets are present in the reaction tube or well. Each set amplifies a specific target or endogenous control. Typically, a probe labeled with FAM™ dye detects the target and a probe labeled with VIC® dye detects the endogenous control.

IMPORTANT! SYBR® Green reagents cannot be used for multiplex PCR.

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



1- vs. 2-Step RT-PCR

You can perform reverse transcription (RT) and PCR in a single reaction (1-step) or in separate reactions (2-step). The reagent configuration you use depends on whether you are performing 1- or 2-step RT-PCR:

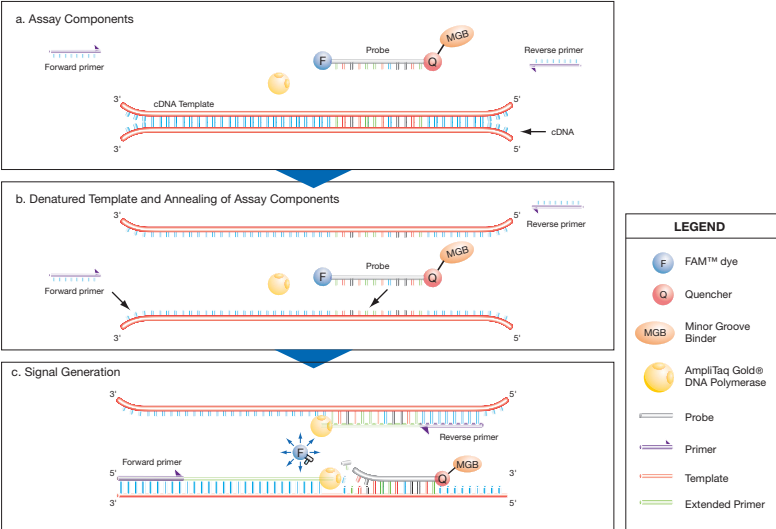
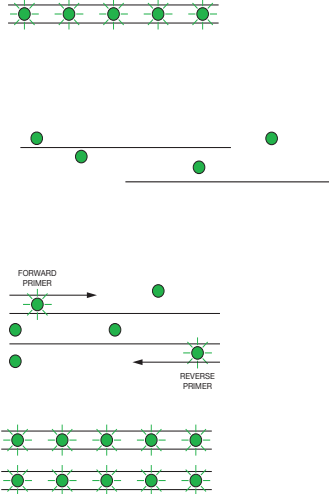
- In 1-step RT-PCR, RT and PCR take place in one buffer system, which provides the convenience of a single-tube preparation for RT and PCR amplification. However, you cannot use Fast PCR master mix or the carryover prevention enzyme, AmpErase® UNG (uracil-N-glycosylase), to perform 1-step RT-PCR.
- 2-step RT-PCR is performed in two separate reactions: First, total RNA is reverse-transcribed into cDNA, then the cDNA is amplified by PCR. This method is useful for detecting multiple transcripts from a single cDNA template or for storing cDNA aliquots for later use. The AmpErase® UNG enzyme can be used to prevent carryover contamination.

Note: For more information on AmpErase® UNG, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*.

Notes

Supported Reagents TaqMan® and SYBR® Green Reagents

Applied Biosystems offers TaqMan® and SYBR® Green reagents for use on the StepOne and StepOnePlus systems. Both reagent types are briefly described in the table below.

Reagent Type	Process
<p>TaqMan® reagents or kits</p> <p>Description</p> <p>TaqMan reagents use a fluorogenic probe to enable detection of a specific PCR product as it accumulates during PCR cycles.</p> <p>Advantages</p> <ul style="list-style-type: none"> • Increased specificity with the addition of a fluorogenic probe. • Provides multiplex capability. • Preformulated assays, optimized to run under universal thermal cycling conditions, are available. • Can be used for either 1- or 2-step RT-PCR. <p>Limitations</p> <p>Requires synthesis of a unique fluorogenic probe.</p>	<p>PCR and Detection of cDNA</p>  <p>LEGEND</p> <ul style="list-style-type: none"> F FAM™ dye Q Quencher MGB Minor Groove Binder AmpliTaq Gold® DNA Polymerase Probe Primer Template Extended Primer
<p>SYBR® Green reagents</p> <p>Description</p> <p>SYBR Green reagents use SYBR® Green I dye, a double-stranded DNA binding dye, to detect PCR products as they accumulate during PCR cycles.</p> <p>Advantages</p> <ul style="list-style-type: none"> • Economical (no probe needed). • Allows for melt curve analysis to measure the T_m of all PCR products. • Can be used for either 1- or 2-step RT-PCR. <p>Limitations</p> <p>Binds nonspecifically to all double-stranded DNA sequences. To avoid false positive signals, check for nonspecific product formation using melt curve or gel analysis.</p>	 <p>Step 1: Reaction setup The SYBR® Green I dye fluoresces when bound to double-stranded DNA.</p> <p>Step 2: Denaturation When the DNA is denatured into single-stranded DNA, the SYBR® Green I dye is released and the fluorescence is drastically reduced.</p> <p>Step 3: Polymerization During extension, primers anneal and PCR product is generated.</p> <p>Step 4: Polymerization completed SYBR® Green I dye binds to the double-stranded product, resulting in a net increase in fluorescence detected by the instrument.</p>

IMPORTANT! Applied Biosystems does not recommend the use of TAMRA dye as a reporter or quencher with the StepOne system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus system.

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Other Reagents

You can use other fluorescent-based reagents on the StepOne and StepOnePlus systems, but note the following:

- You must design your experiment using Advanced Setup instead of the Design Wizard. (See [“Advanced Setup Workflow”](#) on page 116.)
- For Applied Biosystems TaqMan and SYBR Green reagents, the StepOne software automatically calculates reaction volumes in the Reaction Setup screen.

For More Information

For more information on real-time PCR experiments, PCR options, and reagents, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*.

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

By using the example experiment data provided with the StepOne software, you can use this guide as a tutorial for performing a standard curve experiment on a StepOne or StepOnePlus system. Follow the procedures in Chapters 2 to 5:

Chapter	Procedure
2	Design the experiment using the Design Wizard in the StepOne software.
3	Prepare the experiment, using the reagents and volumes calculated by the Design Wizard in Chapter 2.
4	Run the experiment on a StepOne or StepOnePlus instrument (standalone or colocated layout)
5	Analyze the results.

For more information, see [“About the Example Experiment”](#) on page 10.

Using This Guide With Your Own Experiments

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

Notes

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

Workflow	Description	See...
Design Wizard	Set up a new experiment with guidance from the software. The Design Wizard walks 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 116
QuickStart	Run a new experiment with no plate setup information. If desired, you can add all design parameters after the run.	page 117
Template	Set up a new experiment using setup information from a template.	page 119
Export/Import	Import experiment designs from ASCII text files that contain experiment setup information.	page 121

About the Example Experiment

To illustrate how to perform standard curve 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 StepOne or StepOnePlus system.

Description The objective of the standard curve example experiment is to determine the quantity of the RNase P gene in two populations.

In the standard curve example experiment:

- The samples are genomic DNA isolated from two populations.
- The target is the RNase P gene.
- One standard curve is set up for the RNase P gene (target). The standard used for the standard dilution series contains known quantities of the RNase P gene. Because a single target is being studied, only one standard curve is required.

Note: In experiments where multiple targets are being studied, a standard curve is required for each target.

- Three replicates of each sample and each dilution point in the standard curve are performed to ensure statistical significance.

Notes _____

- The experiment is designed for singleplex PCR, where every well contains a primer/probe set for a single target.
- Reactions are set up for 2-step RT-PCR.
- Primer/probe sets are from Applied Biosystems RNase P assay.

Note: The human RNase P FAM™ dye-labeled MGB probe is not available as a TaqMan® Gene Expression Assay. It can be ordered as a Custom TaqMan® Gene Expression Assay (PN 4331348).

IMPORTANT! Applied Biosystems does not recommend the use of TAMRA dye as a reporter or quencher with the StepOne system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus system.

Reaction Plate Layout

The standard curve example experiment was created for a StepOne instrument. For the StepOne instrument, the software displays a 48-well reaction plate layout:

You can create the example experiment for a StepOnePlus instrument; however, your reaction plate layout will differ from the 48-well reaction plate layout shown throughout this guide. For the StepOnePlus instrument, the software displays a 96-well reaction plate layout:

Notes

About the Example Experiment Data

In this getting started guide you will use two files:

- In Chapter 2, you will create a standard curve example experiment file that contains setup data and save it to the experiments folder on your computer:

```
<drive>:\Applied Biosystems\<software name>\experiments\  
Standard Curve Example.eds
```

- In Chapter 5, you will view results in a standard curve example experiment file that contains run data. The data file for the example experiment installs with the StepOne software. You can find the data file for the example experiment on your computer:

```
<drive>:\Applied Biosystems\<software name>\experiments\examples\  
Standard Curve Example.eds
```

where:

- *<drive>* is the computer hard drive on which the StepOne software is installed. The default installation drive for the software is the D drive.
- *<software name>* is the current version of the StepOne software.

Data Files in the Examples Folder

The examples folder contains several data files that you can reference when analyzing your own data, as listed below. The data files install with the StepOne software.

Note: Be sure to use the Standard Curve Example.eds file when performing the tutorial procedures in this guide. The 96-Well Standard Curve Example.eds file is a different example of the standard curve method.

StepOne Instrument	StepOnePlus Instrument
Comparative CT Example.eds	96-Well Comparative CT Example.eds
Multiplex Example.eds	96-Well Multiplex Example.eds
Genotyping Example.eds	96-Well Genotyping Example.eds
Presence Absence Example.eds	96-Well Presence Absence Example.eds
Relative Standard Curve Example.eds	96-Well Relative Standard Curve Example.eds
RNase P Experiment.eds	96-Well RNase P Experiment.eds
Standard Curve Example.eds	96-Well Standard Curve Example.eds
SYBR Example.eds	96-Well SYBR Example.eds

Example Experiment Workflow

The figure on [page 13](#) shows the workflow for the standard curve example experiment.

Notes _____

Start Experiment

Design the Experiment (Chapter 2)

1. Create a new experiment.
2. Define the experiment properties.
3. Define the methods and materials.
4. Set up the targets.
5. Set up the standards.
6. Set up the samples.
7. Set up the run method.
8. Review the reaction setup.
9. Order materials for the experiment.
10. Finish the Design Wizard.

Prepare the Reactions (Chapter 3)

1. Prepare the sample dilutions.
2. Prepare the standard dilution series.
3. Prepare the reaction mix for each target assay.
4. Prepare the reaction plate.

Run the Experiment (Chapter 4)

1. Prepare for the run.
2. (Optional) Enable the notification settings.
3. Start the run.
4. Monitor the run.
5. Unload the instrument and transfer the data.

Analyze the Experiment (Chapter 5)

Section 1, Review Results:

1. Analyze.
2. View the standard curve.
3. View the amplification plot.
4. View the results in a table.
5. Publish the data.

Section 2, Troubleshoot (If Needed):

1. View the analysis settings; adjust the baseline/threshold.
2. View the quality summary.
3. Omit wells.
4. View the multicomponent plot.
5. View the raw data plot.

End Experiment

Notes


Notes _____

2

Design the Experiment

This chapter covers:

■ Chapter Overview	16
■ Create a New Experiment	17
■ Define the Experiment Properties	20
■ Define the Methods and Materials	22
■ Set Up the Targets	25
■ Set Up the Standards	27
■ Set Up the Samples	29
■ Set Up the Run Method	31
■ Review the Reaction Setup	33
■ Order Materials for the Experiment	38
■ Finish the Design Wizard	42

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

Notes

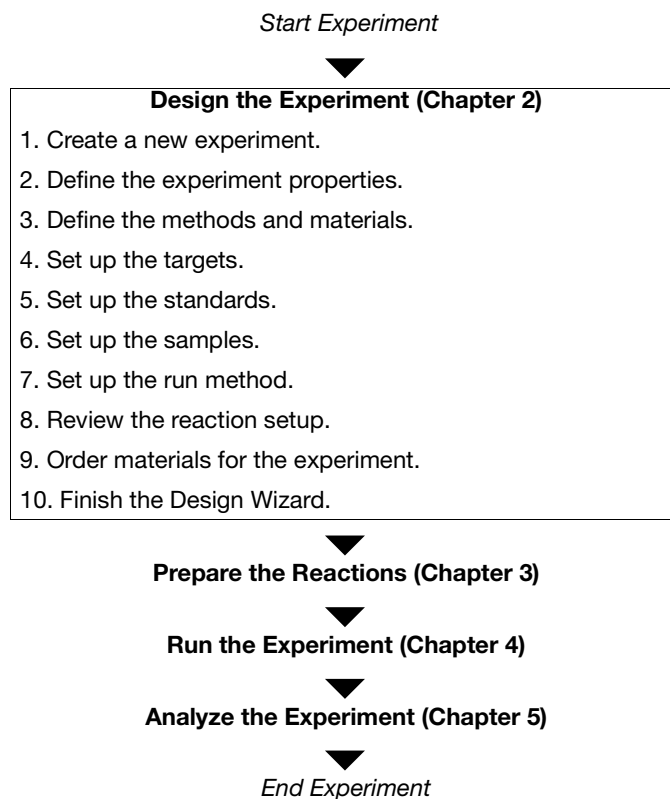
Chapter Overview

This chapter explains how to use the Design Wizard in the StepOne™ software to set up the standard curve example experiment. The Design Wizard walks you through Applied Biosystems recommended best practices as you enter design parameters for the example experiment.

Example Experiment Workflow

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

Note: Design the example experiment using the Design Wizard in the StepOne software. When you design your own experiments, you can select alternate workflows (see [“Using This Guide With Your Own Experiments”](#) on page 9).





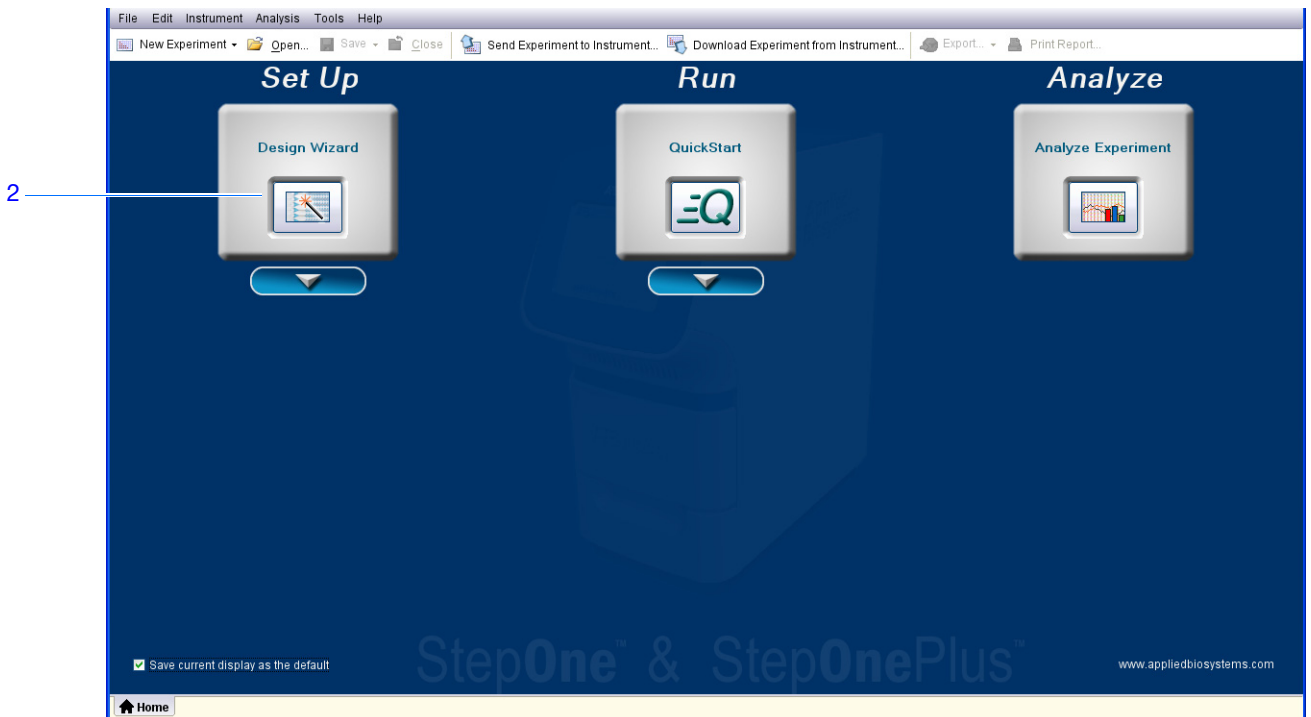
Notes _____

Create a New Experiment

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

Create an Experiment

1. Double-click  (StepOne software shortcut) or select **Start ▶ All Programs ▶ Applied Biosystems ▶ StepOne Software ▶ <software name>** where <software name> is the current version of the StepOne software.
2. From the Home screen, click  **Design Wizard** to open the Design Wizard.



3. See “Software Elements” on page 18 for information on navigating within the Design Wizard.

Notes

Software Elements

The StepOne software elements for the Design Wizard are illustrated below.

1. Menu bar – Displays the menus available in the software:
 - File
 - Edit
 - Instrument
 - Analysis
 - Tools
 - Help
2. Toolbar – Displays the tools available in the software:
 - New Experiment
 - Open
 - Close
 - Send Experiment to Instrument
 - Download Experiment from Instrument
3. Experiment header - Displays the experiment name, experiment type, and reagents for the open experiment.
4. Navigation pane – Provides links to all screens in the Design Wizard:
 - Experiment Properties
 - Methods & Materials
 - Targets
 - Standards
 - Samples
 - Run Method
 - Reaction Setup
 - Materials List

Note: The Design Wizard initially displays the Quantitation - Standard Curve experiment type. The available Design Wizard screens may change when you select a different experiment type. For example, the Relative Quantitation Settings screen is not displayed until you select the relative standard curve or comparative C_T ($\Delta\Delta C_T$) experiment type.

5. Experiment tab(s) – Displays a tab for each open experiment.

Notes _____

1 File Edit Instrument Analysis Tools Help

2 New Experiment Open Save Close Send Experiment to Instrument... Download Experiment from Instrument... Export... Print Report...

3 Design your experiment... Experiment: **Untitled** Type: **Quantitation - Standard Curve** Reagents: **TaqMan® Reagents**

1. Define

- Experiment Properties
- Methods & Materials

2. Set Up

- Targets
- Standards
- Samples
- Run Method
- Reaction Setup

3. Order (Optional)

1A. Define: Experiment Properties Experiment Properties Help

Instructions: Enter an experiment name, select the instrument type, then select the type of experiment to design.

How do you want to identify this experiment? = Required

* Experiment Name:

Barcode (Optional):

User Name (Optional):

Comments (Optional):

Which instrument are you using to run the experiment?

StepOnePlus™ Instrument (96 Wells) StepOne™ Instrument (48 Wells)

Set up, run, and analyze an experiment using a 3-color, 48-well system.

What type of experiment do you want to design?

Quantitation Genotyping Presence / Absence

Design a gene quantitation experiment to determine the amount of target nucleic acid sequence in a sample.

← Previous Finish Designing Experiment Next →

5 Untitled

Notes

Define the Experiment Properties

On 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 standard curve example experiment:

- The experiment is identified as an example.
- The instrument selected to run the experiment is the StepOne instrument.
- A MicroAmp™ Fast Optical 48-Well Reaction Plate is used.
- The experiment type is quantitation.

Complete the Experiment Properties Screen

1. Click the **Experiment Name** field, then enter **Standard Curve Example**.

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

2. Leave the Barcode field empty.

Note: The MicroAmp Fast Optical 48-Well Reaction Plate does not have a barcode.

3. Click the **User Name** field, then enter **Example User**.
4. Click the **Comments** field, then enter **Standard Curve Getting Started Guide Example**.
5. Select **StepOne™ Instrument (48 Wells)**.

Note: The example experiment was created for a StepOne instrument. You can create the example experiment for a StepOnePlus instrument; however, your reaction plate layout will differ from the layout shown in this guide. The software displays a 48-well reaction plate layout for the StepOne instrument and a 96-well reaction plate layout for the StepOnePlus instrument. To create the example experiment for a StepOnePlus instrument, select **StepOnePlus™ Instrument (96 Wells)**.

6. Select **Quantitation** for the experiment type.
7. Click **Next >**.

Notes _____

Design Guidelines

When you design your own standard curve experiment:

- Enter an experiment name:
 - Enter a name that is descriptive and easy to remember. You can enter up to 100 characters in the Experiment Name field.

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

- The experiment name is used as the default file name.
- *(Optional)* If you are using a MicroAmp™ Fast Optical 96-Well Reaction Plate, enter a barcode to identify the barcode on the reaction plate. You can enter up to 100 characters in the Barcode field.

Note: The MicroAmp Fast Optical 48-Well Reaction Plate does not have a barcode.

- *(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.

Notes

- Select the instrument you are using to run the experiment:
 - **StepOne™ Instrument (48 Wells)**
 - **StepOnePlus™ Instrument (96 Wells)**


Note: You can use StepOne Software v2.0 or later to design experiments for both the StepOne and StepOnePlus instruments. 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 **General** tab (default). From the Default Instrument Type dropdown menu, select the appropriate instrument.

- Select **Quantitation** as the experiment type.

For More Information

For more information on:

- Completing the Experiment Properties screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Consumables, see [“Supported Consumables” on page 4](#).
- Quantitation experiments, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*.

Define the Methods and Materials

On the Methods & Materials screen, select the quantitation method, reagents, ramp speed, and PCR template to use for the experiment.

About the Example Experiment

In the standard curve example experiment:

- The standard curve quantitation method is used.
- TaqMan® reagents are used.
- The standard ramp speed is used in the instrument run.
- Purified gDNA (isolated from two populations) is the template type. Before using gDNA template, you must first extract the gDNA from tissue or sample.

Complete the Methods & Materials Screen

1. Select **Standard Curve** as the quantitation method.
2. Select **TaqMan® Reagents** for the reagents.
3. Select **Standard (~ 2 hours to complete a run)** for the ramp speed.
4. Select **gDNA (genomic DNA)** for the template type.
5. Click **Next >**.

Notes _____

1B. Define: Methods & Materials Methods & Materials Help

Instructions: Select the quantitation method, reagents, ramp speed, and type of template for the real-time PCR reactions.

Which quantitation method are you using?

1 Standard Curve Relative Standard Curve Comparative Ct ($\Delta\Delta C_t$)

With the standard curve method, you use standards to determine the absolute quantity of target sequence in a sample.

Which reagents do you want to use to detect the target sequence?

2 TaqMan® Reagents SYBR® Green Reagents

These real-time PCR reactions contain two primers and a TaqMan® probe. The primers are designed to amplify the target sequence. The TaqMan probe is designed to hybridize to the target sequence and generate fluorescence signal when the target sequence is amplified.

Which ramp speed do you want to include in the instrument run?

3 Standard (~ 2 hours to complete a run) Fast (~ 40 minutes to complete a run)

For optimal results using the standard ramp speed, Applied Biosystems recommends standard reagents for your real-time PCR reactions.

What type of template do you want to use in the real-time PCR reactions?

4 cDNA (complementary DNA) RNA gDNA (genomic DNA)

You are adding purified gDNA to the real-time PCR reactions. You have already extracted the gDNA from tissue or sample.

Design Guidelines

When you design your own standard curve experiment:

- Select **Standard Curve** as the quantitation method. The standard curve method is used to determine the absolute target quantity in samples. When setting up your reaction plate, the standard curve method requires targets, standards, and samples.
- Select the reagents you want to use:
 - Select **TaqMan® Reagents** if you want to use TaqMan reagents to detect amplification and quantify the amount of target in the samples. TaqMan reagents consist of two primers and a TaqMan® probe. The primers are designed to amplify the target. The TaqMan probe is designed to hybridize to the target and generate fluorescence signal when the target is amplified.

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

Notes

- Select **SYBR® Green Reagents** if you want to use SYBR Green reagents to detect amplification and quantify the amount of target in the samples. SYBR Green reagents consist of two primers and SYBR Green dye. The primers are designed to amplify the target. The SYBR Green dye generates fluorescence signal when it binds to double-stranded DNA. SYBR Green dye is often part of the SYBR Green master mix added to the reaction. If you use SYBR Green dye: Select the **Include Melt Curve** checkbox to perform melt curve analysis of the amplified target.

Select **Standard** as the ramp speed. A Fast master mix for SYBR Green reagents is not available from Applied Biosystems.

Note: You can use other fluorescent-based reagents on the StepOne and StepOnePlus systems, however, you must design your experiment using Advanced Setup instead of the Design Wizard.


- Select the appropriate ramp speed for the instrument run:
 - Select **Fast (~ 40 minutes to complete a run)** if you are using Fast reagents for the PCR reactions.
 - Select **Standard (~ 2 hours to complete a run)** if you are using standard reagents for the PCR reactions.
- Select the appropriate PCR template:
 - Select **cDNA (complementary DNA)** if you are performing 2-step RT-PCR, and you have already performed reverse transcription to convert the RNA to cDNA. You are adding complementary DNA to the PCR reactions.
 - Select **RNA** if you are performing 1-step RT-PCR. You are adding total RNA or mRNA to the PCR reactions.

Note: To use the Fast ramp speed with RNA templates, you must design your experiment using Advanced Setup instead of the Design Wizard.

- Select **gDNA (genomic DNA)** if you have already extracted the gDNA from tissue or sample. You are adding purified genomic DNA to the PCR reactions.

For More Information

For more information on:

- Completing the Methods & Materials screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Using Advanced Setup, see [“Advanced Setup Workflow” on page 116](#).
- Using other quantitation methods, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments*.
- TaqMan and SYBR Green reagents, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*.
- PCR, including singleplex vs. multiplex PCR and 1-step vs. 2-step RT PCR, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*.

Notes

Set Up the Targets

On the Targets screen, enter the number of targets you want to quantify in the PCR reaction plate, then set up the assays for each target.

About the Example Experiment

In the standard curve example experiment:

- One target is quantified in the reaction plate.
- The Set Up Standards checkbox is selected. When this checkbox is selected, the software automatically displays the Standards screen after you complete the Targets screen. In the Standards screen, you can set up a standard curve for the target assay (see [“Set Up the Standards” on page 27](#)).
- The Target 1 assay is set up for the target you are studying. For the example experiment, this is the RNase P gene.

Complete the Targets Screen

1. Click the **How many targets do you want to quantify in the reaction plate?** field, then enter **1**.

Note: The number of rows in the target assays table updates with the number you entered.

2. Select the **Set Up Standards** checkbox to set up standards for the target assay.

Note: The Set Up Standards checkbox is selected by default.

3. Set up the Target 1 assay:

- a. Click the **Enter Target Name** cell, then enter **RNase P**.
- b. From the Reporter dropdown menu, select **FAM** (default).
- c. From the Quencher dropdown menu, select **NFQ-MGB** (default).
- d. Leave the default in the Color field.

4. Click **Next >**.

Note: Leave the (Optional) Enter Gene Name field blank. You can search for the gene/assay ID when you order your materials (see [“Order Materials for the Experiment” on page 38](#)).

Notes


Design Guidelines

When you design your own standard curve experiment:

- Select the **Set Up Standards** checkbox. Applied Biosystems recommends that you set up a standard curve for each target assay in the reaction plate.
- Identify each target assay with a unique name and color. You can enter up to 100 characters in the Target Name field.
- Select the reporter dye used in the target assay. In the Methods & Materials screen on [page 22](#), if you selected:
 - TaqMan[®] Reagents, select the dye attached to the 5' end of the TaqMan probe.
 - SYBR[®] Green Reagents, select **SYBR**.
- Select the quencher used in the target assay. In the Methods & Materials screen on [page 22](#), if you selected:
 - TaqMan[®] Reagents, select the quencher attached to the 3' end of the TaqMan probe.
 - SYBR[®] Green Reagents, select **None**.

IMPORTANT! Applied Biosystems does not recommend the use of TAMRA dye as a reporter or quencher with the StepOne system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus system.

For More Information

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

Notes _____

Set Up the Standards

On the Standards screen, enter the number of points and replicates for all standard curves in the reaction plate. For each standard curve, enter the starting quantity and select the serial factor.

About the Example Experiment

In the standard curve example experiment:

- One standard curve is set up for the target (RNase P). The standard used for the standard dilution series contains known quantities of the RNase P gene. Because a single gene is being studied, only one standard curve is required.
- Five points are used in the standard curve.
- Three replicates are used for each point. Replicates are identical reactions, containing identical reaction components and volumes.
- The starting quantity is 10,000 copies and the serial factor is 1:2.

Complete the Standards Screen

1. Click the **How many points do you need for each standard curve?** field, then enter **5**.
2. Click the **How many replicates do you need for each point?** field, then enter **3**.
3. Define the range of standard quantities for the RNase P assay:
 - a. Click the **Enter Starting Quantity** field, then enter **10000**.
 - b. From the Select Serial Factor dropdown menu, select **1:2**.
4. Review the Standard Curve Preview pane. The standard curve has the following points: 10000, 5000, 2500, 1250, and 625.
5. Click **Next >**.

Notes


**Design
Guidelines**

When you design your own standard curve experiment:

- Set up a standard curve for each target in the reaction plate. The targets are previously defined on the Targets screen (“Set Up the Targets” on page 25).
- Enter the number of points for each standard curve in the reaction plate. Applied Biosystems recommends at least five dilution points for each standard curve.
- Enter the number of replicates (identical reactions) for each point in the standard curve. Applied Biosystems recommends three replicates for each point.
- Because the range of standard quantities affects the amplification efficiency calculations, carefully consider the appropriate range of standard quantities for your assay:
 - For more accurate measurements of amplification efficiency, use a broad range of standard quantities, spanning between 5 and 6 logs. If you specify a broad range of quantities for the standards, you need to use a PCR product or a highly concentrated template, such as a cDNA clone.
 - If you have a limited amount of cDNA template and/or if the target is a low-copy number transcript, or known to fall within a given range, a narrow range of standard quantities may be necessary.
- The serial factor is used to calculate the quantities in all points of the standard curve. If your starting quantity is the highest quantity, select a dilution factor such as 1:2, 1:3, and so on. If your starting quantity is the lowest quantity, select a concentration factor such as 2X, 3X, and so on.

**For More
Information**

For more information on:

- Completing the Standards screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Amplification efficiency, refer to the *Amplification Efficiency of TaqMan® Gene Expression Assays Application Note*.

Notes _____

Set Up the Samples

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

About the Example Experiment

In the standard curve example experiment:

- Two samples are used: genomic DNA from two populations. The samples contain unknown quantities of the target (RNase P).
- Three replicates are used. The replicates are identical reactions, containing identical reaction components and volumes.
- Three negative controls are used. The negative control reactions contain water instead of sample and should not amplify.

Complete the Samples Screen

1. Click the **How many samples do you want to test in the reaction plate?** field, then enter **2**.

Note: The number of rows in the samples table updates with the number you entered.

2. Click the **How many replicates do you need?** field, then enter **3**.
3. Click the **How many negative controls do you need for each target assay?** field, then enter **3**.
4. Set up Sample 1:
 - a. Click the **Enter Sample Name** field, then enter **pop1** (for population 1).
 - b. Leave the default in the Color field.
5. Set up Sample 2:
 - a. Click the **Enter Sample Name** field, then enter **pop2** (for population 2).
 - b. Leave the default in the Color field.
6. Select **All Sample/Target Reactions** to test all targets in all samples.
7. In the Well Count pane, confirm there are:
 - 6 Unknown wells **U**
 - 15 Standard wells **S**
 - 3 Negative control wells **N**
 - 24 Empty wells
8. In the View Plate Layout tab:
 - a. From the Arrange Plate by dropdown menu, select **Rows** (default).

Notes

- b. From the Place Negative Controls in dropdown menu, select **Upper Left** (default).

9. Click **Next >**.

Design Guidelines

When you design your own standard curve 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 replicates (identical reactions) to set up. Applied Biosystems recommends three replicates for each sample reaction.
- Enter the number of negative control reactions to set up. Applied Biosystems recommends three negative control reactions for each target assay.
- Select which targets to test in the samples:
 - Select **All Sample/Target Reactions** to test all targets in all samples.
 - Select **Specify Sample/Target Reactions** to specify the targets to test in each sample.

Note: When you use the Design Wizard to set up a standard curve experiment, you can set up only singleplex reactions (amplification and detection of one target per well). If you want to set up a standard curve experiment with multiplex reactions (amplification and detection of two or more targets per well), design your experiment using Advanced Setup instead of the Design Wizard.

- If you are running the experiment on a StepOnePlus instrument and plan to edit the Run Method ([page 31](#)) to set a different temperature for one or more of the VeriFlex blocks, you need to:
 - a. Design your experiment using Advanced Setup instead of the Design Wizard.


Notes

- b. In the Plate Setup screen, select the **Assign Targets and Samples** tab, select the **View Plate Layout** tab, then select the **Enable VeriFlex™ Block** checkbox.

IMPORTANT! If you do not select the **Enable VeriFlex™ Block** checkbox in the Plate Setup screen, you will not be able to set a different temperature for one or more of the VeriFlex blocks in the Run Method screen (page 31).

For More Information

For more information on:

- Completing the Samples screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Using Advanced Setup, see [“Advanced Setup Workflow” on page 116](#).

Set Up the Run Method

On 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 Run Method library.

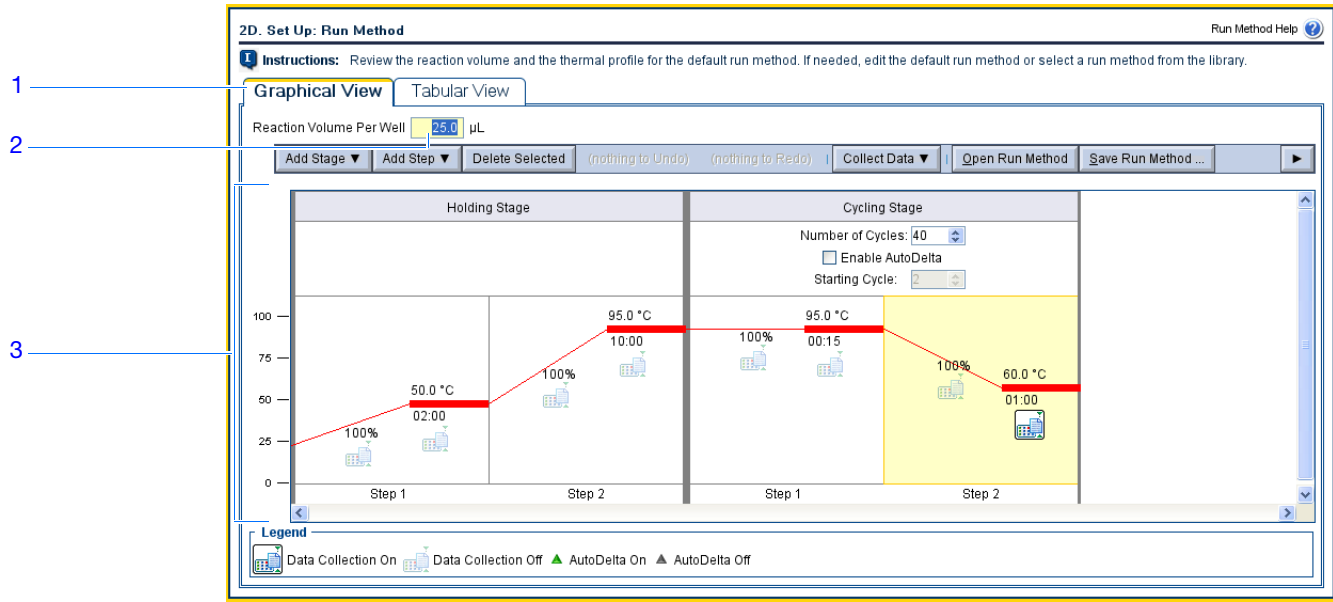
About the Example Experiment

In the standard curve example experiment, the default run method is used with one change: The reaction volume per well is changed from 20 μL to 25 μL .

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 thermal profile displays the holding and cycling stages shown below.
4. Click **Next >**.

Notes



Design Guidelines

When you design your own standard curve experiment:

- Enter a number from 10 to 30 for the reaction volume/well. The StepOne and StepOnePlus systems support reaction volumes from 10 to 30 μL .
- Review the thermal profile:
 - Make sure the thermal profile is appropriate for your reagents.
 - If you are performing 1-step RT-PCR, include a reverse transcription step.

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 StepOne software.

- If you are running the experiment on a StepOnePlus instrument and you want to set a different temperature for one or more of the VeriFlex blocks, you need to:
 - a. Design your experiment using Advanced Setup instead of the Design Wizard.
 - b. In the Plate Setup screen ([page 30](#)), select the **Assign Targets and Samples** tab, select the **View Plate Layout** tab, then select the **Enable VeriFlex™ Block** checkbox.

IMPORTANT! If you do not select the **Enable VeriFlex™ Block** checkbox in the Plate Setup screen, you will not be able to set a different temperature for one or more of the VeriFlex blocks in the Run Method screen.

- c. In the Run Method screen, select the **Graphical View** tab.



Notes _____

- d. For each VeriFlex™ block you want to change, click the temperature, then enter the desired value.

Note: You can set a different temperature for one or more of the VeriFlex blocks, or set each of the VeriFlex blocks to the same temperature. If neighboring VeriFlex blocks are not set to the same temperature, the temperature difference must be between 0.1 and 5.0 °C. The maximum temperature is 99.9 °C.

For More Information

For more information on:

- The Run Method library or on completing the Run Method screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Setting temperatures for the VeriFlex blocks, access the StepOne Software Help by clicking  or pressing **F1**.
- Using Advanced Setup, see “[Advanced Setup Workflow](#)” on page 116.

Review the Reaction Setup

On the Reaction Setup screen, select the assay type (if using TaqMan reagents), then review the calculated volumes for preparing the PCR reactions, standard dilution series, and sample dilutions. If needed, you can edit the reaction volume, excess reaction volume, component concentrations, standard concentration, and/or diluted sample concentration.

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

About the Example Experiment

In the standard curve example experiment:

- Applied Biosystems RNase P assay is used.
- The reaction volume per well is 25 µL.
- The excess reaction volume is 10%.
- The reaction components are:
 - TaqMan® Universal PCR Master Mix (2X) or TaqMan® 2X Universal PCR Master Mix, No AmpErase® UNG
 - RNase P Assay Mix (20X)
 - Sample or standard
 - Water
- The standard concentration in stock is 20,000 copies/µL.
- The diluted sample concentration is 6.6 ng/µL.
- The sample stock concentration is 100 ng/µL.

Notes

Complete the Reaction Setup Screen

Complete the Reaction Mix Calculations Tab

1. Select the **Reaction Mix Calculations** tab (default).
2. From the Select Target pane, select **RNase P** (default).
3. From the Assay Type dropdown menu, select **Inventoried/Made to Order**.
4. Make sure the Reaction Volume Per Well field displays **25 μL** .
5. Make sure the Excess Reaction Volume field displays **10%**.
6. In the Reactions for RNase P pane:
 - a. Make sure the Master Mix Concentration field displays **2.0X**.
 - b. Make sure the Assay Mix Concentration field displays **20.0X**.
 - c. Review the components and calculated volumes for the PCR reactions:

Component	Volume (μL) for 1 Reaction
Master Mix (2.0X)	12.5
Assay Mix (20.0X)	1.3
Sample (10X) or Standard	2.5 [‡]
H ₂ O	8.8
Total Volume	25.0

[‡] The sample or standard volume is limited to 10% of the total reaction volume.

2E. Set Up: Reaction Setup Reaction Setup Help

Instructions: For each target assay in the reaction plate, select the assay type (if using TaqMan reagents), then review the calculated volumes for preparing the standard dilution series, samples, and PCR reactions. If needed, edit the reaction volume, excess reaction volume, component concentrations, and/or stock concentrations. Click "Print Reaction Setup" to print instructions on how to prepare the PCR reactions.

1 **Reaction Mix Calculations** | Sample Dilution Calculations

2 **Select Target** | RNase P

Assay Type: Inventoried/Made to Order | Reaction Volume Per Well: 25 μL | Excess Reaction Volume: 10 % | Print Reaction Setup

3 **Reactions for RNase P**

6a Master Mix Concentration: 2.0 X | Assay Mix Concentration: 20.0 X

6b

Component	Volume (μL) for 1 Reaction
Master Mix (2.0X)	12.5
Assay Mix (20.0X)	1.3
Sample (10X) or Standard	2.5
H ₂ O	8.8
Total Volume	25.0

6c

Standard Dilution Series for RNase P

Standard Concentration in Stock: 100.0 ng per μL

Dilution Point	Source	Source Volume (μL)	Diluent Volume (μL)	Total Volume (μL)	Standard Concentration...
1 (10,000)					

Notes

7. In the Standard Dilution Series for RNase P pane:
 - a. Click the **Standard Concentration in Stock** field, then enter **20000**.
 - b. Click the units field, then enter **copies** per μL .
 - c. Review the calculated volumes for preparing the standard dilution series:

Dilution Point	Source	Source Volume (μL)	Diluent Volume (μL)	Total Volume (μL)	Standard Concentration (copies/ μL)
1 (10,000)	Stock	3.6	14.5	18.2	4000.0
2 (5,000)	Dilution 1	9.1	9.1	18.2	2000.0
3 (2,500)	Dilution 2	9.1	9.1	18.2	1000.0
4 (1,250)	Dilution 3	9.1	9.1	18.2	500.0
5 (625)	Dilution 4	9.1	9.1	18.2	250.0

7a
7b
7c

Complete the Sample Dilution Calculations Tab

1. Select the **Sample Dilution Calculations** tab.
2. Click the **Diluted Sample Concentration (10X for Reaction Mix)** field, then enter **6.6**.
3. From the unit dropdown menu, select **ng/ μL** (default).

Notes

4. Review the calculated volumes for the sample dilutions:

Sample Name	Stock Concentration (ng/ μ L)	Sample Volume (μ L)	Diluent Volume (μ L)	Total Volume of Diluted Sample (μ L)
pop1	100.0	1.0	14.2	15.2
pop2	100.0	1.0	14.2	15.2

2E. Set Up: Reaction Setup > Reaction Mix Calculations Reaction Setup Help ?

Instructions: For each target assay in the reaction plate, select the assay type (if using TaqMan reagents), then review the calculated volumes for preparing the standard dilution series, samples, and PCR reactions. If needed, edit the reaction volume, excess reaction volume, component concentrations, and/or stock concentrations. Click "Print Reaction Setup" to print instructions on how to prepare the PCR reactions.

Reaction Mix Calculations **Sample Dilution Calculations**

Diluted Sample Concentration (10 \times for Reaction Mix): ng/ μ L Print Reaction Setup

Sample Name	Stock Concentration (ng/ μ L)	Sample Volume (μ L)	Diluent Volume (μ L)	Total Volume of Diluted Sample (μ L)
pop1	<input type="text" value="100.0"/>	1.0	14.2	15.2
pop2	<input type="text" value="100.0"/>	1.0	14.2	15.2

Print Reaction Setup Instructions

Print detailed reaction setup instructions, then save the instructions for [Chapter 3](#), "Prepare the Reactions."

1. Click **Print Reaction Setup**.

2E. Set Up: Reaction Setup > Reaction Mix Calculations Reaction Setup Help ?

Instructions: For each target assay in the reaction plate, select the assay type (if using TaqMan reagents), then review the calculated volumes for preparing the standard dilution series, samples, and PCR reactions. If needed, edit the reaction volume, excess reaction volume, component concentrations, and/or stock concentrations. Click "Print Reaction Setup" to print instructions on how to prepare the PCR reactions.

Reaction Mix Calculations **Sample Dilution Calculations**

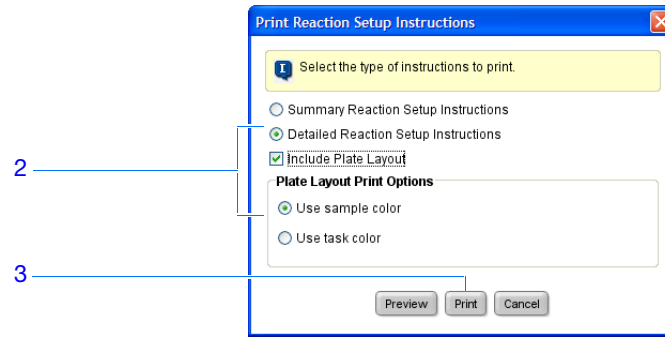
Diluted Sample Concentration (10 \times for Reaction Mix): ng/ μ L Print Reaction Setup 1

2. In the dialog box, select:

- **Detailed Reaction Setup Instructions**
- **Include Plate Layout**
- **Use sample color**

3. Click **Print** to send the reaction setup instructions to your printer.

Notes



4. Click **Next** >.

Design Guidelines

When you design your own standard curve experiment:

- If you are using TaqMan reagents, select the type of assay you are using:
 - Select **Inventoried/Made to Order** if you are using Applied Biosystems TaqMan[®] Gene Expression Assays (Inventoried or Made to Order) or Applied Biosystems Custom TaqMan[®] Gene Expression Assays.
 - Select **Custom** if you are designing your own assays with Primer Express[®] software.
- Enter a number from 10 to 30 for the reaction volume/well. The StepOne and StepOnePlus systems support 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 reaction mix concentrations for each target: If needed:
 - For TaqMan reagents, edit the master mix and assay mix concentrations.
 - For SYBR Green reagents, edit the master mix, forward primer, and reverse primer concentrations.
 - For 1-step RT-PCR, edit the reverse transcriptase concentration.
- Review the reaction mix components for each target:
 - If you are running Fast PCR reactions, make sure you use Fast master mix in the PCR reactions.
 - If you are running standard PCR reactions, make sure you use standard master mix in the PCR reactions.
 - For 1-step RT-PCR, make sure you include reverse transcriptase in the PCR reactions and use a specific buffer.
- Review the standard dilution series calculations for each target. If needed, edit the standard concentration in stock (including units).


Note: For the Standard Concentration in Stock units field, you can select **ng** or **μg** from the dropdown menu or you can enter another unit in the field (for example, **copies**, **IU**, [International Units], **nmol**, **pg**, and so on). The table updates according to your entry.

Notes

- Review the sample dilution calculations for each sample. If needed, edit the diluted sample concentration (including units) and stock concentration.

For More Information

For more information on:

- Completing the Reaction Setup screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Applied Biosystems assays, refer to the:
 - *TaqMan[®] Gene Expression Assays Protocol*
 - *Custom TaqMan[®] Gene Expression Assays Protocol*

Order Materials for the Experiment

On 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 StepOne 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 standard curve example experiment, the recommended materials are:

- MicroAmp[™] Fast Optical 48-Well Reaction Plate
- MicroAmp[™] 48-Well Optical Adhesive Film
- MicroAmp[™] 96-Well Support Base
- TaqMan[®] Universal PCR Master Mix (2X) or TaqMan[®] 2X Universal PCR Master Mix, No AmpErase[®] UNG

Notes _____

- Applied Biosystems RNase P assay

Note: The human RNase P FAM™ dye-labeled MGB probe is not available as a TaqMan® Gene Expression Assay. It can be ordered as a Custom TaqMan® Gene Expression Assay (PN 4331348).

IMPORTANT! Applied Biosystems does not recommend the use of TAMRA dye as a reporter or quencher with the StepOne system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus system.

Note: The example experiment was created for a StepOne instrument. If you selected the StepOnePlus instrument in the Experiment Properties screen (page 20), the 96-well consumables (for example, the MicroAmp™ Fast Optical 96-Well Reaction Plate) are listed in place of the 48-well consumables.

Complete the Materials List Screen

1. For the example experiment, leave the Find Assay pane empty.

The human RNase P FAM dye-labeled MGB probe is not available as a TaqMan Gene Expression Assay and cannot be ordered from the Order Materials List screen. If desired, you can order it as a Custom TaqMan Gene Expression Assay (PN 4331348). Refer to the *Custom TaqMan® Gene Expression Assays Protocol*.

Note: When you design your own standard curve experiment, see “[Design Guidelines](#)” on page 41 for information on how to complete the Find Assay pane.

2. From the Display dropdown menu, 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 on Applied Biosystems Store. To access the Applied Biosystems Store, you need to have an Internet connection.

3. (Optional) Click **Print Materials List** to send the materials list to your printer.

4. (Optional) Create a shopping list:

- a. Select the checkbox next to each of the following items:

- MicroAmp™ Fast Optical 48-Well Reaction Plate
- MicroAmp™ 48-Well Optical Adhesive Film
- MicroAmp™ 96-Well Support Base
- TaqMan® Universal PCR Master Mix (2X) or TaqMan® 2X Universal PCR Master Mix, No AmpErase® UNG

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

Notes

5. (Optional) Create a shopping basket on 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**.

3A. Order: Materials List (Optional) Materials List Help

1 Instructions: Review the list of materials recommended to prepare the PCR reaction plate. To create a shopping basket on the Applied Biosystems Store, add items to the shopping list, enter a name for the shopping basket, click "Order Materials in List," then log in.

Find Assay

Enter Gene Name Enter a gene name, then click "Find Assay" to search the Applied Biosystems Store for a gene expression assay.

Experiment Materials List

Display: All Items

<input type="checkbox"/> Check All	Item	Part Number	Description
<input checked="" type="checkbox"/>	MicroAmp™ Fast Optical 48-Well Reaction Plate...	4375816	The MicroAmp™ Fast Optical 48-Well Reaction Plate, constructed from a single rigid piece of polypropylene in a 48-well format. Increased thermal contact for faster, more uniform heating. An optically clear adhesive film used to seal the samples into the...

Experiment Shopping List (2 items)

Shopping Basket Name: Standard Curve Example StepOne

<input type="checkbox"/> Check All	Item	Part Number	Quantity
<input type="checkbox"/>	MicroAmp™ Fast Optical 48-Well Reaction P...	4375816	<input type="text" value="1"/>
<input type="checkbox"/>	MicroAmp™ 96-Well Support Base (10 bases)	4379590	<input type="text" value="1"/>

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

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

Order Materials - Log In

Log into the Applied Biosystems Store to place the selected items in your shopping basket. If you do not have a user name and password, click "Register Now" to create a new account.

Store Log In

To log into the Applied Biosystems Store, enter your user name and password then click "Log In and Submit".

User Name:

Password:

OR

Register

If you do not have an Applied Biosystems account, click the link below to create a new account.

[Register Now](#)

Remember my user name and password for future orders

Notes

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

6. Go to “Finish the Design Wizard” on page 42.

Design Guidelines

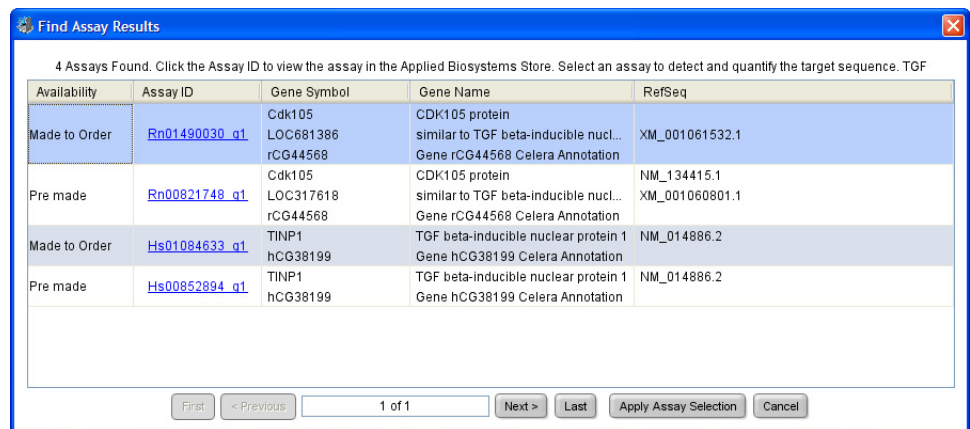
When you design your own standard curve experiment:

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

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

Note: Make sure that cookies and Java Script are turned on for the web site to function correctly.

- To find your assay on the Applied Biosystems Store, complete the Find Assay pane:
 - a. Click the **Enter Gene Name** field, enter the gene name, then click **Find Assay**.
 - b. In the Find Assay Results dialog box, select your assay.
 - c. Click **Apply Assay Selection**.



For More Information

For more information on completing the Materials List screen, access the StepOne 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 StepOne software automatically selects locations for the wells in the reaction plate. In the standard curve example experiment:

- The wells are arranged as shown below.

	1	2	3	4	5	6	7	8
A	N RNase P	N RNase P	N RNase P	pop1 RNase P	pop1 RNase P	pop1 RNase P	pop2 RNase P	pop2 RNase P
B	pop2 RNase P	S RNase P 1E4	S RNase P 1E4	S RNase P 1E4	S RNase P 5E3	S RNase P 5E3	S RNase P 5E3	S RNase P 2.5E3
C	S RNase P 2.5E3	S RNase P 2.5E3	S RNase P 1.25E3	S RNase P 1.25E3	S RNase P 1.25E3	S RNase P 625	S RNase P 625	S RNase P 625
D								
E								
F								

Note: The example experiment was created for a StepOne instrument. If you selected the StepOnePlus instrument in the Experiment Properties screen ([page 20](#)), your reaction plate layout will differ from the layout shown above. The software displays a 96-well reaction plate layout for the StepOnePlus instrument. For an example of the 96-well reaction plate layout, see [page 11](#).

- The experiment is saved as is and closed.

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

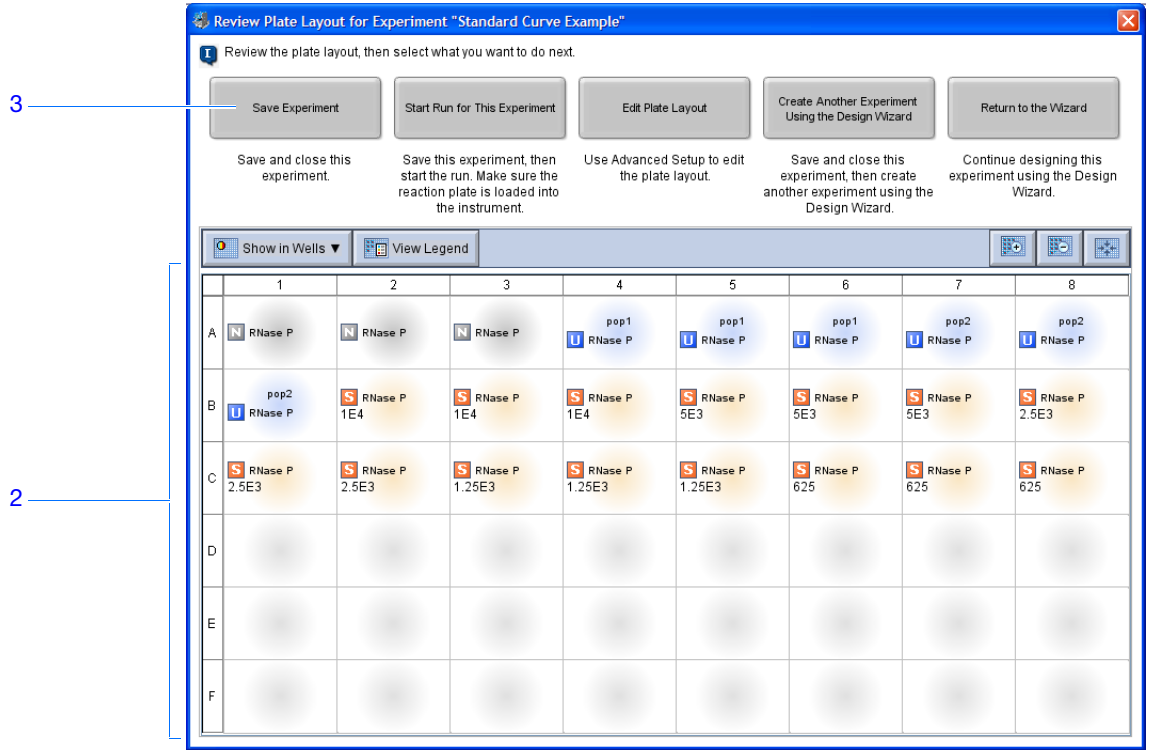
Finish the Design Wizard

- At the bottom of the StepOne software screen, click **Finish Designing Experiment**.
- In the Review Plate for Experiment window, review the plate layout. Make sure there are:
 - 6 Unknown wells **U**
 - 15 Standard wells **S**
 - 3 Negative control wells **N**
 - 24 Empty wells

Note: If the plate layout is incorrect, click **Return to the Wizard** and check your entered values.

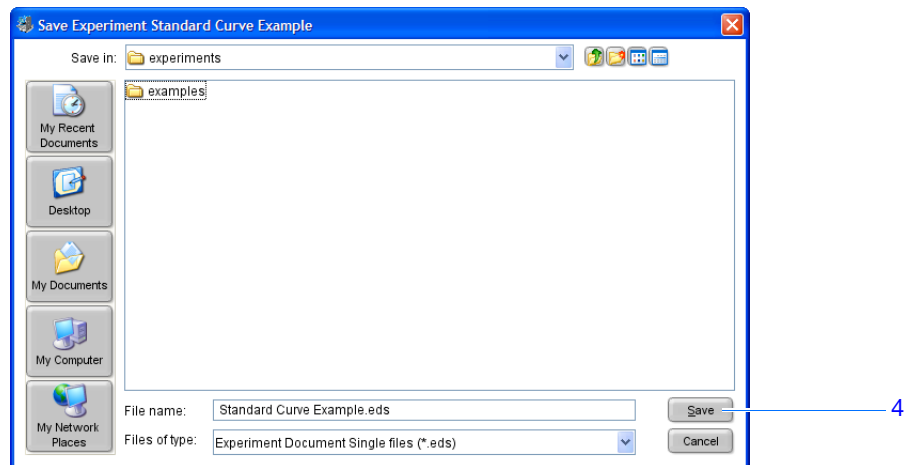
Notes

3. Click **Save Experiment**.



4. In the Save Experiment dialog box, click **Save** to accept the default file name and location. The example experiment is saved and closed, and you are returned to the Home screen.

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 standard curve experiment:

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

Click	If you want 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	<ul style="list-style-type: none"> • use advanced setup to edit the plate layout. • (<i>StepOnePlus instrument only</i>) set a different temperature for one or more of the VeriFlex blocks using Advanced Setup.
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 the <drive>:\Applied Biosystems\<software name>\experiments folder. 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 **General** tab (default). In the Default Data Folder field, browse to the desired location.

For More Information

For more information on using Advanced Setup, see [“Advanced Setup Workflow”](#) on page 116.


Notes _____

3

Prepare the Reactions

This chapter covers:

■ Chapter Overview	46
■ Prepare the Sample Dilutions	47
■ Prepare the Standard Dilution Series	49
■ Prepare the Reaction Mix	51
■ Prepare the Reaction Plate	53

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

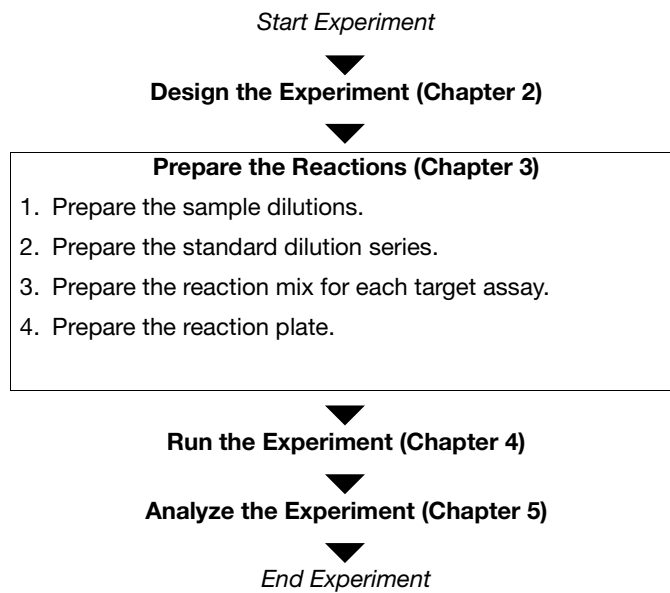
Notes

Chapter Overview

This chapter explains how to prepare the PCR reactions for the standard curve example experiment and provides guidelines for how to prepare the PCR reactions for your own standard curve 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 Sample Dilutions

Perform sample dilutions before adding the samples to the final reaction mix. Dilute the samples using the volumes that were calculated by the StepOne™ software (“[Complete the Sample Dilution Calculations Tab](#)” on page 35).

About the Example Experiment

For the standard curve example experiment:

- Sample dilutions are necessary because the sample volume is limited to 10% of the total reaction volume in the StepOne software. The total reaction volume is 25 µL/reaction, so the sample volume is 2.5 µL/reaction.
- The stock concentration is 100 ng/µL. After diluting the sample according to the Sample Dilutions Calculations table, the sample will have a concentration of 6.6 ng/µL. This is a 10× concentration when adding 2.5 µL to the final reaction mix volume of 25 µL. You will have a 1× concentration in the final reaction.
- 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)
pop1	100.0	1.0	14.2	15.2
pop2	100.0	1.0	14.2	15.2

Required Materials

- Water to dilute the sample
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Sample stock
- Vortexer
- Centrifuge

Prepare the Sample Dilutions

1. Label a separate microcentrifuge tube for each diluted sample:
 - **Population 1**
 - **Population 2**
2. Add the required volume of water (diluent) to each empty tube:

Tube	Sample Name	Diluent Volume (µL)
1	Population 1	14.2
2	Population 2	14.2

Notes _____

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

Tube	Sample Name	Sample Volume (µL)
1	Population 1	1.0
2	Population 2	1.0

4. Vortex each diluted sample for 3 to 5 seconds, then centrifuge the tubes briefly.
5. Place the diluted samples on ice until you prepare the reaction plate.

Preparation Guidelines

When you prepare your own standard curve experiment:

- Sample dilutions may be necessary because the sample volume is limited to 10% of the total reaction volume in the StepOne software. You must perform the sample dilutions before adding the samples to the final reaction mix.
- For optimal performance of TaqMan[®] Gene Expression Assays or Custom TaqMan[®] Gene Expression Assays, use 10 to 100 ng of cDNA template per 20-µL reaction. For Fast reagents, Applied Biosystems recommends 10 ng.
- Use TE buffer or water to dilute the sample.

For More Information

For more information on Applied Biosystems assays, refer to the:

- *TaqMan[®] Gene Expression Assays Protocol*
- *Custom TaqMan[®] Gene Expression Assays Protocol*

Notes _____

Prepare the Standard Dilution Series

Prepare the standard dilution series using the volumes that were calculated by the StepOne software (“[Complete the Reaction Mix Calculations Tab](#)” on page 34).

About the Example Experiment

For the standard curve example experiment:

- The standard concentration in stock is 20,000 copies/ μL .
- The volumes calculated in the software are:

Dilution Point	Source	Source Volume (μL)	Diluent Volume (μL)	Total Volume (μL)	Standard Concentration (copies/ μL)
1 (10,000)	Stock	3.6	14.5	18.2	4000.0
2 (5,000)	Dilution 1	9.1	9.1	18.2	2000.0
3 (2,500)	Dilution 2	9.1	9.1	18.2	1000.0
4 (1,250)	Dilution 3	9.1	9.1	18.2	500.0
5 (625)	Dilution 4	9.1	9.1	18.2	250.0

Required Materials

- Water to dilute the standards
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Standard stock
- Vortexer
- Centrifuge

Prepare the Standard Dilution Series for the RNase P Assay

1. Label a separate microcentrifuge tube for each standard:
 - RNase P Std. 1
 - RNase P Std. 2
 - RNase P Std. 3
 - RNase P Std. 4
 - RNase P Std. 5
2. Add the required volume of water (diluent) to each empty tube:

Tube	Standard Name	Volume of Diluent to Add (μL)
1	RNase P Std. 1	14.5
2	RNase P Std. 2	9.1
3	RNase P Std. 3	9.1

Notes

Tube	Standard Name	Volume of Diluent to Add (μL)
4	RNase P Std. 4	9.1
5	RNase P Std. 5	9.1

3. Prepare dilution 1 in the RNase P Std. 1 tube:
 - a. Vortex the stock for 3 to 5 seconds, then centrifuge the tube briefly.
 - b. Using a new pipette tip, add 3.6 μL of stock to the RNase P Std. 1 tube.
 - c. Vortex Std. 1 for 3 to 5 seconds, then centrifuge the tube briefly.
4. Prepare dilution 2 in the RNase P Std. 2 tube:
 - a. Using a new pipette tip, add 9.1 μL of dilution 1 to the RNase P Std. 2 tube.
 - b. Vortex Std. 2 for 3 to 5 seconds, then centrifuge the tube briefly.
5. Prepare dilution 3 in the RNase P Std. 3 tube:
 - a. Using a new pipette tip, add 9.1 μL of dilution 2 to the RNase P Std. 3 tube.
 - b. Vortex Std. 3 for 3 to 5 seconds, then centrifuge the tube briefly.
6. Prepare dilution 4 in the RNase P Std. 4 tube:
 - a. Using a new pipette tip, add 9.1 μL of dilution 3 to the RNase P Std. 4 tube.
 - b. Vortex Std. 4 for 3 to 5 seconds, then centrifuge the tube briefly.
7. Prepare dilution 5 in the RNase P Std. 5 tube:
 - a. Using a new pipette tip, add 9.1 μL of dilution 4 to the RNase P Std. 5 tube.
 - b. Vortex Std. 5 for 3 to 5 seconds, then centrifuge the tube briefly.
8. Place the standards on ice until you prepare the reaction plate.

Preparation Guidelines

When you prepare your own standard curve experiment:

- Standards are critical for accurate analysis of run data.
- Any mistakes or inaccuracies in making the dilutions directly affect the quality of results.
- The quality of pipettors and tips and the care used in measuring and mixing dilutions affect accuracy.
- Use TE buffer or water to dilute the standards.

Notes _____

Prepare the Reaction Mix

Prepare the reaction mix using the components and volumes that were calculated by the StepOne software (“[Complete the Reaction Mix Calculations Tab](#)” on page 34).

Note: The software calculates the volumes for all components for the PCR reactions. However, when you prepare the reaction mix in this section, include only the master mix, assay mix, and water. Add the sample or standard when you prepare the reaction plate (see “[Prepare the Reaction Plate](#)” on page 53).

About the Example Experiment

For the standard curve example experiment:

- The reaction mix components are:
 - TaqMan[®] Universal PCR Master Mix (2X) or TaqMan[®] 2X Universal PCR Master Mix, No AmpErase[®] UNG
 - RNase P Assay Mix (20X)
 - Water
- The volumes calculated in the software are:

Component	Volume (µL) for 1 Reaction
Master Mix (2.0X)	12.5
Assay Mix (20.0X)	1.3
H ₂ O	8.8
Total Volume	22.6


Note: The sample or standard is not added at this time.


Required Materials

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

Notes

Prepare the
Reaction Mix

 **CAUTION** **CHEMICAL HAZARD.** TaqMan® Universal PCR Master Mix (2X) 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® 2X 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.

1. Label an appropriately sized tube for the reaction mix: **RNase P Reaction Mix.**
2. For the RNase P assay, add the required volumes of each component to the tube:

Component	Volume (µL) for 1 Reaction	Volume (µL) for 24 Reactions (Plus 10% Excess)
TaqMan® Universal PCR Master Mix (2X) or TaqMan® 2X Universal PCR Master Mix, No AmpErase® UNG	12.5	330.0
RNase P Assay Mix (20X)	1.3	34.3
Water	8.8	232.3
Total Reaction Mix Volume	22.6	596.6

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

Preparation
Guidelines

When you prepare your own standard curve experiment:

- If your experiment includes more than one target assay, prepare the reaction mix for each target assay separately.
- Include excess volume in your calculations to provide excess volume for the loss that occurs during reagent transfers. Applied Biosystems recommends an excess volume of at least 10%.
- Include all required components.
- Prepare the reagents according to the manufacturer's instructions.
- Keep the assay mix 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.

Notes

- Thaw any frozen samples by placing them on ice. When thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.

For More Information

For more information on preparing the reaction mix, refer to the protocol appropriate for the reagents you are using in the PCR reactions:

- *TaqMan[®] Gene Expression Assays Protocol*
- *Custom TaqMan[®] Gene Expression Assays Protocol*

Prepare the Reaction Plate

Prepare the reactions for each replicate group, then transfer them to the reaction plate. Use the plate layout displayed in the StepOne software.

About the Example Experiment

For the standard curve example experiment:

- A MicroAmp[™] Fast Optical 48-Well Reaction Plate is used.
- The reaction volume is 25 μ L/well.
- The reaction plate contains:
 - 6 Unknown wells **U**
 - 15 Standard wells **S**
 - 3 Negative control wells **N**
 - 24 Empty wells
- The plate layout automatically generated by the StepOne software is used:

Notes

	1	2	3	4	5	6	7	8
A	RNase P	RNase P	RNase P	pop1 RNase P	pop1 RNase P	pop1 RNase P	pop2 RNase P	pop2 RNase P
B	pop2 RNase P	RNase P 1E4	RNase P 1E4	RNase P 1E4	RNase P 5E3	RNase P 5E3	RNase P 5E3	RNase P 2.5E3
C	RNase P 2.5E3	RNase P 2.5E3	RNase P 1.25E3	RNase P 1.25E3	RNase P 1.25E3	RNase P 625	RNase P 625	RNase P 625
D								
E								
F								

Note: The example experiment was created for a StepOne instrument. If you selected the StepOnePlus instrument in the Experiment Properties screen ([page 20](#)), your reaction plate layout will differ from the layout shown above. The software displays a 96-well reaction plate layout for the StepOnePlus instrument. For an example of the 96-well reaction plate layout, see [page 11](#).

Required Materials

- Microcentrifuge tubes
- Pipettors
- Pipette tips
- RNase P reaction mix (from [page 52](#))
- Water
- Standards (from [page 49](#))
- Samples (from [page 47](#))
- MicroAmp™ Fast Optical 48-Well Reaction Plate
- MicroAmp™ 48-Well Optical Adhesive Film
- Centrifuge

Prepare the Reaction Plate

1. Prepare the negative control reactions for the target:
 - a. To an appropriately sized tube, add the volumes of reaction mix and water listed below.

Tube	Reaction Mix	Reaction Mix Volume (μL)	Water Volume (μL)
1	RNase P reaction mix	74.6	8.3

- b. Mix the reaction by gently pipetting up and down, then cap the tube.

Notes _____

- c. Centrifuge the tube briefly to remove air bubbles.
 - d. Add 25 μL of the negative control reaction to the appropriate wells in the reaction plate.
2. For each replicate group, prepare the standard reactions:
- a. To appropriately sized tubes, add the volumes of reaction mix and standard listed below.

Tube	Standard Reaction	Reaction Mix	Reaction Mix Volume (μL)	Standard	Standard Volume (μL)
1	RNase P Std 1	RNase P reaction mix	74.6	RNase P Std 1	8.3
2	RNase P Std 2	RNase P reaction mix	74.6	RNase P Std 2	8.3
3	RNase P Std 3	RNase P reaction mix	74.6	RNase P Std 3	8.3
4	RNase P Std 4	RNase P reaction mix	74.6	RNase P Std 4	8.3
5	RNase P Std 5	RNase P reaction mix	74.6	RNase P Std 5	8.3

- b. Mix the reactions by gently pipetting up and down, then cap the tubes.
 - c. Centrifuge the tubes briefly to remove air bubbles.
 - d. Add 25 μL of the standard reaction to the appropriate wells in the reaction plate.
3. For each replicate group, prepare the reactions for the unknowns:
- a. To appropriately sized tubes, add the volumes of reaction mix and sample listed below.

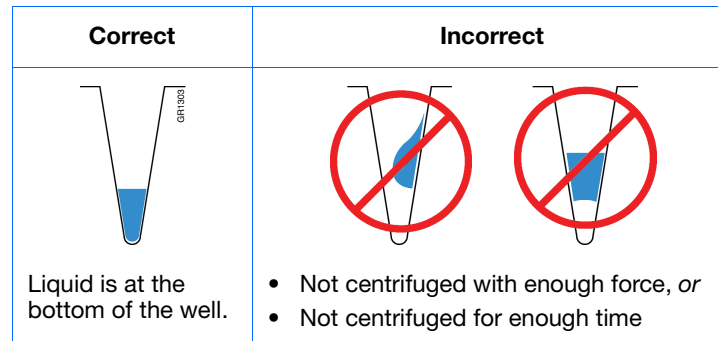
Tube	Unknown Reaction	Reaction Mix	Reaction Mix Volume (μL)	Sample	Sample Volume (μL)
1	RNase P pop1	RNase P reaction mix	74.6	pop1	8.3
2	RNase P pop2	RNase P reaction mix	74.6	pop2	8.3

- b. Mix the reactions by gently pipetting up and down, then cap the tubes.
- c. Centrifuge the tubes briefly to remove air bubbles.
- d. Add 25 μL of the unknown (sample) reaction to the appropriate wells in the reaction plate.

Notes

4. Seal the reaction plate with optical adhesive film.
5. Centrifuge the reaction plate briefly to remove air bubbles.
6. 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(s) and cause an abnormally high background signal.


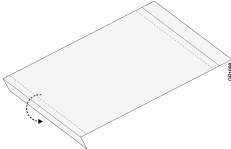


7. Until you are ready to perform the run, place the reaction plate on ice in the dark.

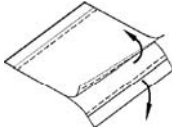
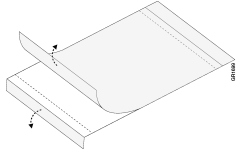
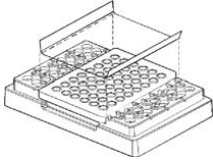
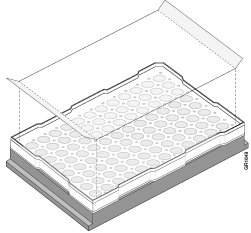
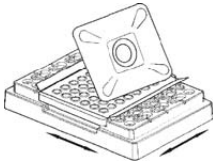
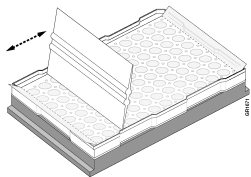
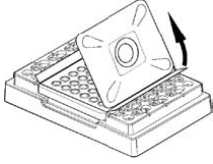
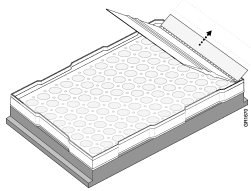
Preparation Guidelines

When you prepare your own standard curve experiment:

- Make sure you use the appropriate consumables.
- Make sure the arrangement of the PCR reactions matches the plate layout displayed in the StepOne software. You can either:
 - Accept the plate layout automatically generated by the software.
 - or*
 - Use Advanced Setup to change the plate layout in the software.
- If you use optical adhesive film to seal your reaction plates, seal each reaction plate as follows:

Action	Example	
	StepOne™ System	StepOnePlus™ System
1. Place the reaction plate onto the center of the 96-well base. Be sure the reaction plate is flush with the top surface of the 96-well base.		
2. Load the reaction plate as desired.		
3. Remove a single optical adhesive film (film) from the box. <ul style="list-style-type: none"> • <i>For the StepOne system reaction plate</i>, bend both end-tabs upward. Hold the film backing side up. • <i>For the StepOnePlus system reaction plate</i>, fold back one of the end-tabs. Hold the film backing side up. 		

Notes _____

Action	Example	
	StepOne™ System	StepOnePlus™ System
<p>4. In one swift movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.</p> <p>IMPORTANT! Improper peeling of the optical adhesive film may result in haziness, but will not affect results. Haziness will disappear when the film comes into contact with the heated cover in the instrument.</p>		
<p>5. Holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure the film is completely covering all wells of the reaction plate.</p>		
<p>6. 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.</p>		
<p>7. 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.</p>		
<p>8. To ensure a tight, evaporation-free seal:</p> <ol style="list-style-type: none"> Repeat step 6. While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film. <p>Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight, evaporation-free seal.</p>		
<p>9. 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.</p>		

For More Information

For more information on:

- Preparing the reaction plate, refer to the protocol appropriate for the reagents you are using in the PCR reactions:
 - *TaqMan® Gene Expression Assays Protocol*
 - *Custom TaqMan® Gene Expression Assays Protocol*
- Consumables, see [“Supported Consumables” on page 4](#).
- Using Advanced Setup to change the plate layout, see [“Advanced Setup Workflow” on page 116](#).

Notes


Notes _____

4

Run the Experiment

This chapter covers:

- Chapter Overview 60
- Prepare for the Run 61
- (Optional) Enable the Notification Settings 63
- Start the Run 65
- Monitor the Run 69
- Unload the Instrument and Transfer the Data 76

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

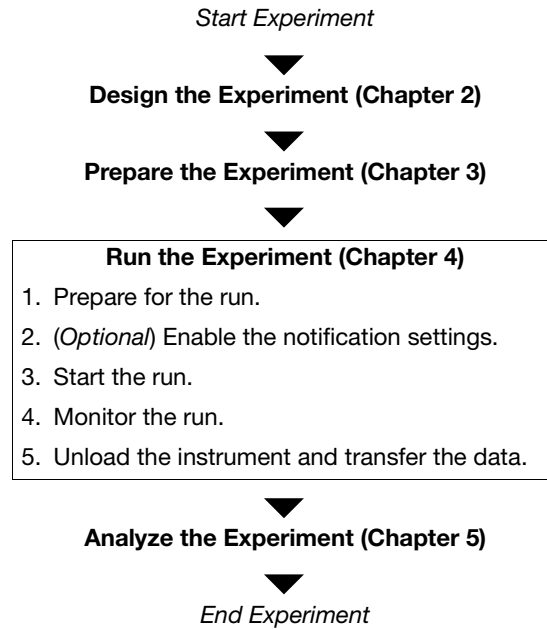
Notes

Chapter Overview

This chapter explains how to perform a run on the Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems.

Example Experiment Workflow

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




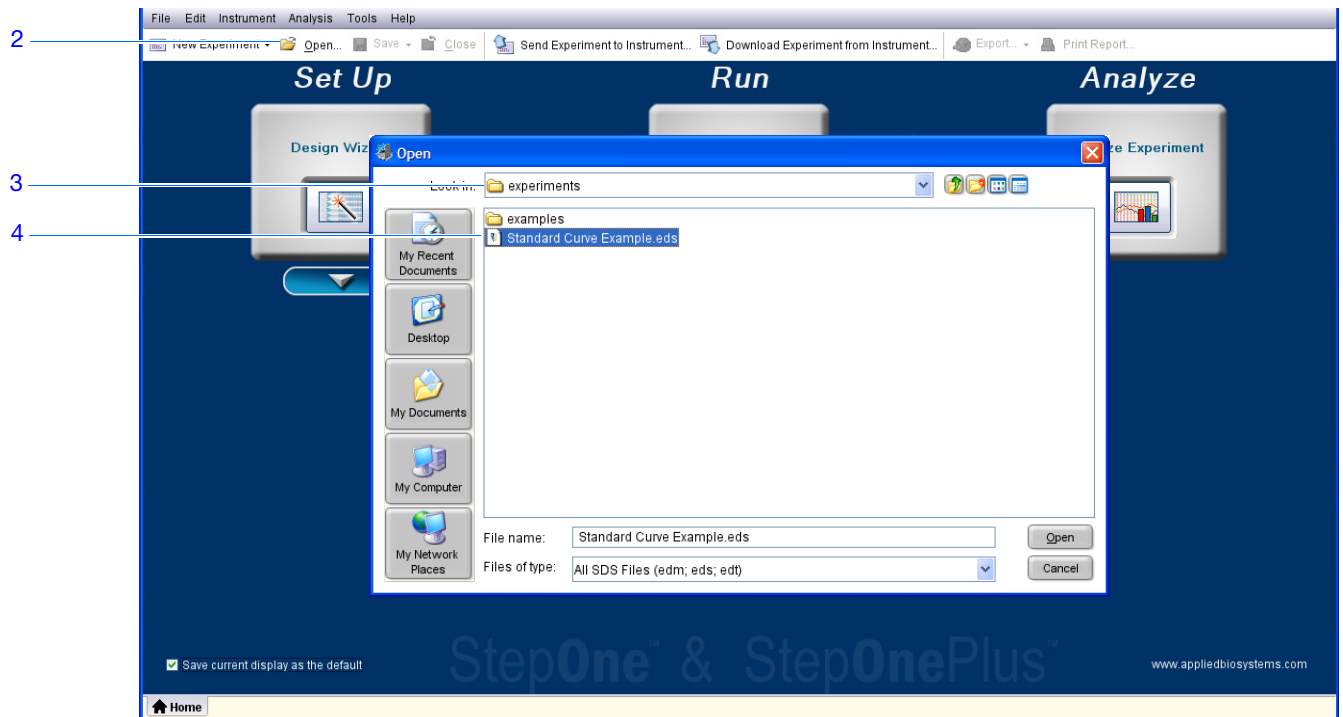
Notes _____

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 StepOne™ or StepOnePlus™ instrument.

Open the Example Experiment


1. Double-click  (StepOne software shortcut) or select **Start ▶ All Programs ▶ Applied Biosystems ▶ StepOne Software ▶ <software name>** where <software name> is the current version of the StepOne software.
2. From 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 **Standard Curve Example** to open the example experiment file you created in [Chapter 2](#).



Notes

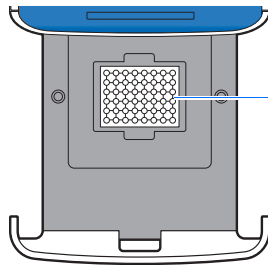
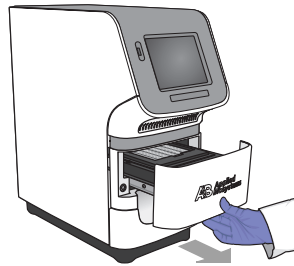
Load the Reaction Plate Into the Instrument



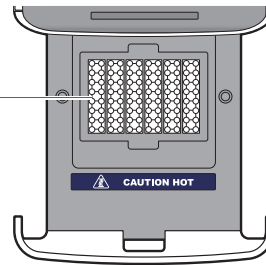
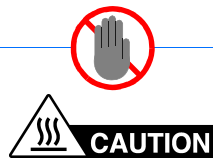
 **CAUTION** **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the sample block(s) can exceed 100 °C. If the instrument has been used recently, keep your hands away until the sample block(s) reaches room temperature.

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

1. Open the instrument drawer.



StepOne instrument
sample block



StepOnePlus
instrument VeriFlex™
Sample Blocks

2. Place the reactions in the sample block(s):
 - If using a reaction plate: Place the reaction plate in the sample block(s) with well A1 at the back-left corner.
 - If using reaction tube strips: Place the tray containing the tube strips in the sample block(s).
 - If using reaction tubes: Place the tray containing the tubes in the sample block(s).



Notes _____

IMPORTANT! For optimal performance with partial loads:

StepOnePlus instruments - Load at least 16 tubes and arrange them in:

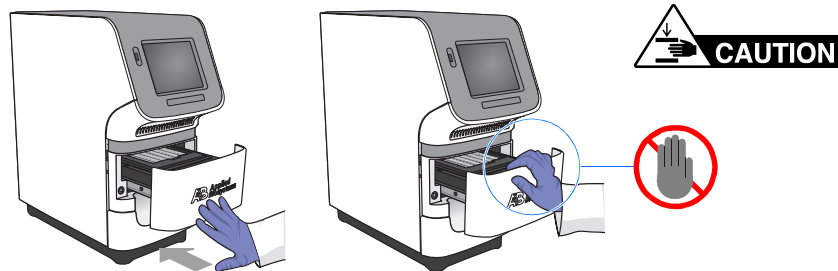
- Adjacent columns of 8 tubes, using rows A through H. For example, fill wells in column 1 (rows A through H) and column 2 (rows A through H).

or

- Adjacent rows of 8 tubes, using columns 3 through 10. For example, fill wells in row A (columns 3 through 10) and row B (columns 3 through 10).

StepOne instruments - Load at least 4 tubes in the sample block.

3. Close the instrument drawer carefully.



(Optional) Enable the Notification Settings

Enable the notification settings so that the StepOne software alerts you via email when the StepOne or StepOnePlus 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 StepOne™ and StepOnePlus™ systems or the duration of the run.

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

Note: The notification system is also available to computers that are monitoring a StepOne or StepOnePlus instrument remotely. For more information, see [“Remote Monitor” on page 73](#).



About the Example Experiment

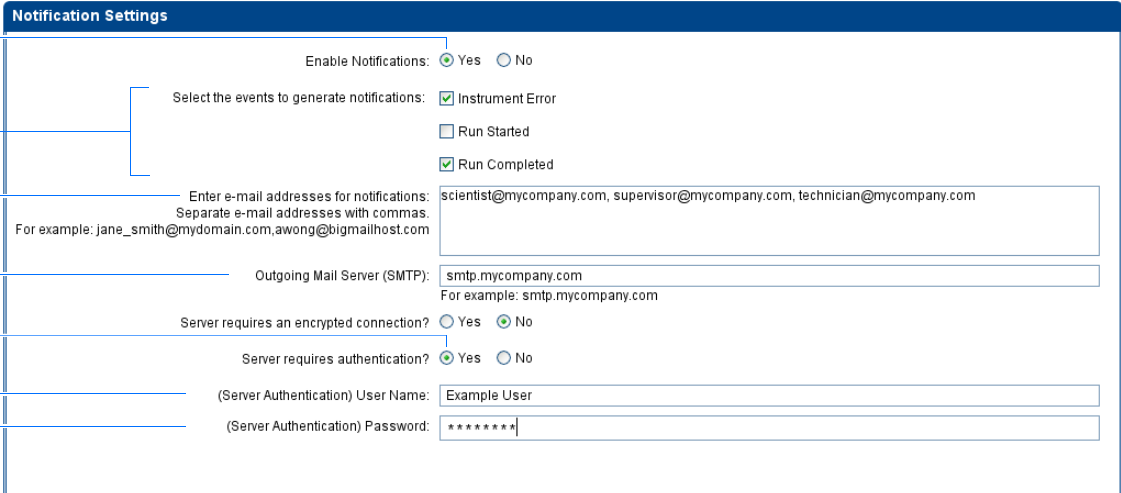
In the example experiment:

- The StepOne software is set up to send notifications to three users (scientist, supervisor, and technician at mycompany.com) when the StepOne or StepOnePlus 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.

Notes

Set Up the Notification Settings

1. In the StepOne 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**.



The screenshot shows the 'Notification Settings' dialog box with the following configuration:

- 3:** Enable Notifications: Yes No
- 4:** Select the events to generate notifications:
 - Instrument Error
 - Run Started
 - Run Completed
- 5:** Enter e-mail addresses for notifications: scientist@mycompany.com, supervisor@mycompany.com, technician@mycompany.com
- 6:** Outgoing Mail Server (SMTP): smtp.mycompany.com
- 7a:** Server requires an encrypted connection? Yes No
- 7b:** Server requires authentication? Yes No
- 7c:** (Server Authentication) User Name: Example User
- (Server Authentication) Password: *****

Run Guidelines

When you set up the StepOne or StepOnePlus system for automatic notification:

- Your system must be set up for network use. Refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking, and Maintenance Guide*.
- Select the events for which you want to receive e-mail notifications:
 - **Instrument Error** – When selected, recipients are e-mailed all errors encountered by the instrument during each run.
 - **Run Started** – When selected, recipients are e-mailed every time the instrument starts a run.

Notes _____

- **Run Completed** – When selected, recipients are e-mailed every time 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)

Start the Run

Start the run according to the layout of your StepOne or StepOnePlus system:

Layout	Description	See...
Colocated	The yellow cable connects the computer to the instrument	“Colocated Startup” below
Standalone	<ul style="list-style-type: none"> • The computer and the instrument are not connected, or • The computer and the instrument are connected to the same network. 	“Standalone Startup” on page 66

Colocated Startup

Perform this procedure if your computer is directly connected to your StepOne or StepOnePlus instrument by the yellow cable.

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




Notes

Standalone Startup

Perform these procedures if your computer and StepOne or StepOnePlus instrument *are not* directly connected by the yellow cable. Start with:

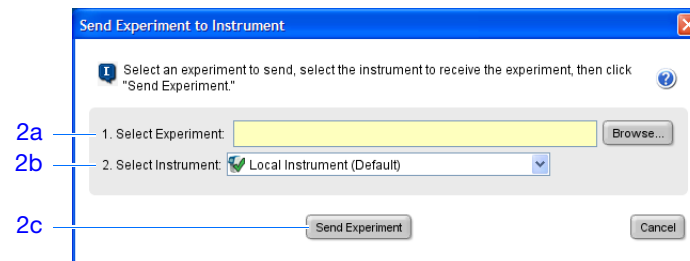
- “[Send the Experiment to the Instrument Over the Network](#)” on page 66 if your computer and instrument are connected to the same network.
- or*
- “[Transfer the Experiment to the Instrument Using a USB Drive](#)” on page 66 if your computer and instrument are not connected to the same network.

Send the Experiment to the Instrument Over the Network

1. In the StepOne software, click  **Send Experiment to Instrument**.
2. In the Send Experiment to Instrument dialog box:
 - a. Click **Browse**, navigate to the example experiment file, then click **Open**.
 - b. Select the instrument to receive the experiment file.


Note: If your instrument is not listed, set up the instrument for monitoring as explained in the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking, and Maintenance Guide*.

- c. Click **Send Experiment** to send the experiment to the your instrument over the network.



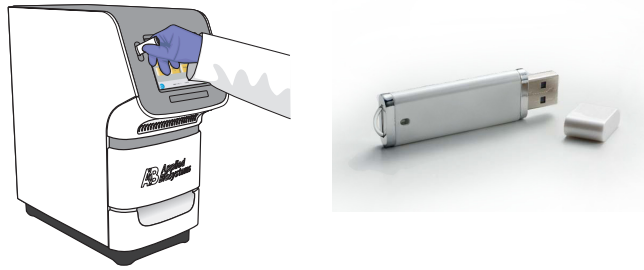
3. When prompted, click **OK** to close the confirmation.
4. Go to “[Start the Instrument Run Using the Touchscreen](#)” on page 67.

Transfer the Experiment to the Instrument Using a USB Drive

1. Connect the USB drive to one of the USB ports on the computer.
2. In the StepOne software, select  **Save ▶ Save As**.
3. In the Save dialog box, navigate to the USB drive, then click **Save**.

Notes _____


4. Remove the USB drive from your computer, then connect it to the USB port of your StepOne or StepOnePlus instrument.





5. Go to [“Start the Instrument Run Using the Touchscreen”](#) below.

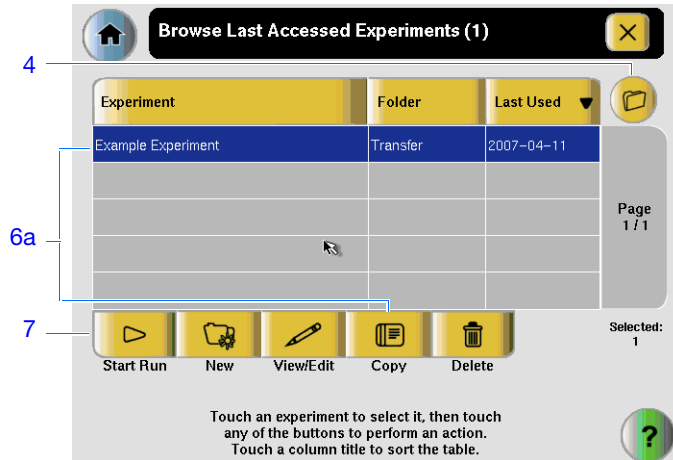
Start the Instrument Run Using the Touchscreen

1. Touch the StepOne or StepOnePlus instrument touchscreen to awaken it.

Note: If the touchscreen is not at the Main Menu screen, touch .

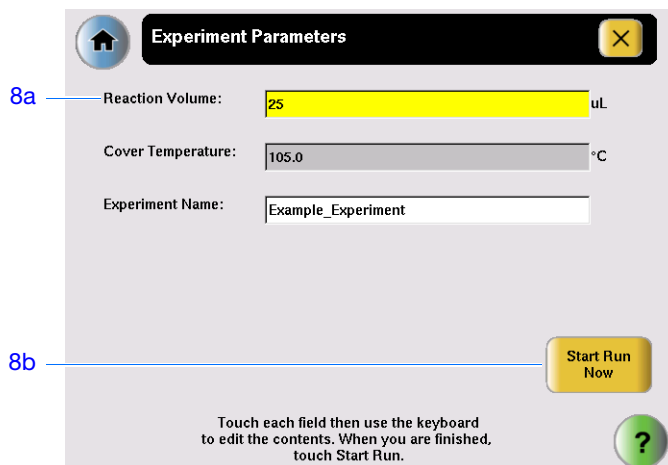
2. Wait for the USB sign to appear on the touchscreen.
3. In the Main Menu screen, touch **Browse/New Experiments**.
4. In the Browse screen, touch  **Folders**.
5. In the Choose an Experiment Folder screen:
 - Touch **USB** if you transferred the experiment on a USB drive.
 - Touch **Default** if you sent the experiment over a network connection.
6. Before starting the run, save the example experiment to your instrument:
 - a. In the Browse screen, touch the example experiment name, then touch **Copy**.
 - b. In the Save Experiment screen, navigate to a destination folder, then click **Save & Exit**.
7. In the Browse screen, touch the example experiment name, then touch  **Start Run**.

Notes



8. In the Run Parameters screen:

- a. Touch the **Reaction Volume** field, use the keypad to enter the reaction volume for the example experiment, then touch **Done**.
- b. Touch **Start Run Now**.



Notes _____




Monitor the Run

Monitor the run according to the layout of your StepOne or StepOnePlus system:

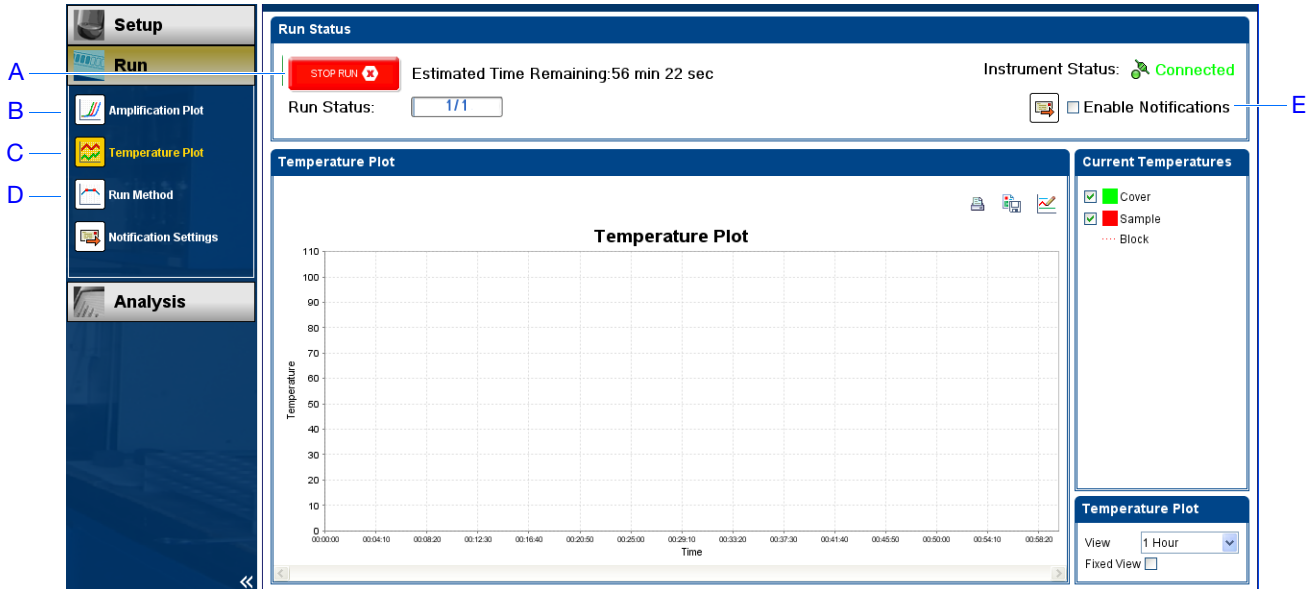
Layout	Description	See...
Colocated	The yellow cable connects the computer to the instrument.	“Colocated Monitoring” below
Standalone (Networked)	The computer and the instrument are connected to the same network.	“Remote Monitor” on page 73
Standalone (Basic)	The computer and the instrument are not connected.	“Standalone Monitoring” on page 75

Colocated Monitoring

If your computer is directly connected to your StepOne or StepOnePlus instrument by the yellow cable, you can view the progress of the run in realtime as described below. During the run, periodically view all three plots available from the StepOne software for potential problems.

#	To...	Action
A	Stop the run	<ol style="list-style-type: none"> In the StepOne software, click STOP RUN. In the Stop Run dialog, click one of the following: <ul style="list-style-type: none"> 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.
B	View amplification data in realtime	Select  Amplification Plot . See “About the Amplification Plot Screen” on page 70.
C	View temperature data for the run in realtime	Select  Temperature Plot . See “About the Temperature Plot Screen” on page 71.
D	View progress of the run in the Run Method screen	Select  Run Method . See “About the Run Method Screen” on page 72.
E	Enable/disable the Notification Settings	Select or deselect Enable Notifications . See “(Optional) Enable the Notification Settings” on page 63.

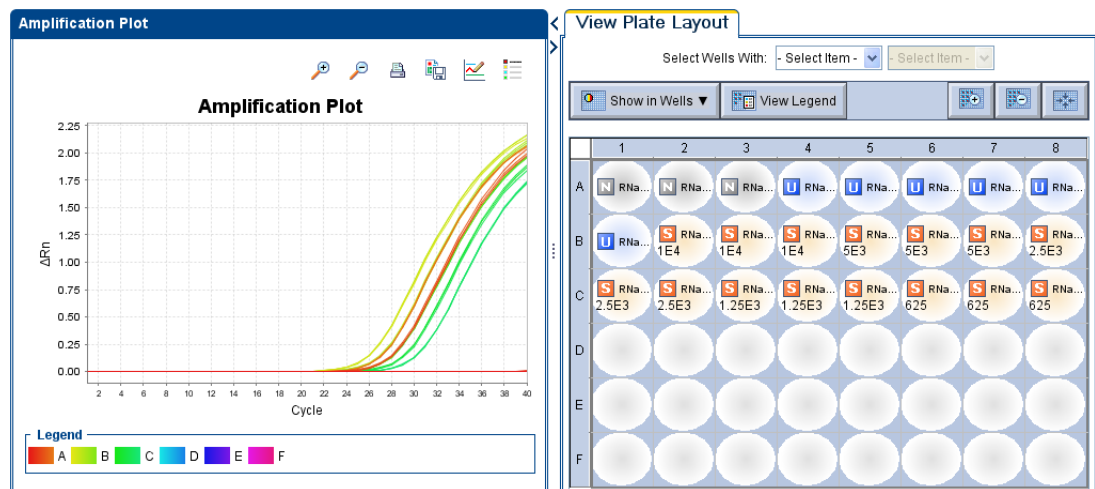
Notes _____



About the Amplification Plot Screen

The Amplification Plot screen allows you to view 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 (ΔR_n) 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.




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

- Increased fluorescence in negative control wells.

Notes _____

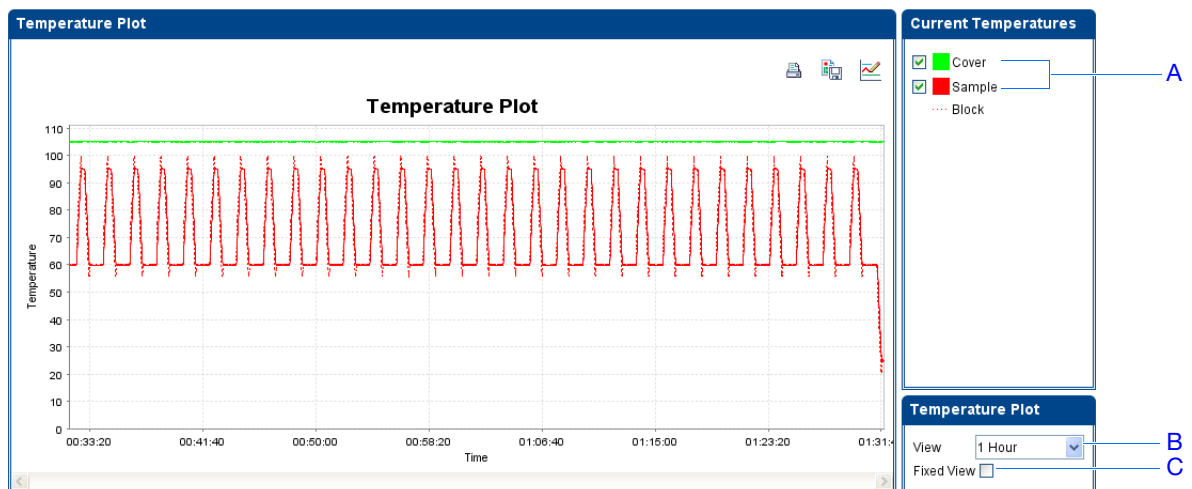
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

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

About the Temperature Plot Screen

During a run, the Temperature Plot screen displays the temperatures of the sample block(s), the heated cover, and samples (calculated) in realtime. The figure below shows the Temperature Plot screen as it appears during the example experiment.


	To...	Action
A	Add/remove temperature plots	Select Cover or Sample Block to toggle the presence of the associated data in the plot.
B	Change the time displayed by plot	From the View dropdown menu, select the amount of time to display in the plot.
C	Display a fixed time window during the instrument run If the entire plot does not fit in the screen, the screen is not updated as the run progresses. For example, if you select 10 minutes from the View dropdown menu, the plot will show data for 10 minutes. If the run lasts more than 10 minutes: <ul style="list-style-type: none"> • The plot updates as the run progresses with Fixed View deselected. • The plot does not update as the run progresses with Fixed View selected. 	Select Fixed View .



The Temperature Plot screen can be useful for identifying hardware failures. When monitoring the Temperature Plot screen, observe the Sample and Block plots for abnormal behavior.

Notes

- In general, the Sample and Block plots should mirror each other approximately. A significant deviation of the plots may indicate a problem.
- The Cover plot should maintain the constant temperature specified in the method. A departure from the **constant** temperature may indicate a problem.

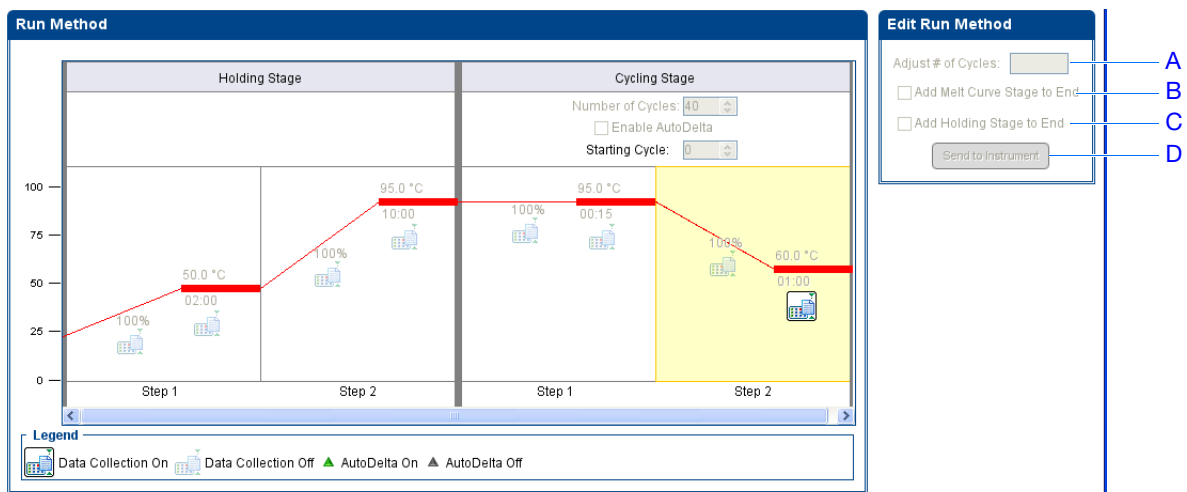
If you notice an abnormal temperature plot, troubleshoot the error as explained in the StepOne Software Help (click  or press **F1**).


Note: The Sample temperature displayed in the Current Temperatures group is an estimated value.

About the Run Method Screen

The Run Method 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 Adjust # of Cycles field, enter the number of cycles to apply to the Cycling Stage.
B	Add a melt curve stage to the end of the run	Select Add Melt Curve Stage to End.
C	Add a Hold stage to the end of the run	Select Add Holding Stage to End.
D	Apply your changes	Click Send to Instrument.



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

Notes

Remote Monitor If your StepOne or StepOnePlus instrument is connected to a network, you can use the Remote Monitor in the StepOne software to view the progress of the run in realtime from any computer on the network.

IMPORTANT! Networked computers cannot control the StepOne or StepOnePlus instrument, only monitor it.

To monitor your instrument remotely:

1. In the StepOne software, select **Instrument ▶ Remote Monitor**.
2. In the navigation pane, select your instrument.

If the navigation pane does not list your instrument:

- a. Click **Add Instrument**.
- b. Enter a name for the instrument profile within the Remote Monitor.

Note: Enter any name that helps you identify the instrument. The profile name you enter will be displayed in the Remote Monitor and in the instrument dropdown menus when you send experiments, download experiments, or monitor instruments.

- c. In the Instrument Name, Host Name, or IP Address field:
 - If you know the host name, enter the host name.
 - If you do not know the host name, enter the instrument name or IP address.

Note: The instrument name and IP address are displayed on the instrument touchscreen. Go to **Settings Menu ▶ Admin Menu ▶ Set Instrument Name** or **Set IP Address**. Contact your systems administrator or information technology department for the host name.

- d. Click **Save & Exit**.


2b — Profile Name

2c — Instrument Name, Host Name, or IP Address

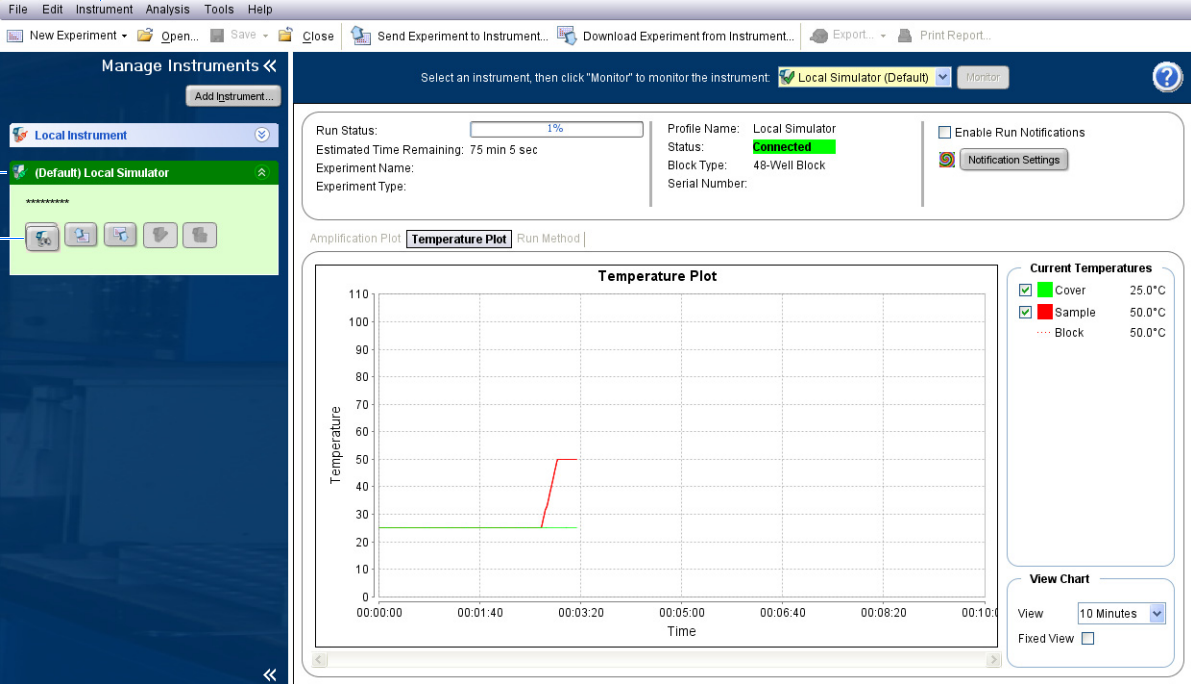
2d — Save & Exit

Note: For more information on configuring the StepOne or StepOnePlus instrument for network use or for the Remote Monitor feature, refer to the *Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking, and Maintenance Guide*.

Notes

3. Click  **Start monitoring the instrument** for your instrument. It may take several minutes for the instrument to send the information to your computer.

1



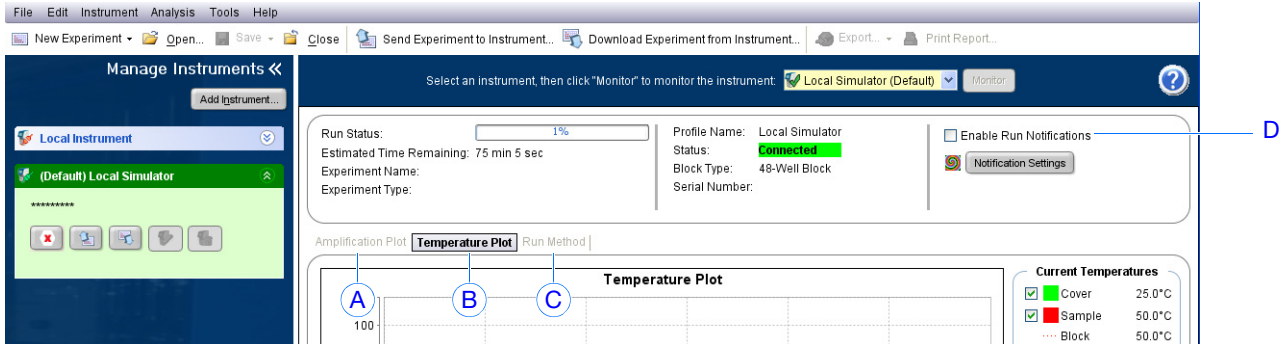
2

3

4. View data as described below.

#	To...	Action
A	View amplification data in realtime	Click Amplification Plot . See “About the Amplification Plot Screen” on page 70.
B	View temperature data for the run in realtime	Click Temperature Plot . See “About the Temperature Plot Screen” on page 71.
C	View progress of the run in the Run Method screen	Click Run Method . See “About the Run Method Screen” on page 72.
D	Enable/disable the Notification Settings	Select or deselect Enable Notifications . See “(Optional) Enable the Notification Settings” on page 63.

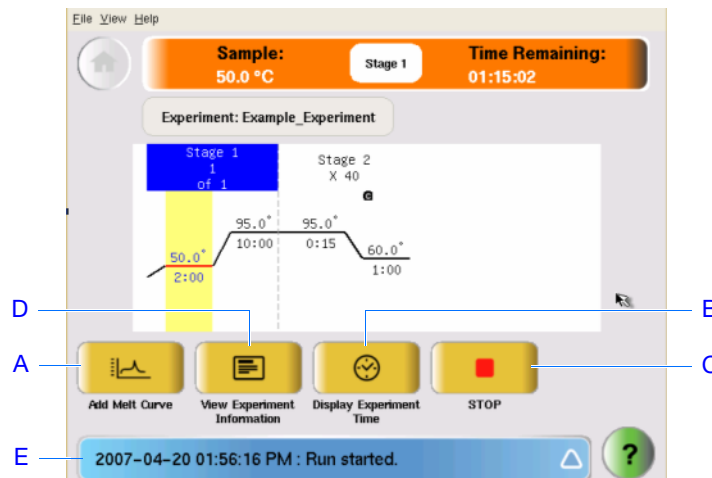
Notes _____



Standalone Monitoring

If you started the run from your StepOne or StepOnePlus instrument, you can view the progress of the run from the touchscreen. The Run Method screen displays the method for the experiment and highlights the thermal profile steps as the instrument performs them.

#	To...	Action
A	Add a melt curve stage to the run	Touch Add Melt Curve , then touch OK .
B	Display the time remaining in the run	Touch Display Experiment Time , then touch X to return to the Run Method screen.
C	Stop the run	Touch STOP , then touch: <ul style="list-style-type: none"> • Stop to stop the run after the instrument completes the current cycle or hold. • Abort to stop the run immediately. • X to continue the run with no changes.
D	View experiment information	Touch View Experiment Information , then touch X to return to the Run Method screen.
E	View the Error Log	Touch the status bar to display the error log.




Notes


Unload the Instrument and Transfer the Data

When your StepOne or StepOnePlus instrument displays the Main Menu screen, unload the reaction plate from the instrument and transfer the experiment data to the computer for analysis.

Unload the Reaction Plate

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

Note: When the StepOne or StepOnePlus instrument completes a run, the system saves the details of the run to the run history, which remains present in the system until the instrument completes another run.

1. When the Run Report screen appears in the StepOne or StepOnePlus instrument touchscreen, touch .
2. Open the instrument drawer.
3. Remove the reaction plate from the sample block(s).
4. Carefully close the instrument drawer.



Select a Data Transfer Method

Transfer the experiment to your computer for analysis according to the layout of your StepOne or StepOnePlus system:

Layout	Description	See...
Colocated	The yellow cable connects the computer and the instrument.	“Colocated Data Transfer” below
Standalone (Networked)	The computer and the instrument are connected to the same network.	“Remote Data Transfer” on page 77
Standalone (Basic)	The computer and the instrument are not connected.	“Standalone Data Transfer” on page 78

Notes _____


Colocated Data Transfer

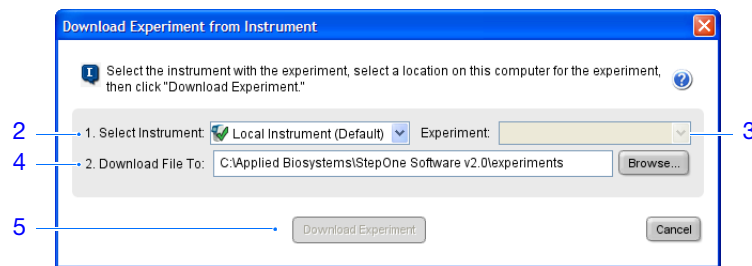
If your computer is directly connected to your StepOne or StepOnePlus instrument by the yellow cable, no action is necessary. The StepOne software automatically transfers the experiment data from the instrument to the computer after the run.

Note: In a colocated layout, you can start the run from the computer or from the instrument touchscreen. However, the StepOne software only transfers the experiment data automatically when a run is started from the computer (see “Colocated Startup” on page 65).

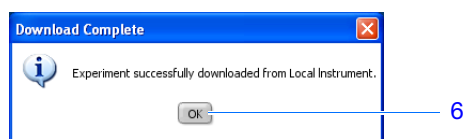
Remote Data Transfer

If your computer and StepOne or StepOnePlus instrument are connected to the same Ethernet network, download the experiment from the instrument over the network:

1. In the StepOne software, click  **Download Experiment from Instrument** to open the Download Experiment from Instrument dialog box.
2. From the Select Instrument dropdown menu, select your instrument.
3. From the Experiment dropdown menu, select the example experiment file.
4. In the Download File To field:
 - a. Click **Browse**.
 - b. Navigate to:
`<drive>:\Applied Biosystems\<software name>\experiments\`
 where:
`<drive>` is the computer hard drive on which the StepOne software is installed. The default installation drive for the software is the D drive.
`<software name>` is the current version of the StepOne software.
 - c. Click **Select**.
5. Click **Download Experiment** to download the example experiment file from your instrument to your computer over the network.



6. When prompted, click **OK** to close the confirmation.

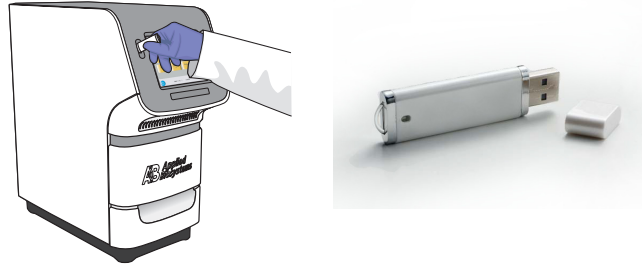


Notes


Standalone Data Transfer

If your computer is not connected to your StepOne or StepOnePlus instrument, use the USB drive to transfer the experiment from the instrument to the computer:

1. If not already connected to the instrument, connect a USB drive to the USB port.



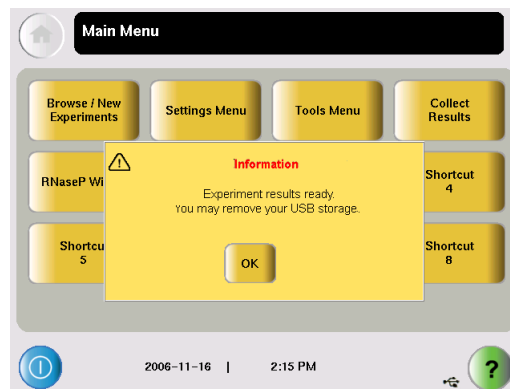
2. Touch the StepOne or StepOnePlus instrument touchscreen to awaken it.

Note: If the touchscreen is not at the Main Menu screen, touch .

3. Wait for the USB sign to appear on the touchscreen.
4. In the Main Menu, touch **Collect Results** to save the data to the USB drive.

Note: If your instrument cannot find the USB drive, remove the USB drive, then try again. If the instrument still does not recognize the USB drive, try another USB drive.

5. When prompted that the data has been transferred successfully, touch **OK**.



6. Remove the USB drive from your instrument, then connect it to one of the USB ports on your computer.
7. In the computer desktop, use the Windows explorer open the USB drive.

Notes _____

8. Copy the example experiment file to:

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

where:

- `<drive>` is the computer hard drive on which the StepOne software is installed. The default installation drive for the software is the D drive.
- `<software name>` is the current version of the StepOne software.

Notes


Notes _____

5

Analyze the Experiment

This chapter covers:

■ Chapter Overview	82
■ Section 5.1 Review Results	83
■ Section 5.2 Troubleshoot (If Needed)	103

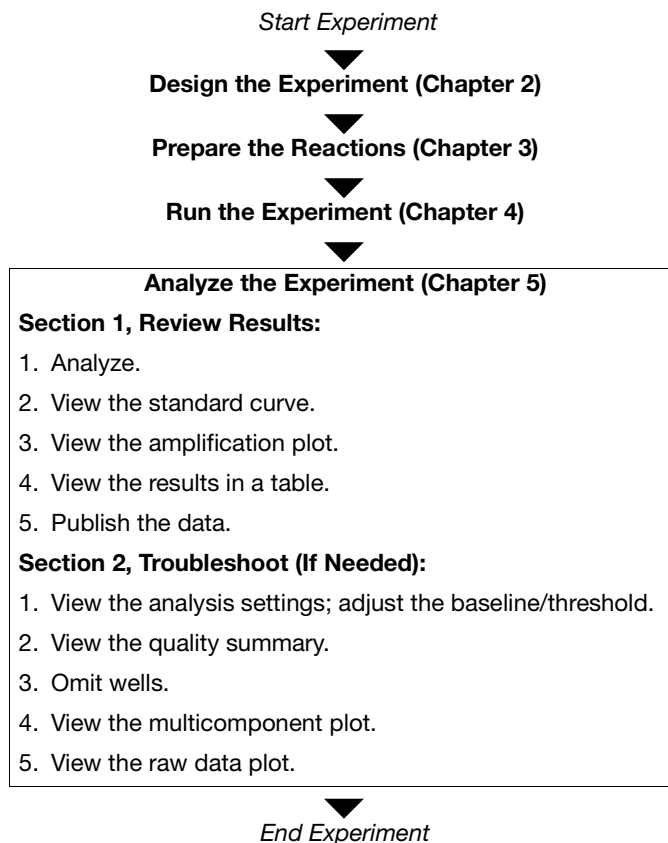
Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems StepOne™ Real-Time PCR Software v2.0 by pressing **F1**, clicking  in the toolbar, or selecting **Help ▶ StepOne Software Help**.

Chapter Overview

The StepOne™ software analyzes the data using the standard curve quantitation method. Section 1 of this chapter explains how to review the analyzed data using several of the analysis screens and how to publish the 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 84
- View the Standard Curve 90
- View the Amplification Plot 92
- View the Well Table 99
- Publish the Data 102

Notes _____

Analyze the Experiment

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

About the Example Experiment


For the standard curve example experiment, use the data file that installs with the StepOne software. The data file was created with the same design parameters provided in [Chapter 2](#), then run and analyzed on a StepOne™ instrument. You can find the data file for the example experiment on your computer:

```
<drive>:\Applied Biosystems\<software name>\experiments\examples\  
Standard Curve Example.eds
```

where:

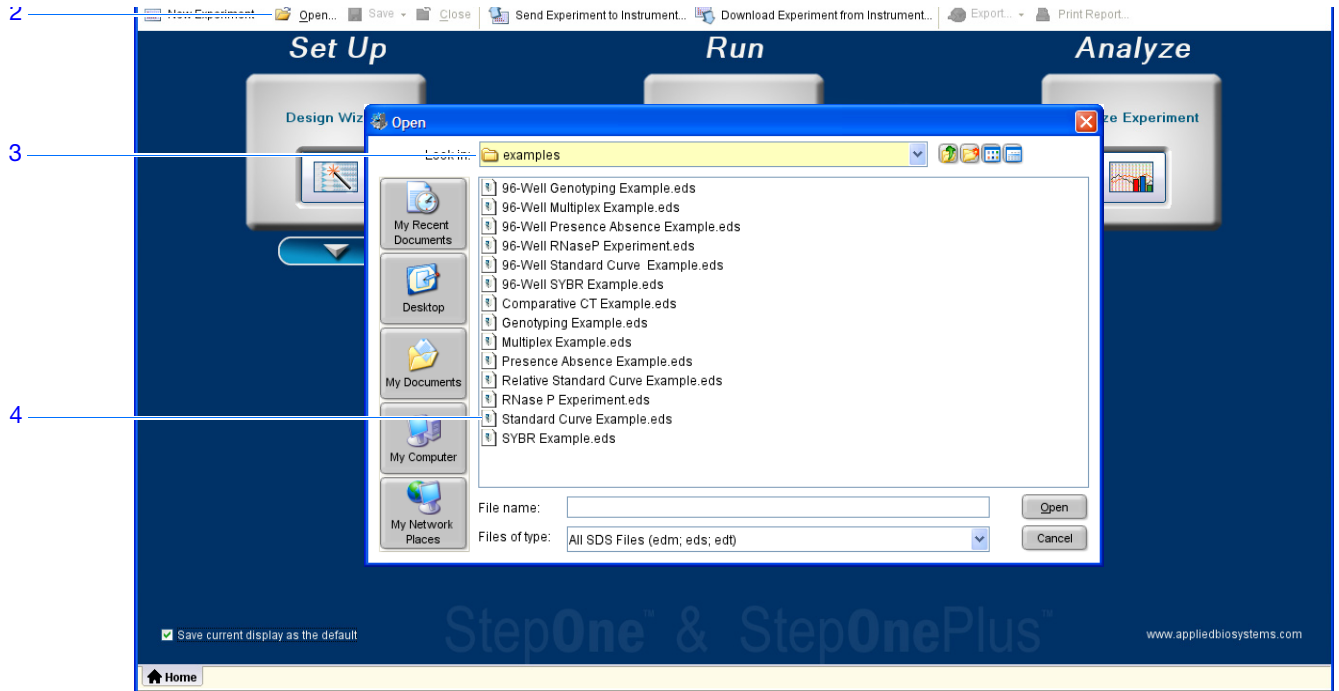
- *<drive>* is the computer hard drive on which the StepOne software is installed. The default installation drive for the software is the D drive.
- *<software name>* is the current version of the StepOne software.

Analyze the Example Experiment

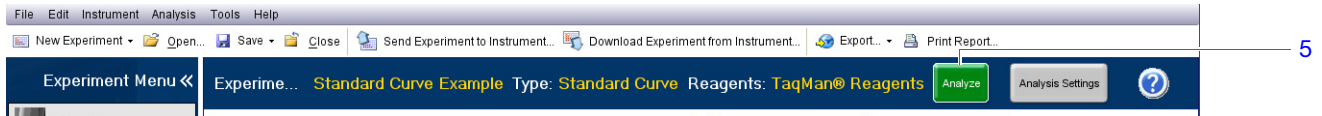
1. Double-click  (StepOne software shortcut) or select **Start ▶ All Programs ▶ Applied Biosystems ▶ StepOne Software ▶ *<software name>***
where *<software name>* is the current version of the StepOne software.
2. From the Home screen, click **Open**.
3. In the Open dialog box, navigate to the **examples** folder:
<drive>:\Applied Biosystems<software name>*\experiments\examples*
4. Double-click **Standard Curve Example** to open the example experiment data file.

Note: The examples folder contains several data files; be sure to select **Standard Curve Example**. For information on the other data files, see [“Data Files in the Examples Folder”](#) on page 12.

Notes _____



5. Click **Analyze**. The software analyzes the data using the default analysis settings.



6. See “[Software Elements](#)” below and “[Navigation Tips](#)” on page 88 for information on navigating within the analysis screens.

Guidelines When you analyze your own standard curve experiment:

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

Software Elements

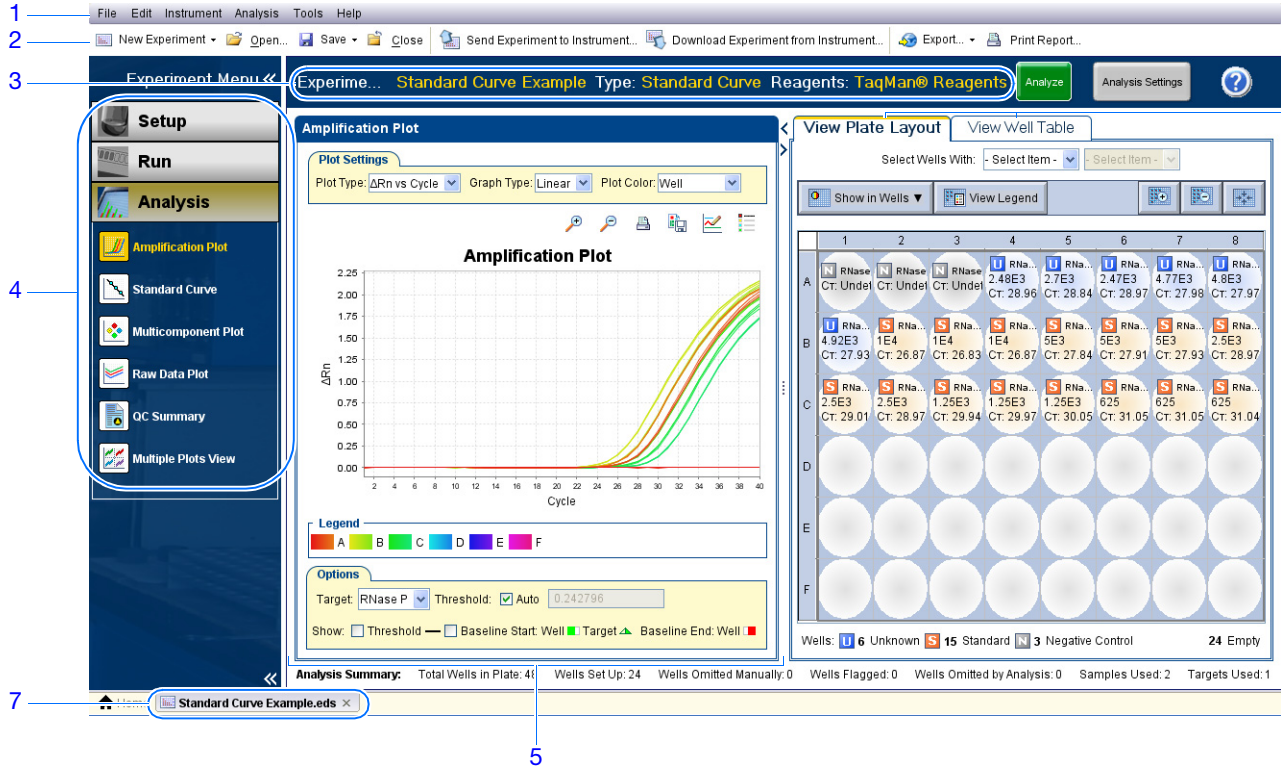
The StepOne software elements for the analysis screens are illustrated below.

1. Menu bar – Displays the menus available in the software:
 - File
 - Edit
 - Instrument
 - Analysis
 - Tools
 - Help

Notes

2. Toolbar – Displays the tools available in the software:
 - New Experiment
 - Open
 - Save
 - Close
 - Send Experiment to Instrument
 - Download Experiment from Instrument
 - Export
 - Print Report
3. Experiment header - Displays the experiment name, experiment type, and reagents for the open experiment.
4. Experiment Menu pane – Provides links to the following software screens:
 - Setup screens
 - Run screens
 - Analysis screens:
 - Amplification Plot
 - Standard Curve
 - Multicomponent Plot
 - Raw Data Plot
 - QC Summary
 - Multiple Plots View
5. Plot pane – Displays the selected analysis screen for the open experiment.
6. View tabs – Displays the plate layout or Well Table for the open experiment
7. Experiment tab(s) – Displays a tab for each open experiment.

Notes _____



Notes

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 dropdown menus: Select **Sample**, **Target**, or **Task**, then select the sample, target, or task name.
2. To select a single well, click the well in the plate layout.
3. To select multiple wells, click and drag over the desired wells, press **CTRL+click**, or press **Shift+click** in the plate layout.
4. To select all 48 wells, click the upper left corner of the plate layout.

The screenshot shows the 'View Plate Layout' tab in a software interface. At the top, there are two tabs: 'View Plate Layout' (selected) and 'View Well Table'. Below the tabs, there is a 'Select Wells With:' dropdown menu set to 'Sample', with a secondary dropdown menu open showing 'pop1' and 'pop2'. To the left of the dropdowns are 'Show in Wells' and 'View Legend' buttons. The main area is a 6x8 grid of wells. Wells A1-A3 are 'Undetermined'. Wells A4-A8, B1-B8, and C1-C8 contain 'RNase P' data with Ct values. Wells D-F are empty. A legend at the bottom indicates: 6 Unknown, 15 Standard, 3 Negative Control, and 24 Empty wells. A blue box highlights the upper left corner of the plate layout, and a blue arrow points to the 'View Plate Layout' tab.





	1	2	3	4	5	6	7	8
A	Unknown Ct: Undetermined	Unknown Ct: Undetermined	Unknown Ct: Undetermined	Unknown 2.48E3 Ct: 28.96	Unknown 2.7E3 Ct: 28.84	Unknown 2.47E3 Ct: 28.97	Unknown 4.77E3 Ct: 27.98	Unknown 4.8E3 Ct: 27.97
B	Unknown 4.92E3 Ct: 27.93	Standard 1E4 Ct: 26.87	Standard 1E4 Ct: 26.83	Standard 1E4 Ct: 26.87	Standard 5E3 Ct: 27.84	Standard 5E3 Ct: 27.91	Standard 5E3 Ct: 27.93	Standard 2.5E3 Ct: 28.97
C	Standard 2.5E3 Ct: 29.01	Standard 2.5E3 Ct: 28.97	Standard 1.25E3 Ct: 29.94	Standard 1.25E3 Ct: 29.97	Standard 1.25E3 Ct: 30.05	Standard 625 Ct: 31.05	Standard 625 Ct: 31.05	Standard 625 Ct: 31.04
D	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
F	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Wells: 6 Unknown 15 Standard 3 Negative Control 24 Empty

Notes _____

How To Display Multiple Plots

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

1. From the Experiment Menu 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 dropdown menu above each plot display.



Notes _____

View the Standard Curve


The Standard Curve screen displays the standard curve for samples designated as standards. The StepOne software calculates the quantity of an unknown target from the standard curve.

About the Example Experiment


In the standard curve example experiment, you review the Standard Curve screen for the following regression coefficient values:

- Slope/amplification efficiency
- R² value (correlation coefficient)
- C_T values

View the Standard Curve

1. From the Experiment Menu pane, select **Analysis** ▶  **Standard Curve**.

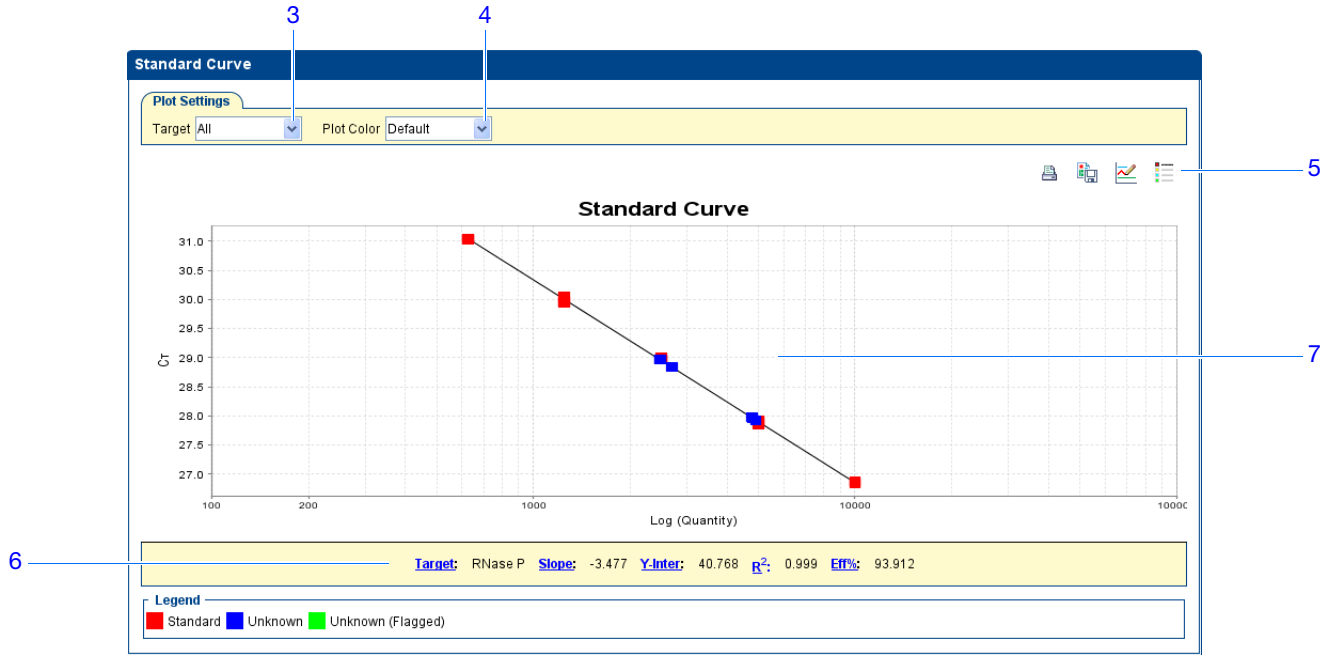
Note: If no data are displayed, click **Analyze**.

2. Display all 48 wells in the Standard Curve screen by clicking the upper left corner of the plate layout in the View Plate Layout tab.
3. From the Target dropdown menu, select **All**.
4. From the Plot Color dropdown menu, select **Default**.
5. Click  **Show a legend for the plot** (default).

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

6. View the values displayed below the standard curve. In the example experiment, the values for the target (RNase P) fall within the acceptable ranges:
 - The slope is -3.477 .
 - The R² value is 0.999.
 - The amplification efficiency (Eff%) is 93.912%.
7. Check that all samples are within the standard curve. In the example experiment, all samples (blue dots) are within the standard curve (red dots).

Notes _____



8. Check the C_T values:

- a. Click the **View Well Table** tab.
- b. From the Group By dropdown menu, select **Replicate**.
- c. Look at the values in the C_T column. In the example experiment, the C_T values fall within the expected range (>8 and <35).

The screenshot shows the 'View Well Table' tab with a table of experimental data. The table has columns for Well, Omit, Flag, Sample Name, Target Name, Task, Dyes, Ct, Ct Mean, Ct SD, Quantity, Quantity Mean, and Quantity SE. The data is grouped by standard concentration. The Ct values for the standards are: 10,000.0 (Ct ~26.8), 1,250.0 (Ct ~29.9), 2,500.0 (Ct ~28.9), and 5,000.0 (Ct ~27.9). The NTC (No Template Control) wells show 'Undetermi...' in the Ct column.

#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity Mean	Quantity SE
RNase P - NTC													
1	A1	<input type="checkbox"/>		RNase P	NTC	FAM-NFQ...	Undetermi...						
2	A2	<input type="checkbox"/>		RNase P	NTC	FAM-NFQ...	Undetermi...						
3	A3	<input type="checkbox"/>		RNase P	NTC	FAM-NFQ...	Undetermi...						
RNase P - STANDARD - 10000.0													
4	B2	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	26.874498	26.85865	0.022	10,000			
5	B3	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	26.834158	26.85865	0.022	10,000			
6	B4	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	26.867296	26.85865	0.022	10,000			
RNase P - STANDARD - 1250.0													
7	C3	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	29.93595	29.985449	0.059	1,250			
8	C4	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	29.9701	29.985449	0.059	1,250			
9	C5	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	30.050293	29.985449	0.059	1,250			
RNase P - STANDARD - 2500.0													
10	B8	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	28.973732	28.981377	0.021	2,500			
11	C1	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	29.005375	28.981377	0.021	2,500			
12	C2	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	28.965023	28.981377	0.021	2,500			
RNase P - STANDARD - 5000.0													
13	B5	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	27.843782	27.894386	0.045	5,000			
14	B6	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	27.907658	27.894386	0.045	5,000			
15	B7	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	27.931719	27.894386	0.045	5,000			
RNase P - STANDARD - 625.0													
16	C6	<input type="checkbox"/>		RNase P	STANDARD	FAM-NFQ...	31.05255	31.04659	0.01	625			

Notes

Analysis Guidelines

When you analyze your own standard curve experiment, look for:


- **Slope/amplification efficiency values** – The amplification efficiency is calculated using the slope of the regression line in the standard curve. A slope close to -3.3 indicates optimal, 100% PCR amplification efficiency. Factors that affect amplification efficiency:
 - Range of standard quantities – For more accurate and precise efficiency measurements, use a broad range of standard quantities, 5 to 6 logs (10^5 to 10^6 fold).
 - Number of standard replicates – For more accurate efficiency measurements, include replicates to decrease the effects of pipetting inaccuracies.
 - PCR inhibitors – PCR inhibitors in the reaction can reduce amplification efficiency.
- **R² values (correlation coefficient)** – The R² value is a measure of the closeness of fit between the regression line and the individual C_T data points of the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points. An R² value >0.99 is desirable.
- **C_T values** – The threshold cycle (C_T) is the PCR cycle number at which the fluorescence level meets the threshold. A C_T value >8 and <35 is desirable. A C_T value <8 indicates that there is too much template in the reaction. A C_T value >35 indicates a low amount of target in the reaction; for C_T values >35 , expect a higher standard deviation.

If your experiment does not meet the guidelines above, troubleshoot as follows:

- Omit wells (see [“Omit Wells from the Analysis”](#) on page 108).
- Or*
- Rerun the experiment.

For More Information

For more information on:

- The Standard Curve screen, access the StepOne Software Help by clicking  or pressing **F1**.
- Amplification efficiency, refer to the *Amplification Efficiency of TaqMan[®] Gene Expression Assays Application Note*.

View the Amplification Plot

The Amplification Plot screen displays amplification of all samples in the selected wells. There are three plots available:

- **ΔRn vs Cycle** – ΔRn is the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. 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.

Notes _____

- **R_n vs Cycle** – R_n is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference. This plot displays R_n 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 meets 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).


Each plot can be viewed as the following graph types: linear or log10.

About the Example Experiment

In the standard curve example experiment, you review the target in the Amplification Plot screen for:

- Correct baseline and threshold values
- Outliers

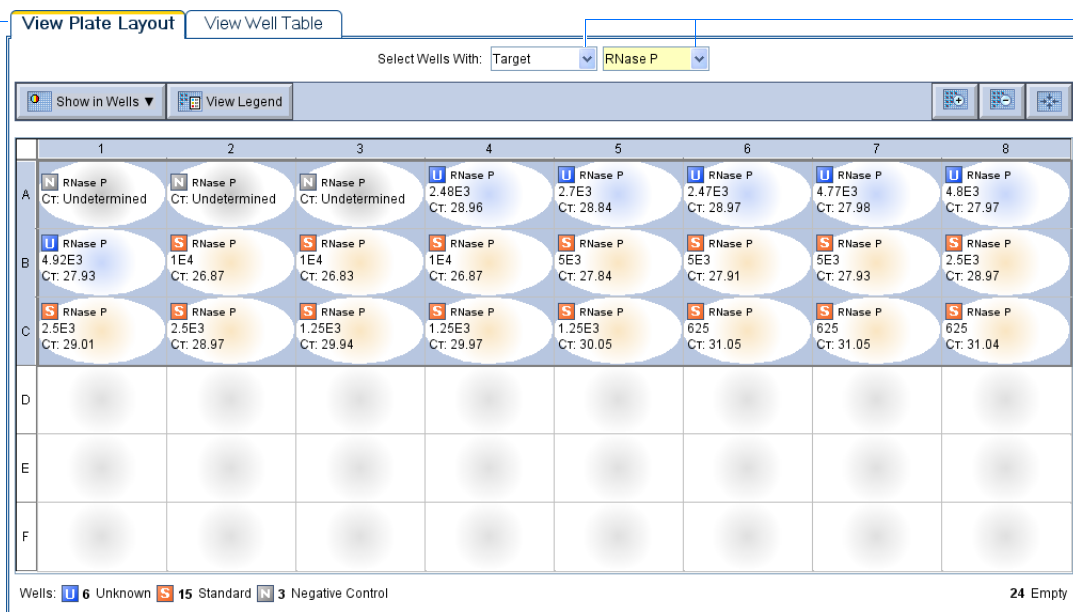
View the Amplification Plot

1. From the Experiment Menu pane, select **Analysis** ▶  **Amplification Plot**.

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

2. Display the RNase P wells in the Amplification Plot screen:
 - a. Click the **View Plate Layout** tab.
 - b. From the Select Wells With dropdown menus, select **Target**, then **RNase P**.

2a



2b

3. In the Amplification Plot screen:
 - a. From the Plot Type dropdown menu, select **ΔR_n vs Cycle** (default).
 - b. From the Plot Color dropdown menu, select **Well** (default).

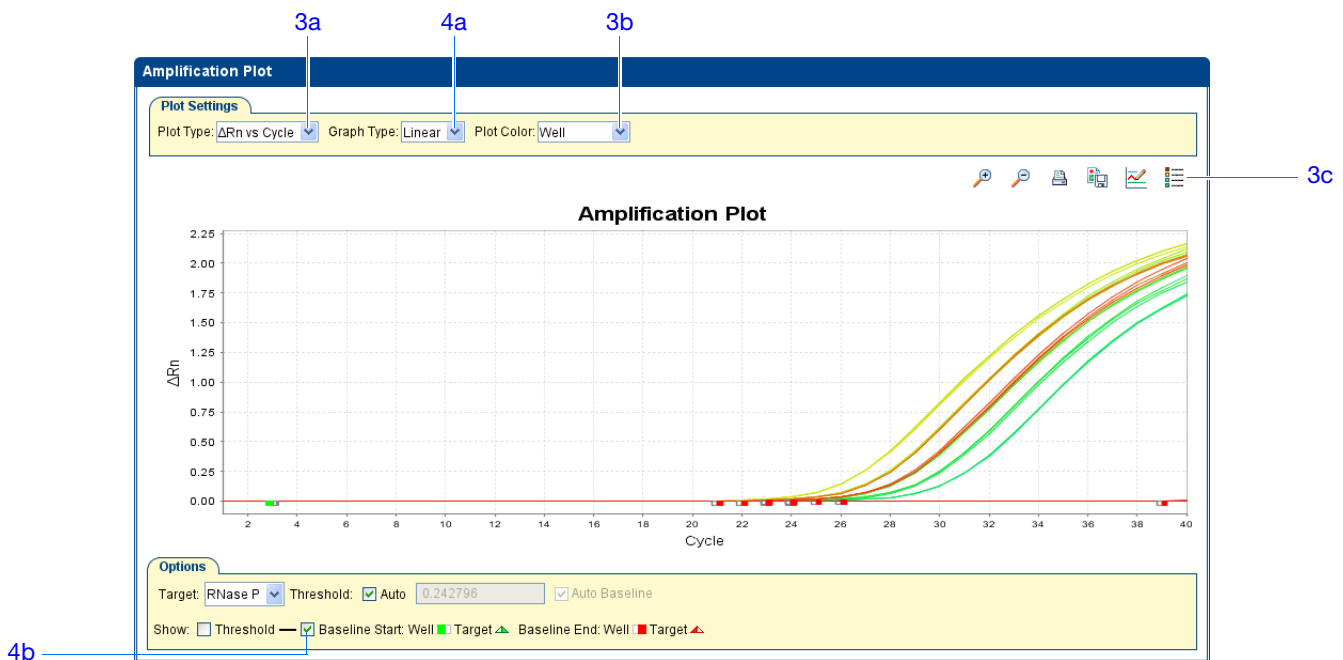
Notes

- c. Click  **Show a legend for the plot** (default).

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

4. View the baseline values:

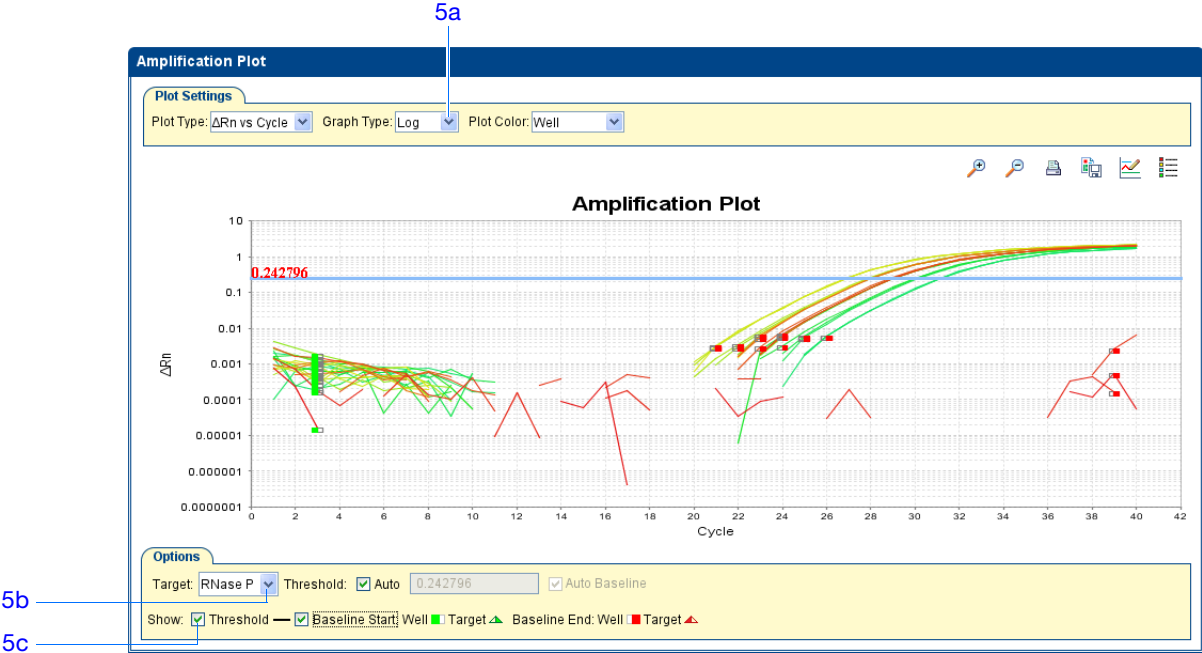
- From the Graph Type dropdown menu, select **Linear**.
- Select the **Baseline** checkbox to show the start cycle and end cycle.
- Verify that the baseline is set correctly: The end cycle should be set a few cycles before the cycle number where significant fluorescent signal is detected. In the example experiment, the baseline is set correctly.



5. View the threshold values:

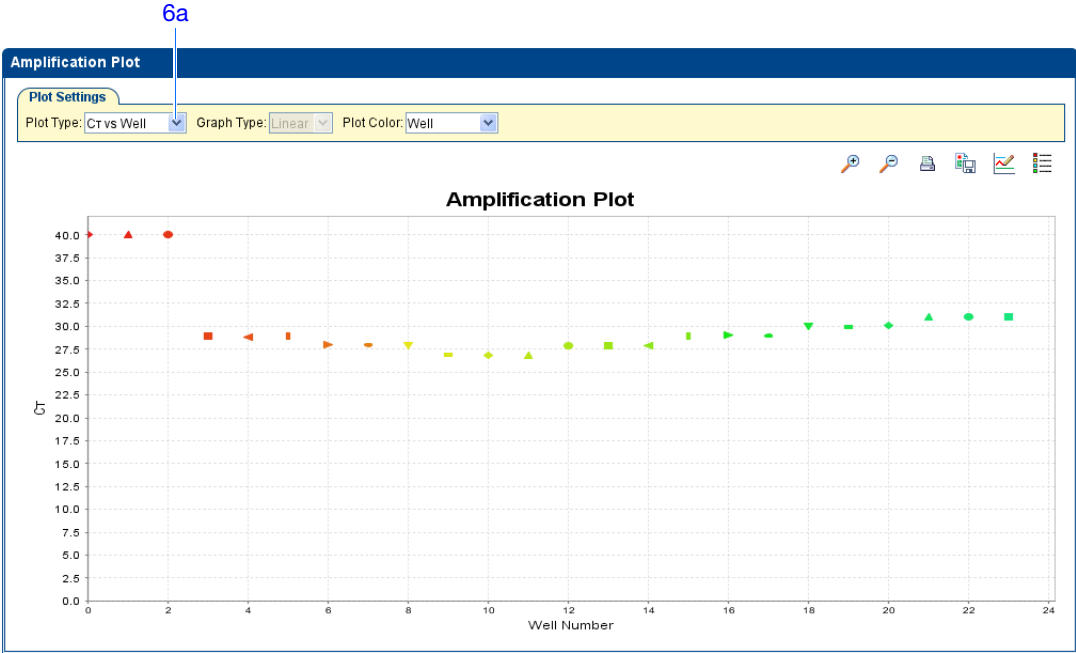
- From the Graph Type dropdown menu, select **Log**.
- From the Target dropdown menu, select **RNase P**.
- Select the **Threshold** checkbox to show the threshold.
- Verify that the threshold is set correctly. In the example experiment, the threshold is in the exponential phase.

Notes _____



6. Locate any outliers:

- a. From the Plot Type dropdown menu, select **C_T vs Well**.
- b. Look for outliers from the amplification plot. In the example experiment, there are no outliers for RNase P.

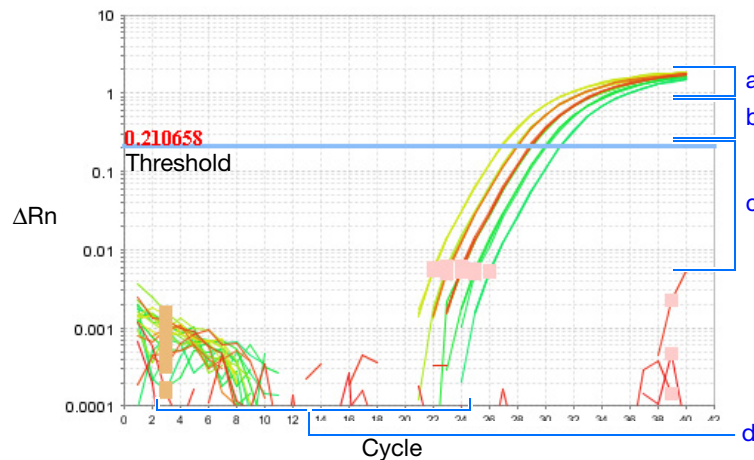


Notes

Analysis Guidelines

When you analyze your own standard curve experiment, look for:

- Outliers
- A typical amplification plot – The StepOne software automatically calculates baseline and threshold values based on the assumption that the data exhibit a *typical* amplification plot. 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 StepOne software. Therefore, Applied Biosystems recommends that you examine the Amplification Plot screen 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 97](#) and the baseline examples on [page 98](#).

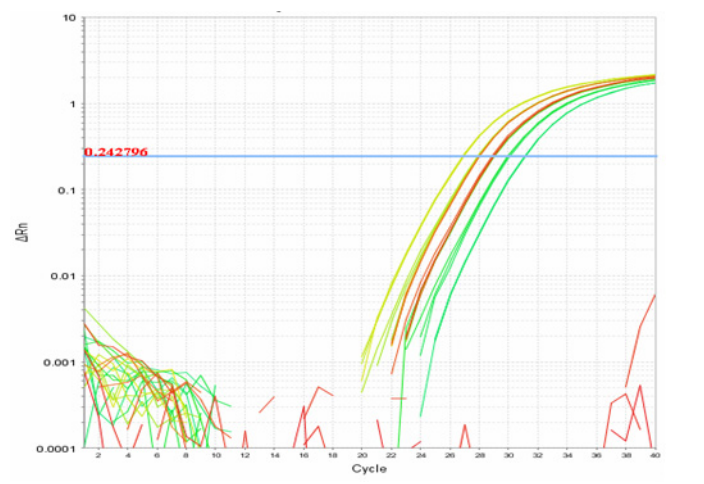
Notes _____

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.

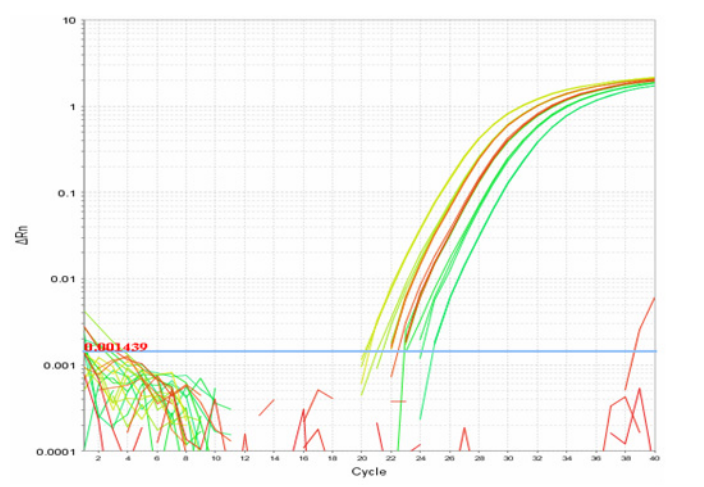
Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity ...	Quantity ...	Comm
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	Undetermi...						
UNKNOWN	FAM-NFQ...	28.96287	28.923796	0.074	2,484.31	2,551.476	126.2	
UNKNOWN	FAM-NFQ...	28.838797	28.923796	0.074	2,697.054	2,551.476	126.2	
UNKNOWN	FAM-NFQ...	28.96972	28.923796	0.074	2,473.064	2,551.476	126.2	



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.

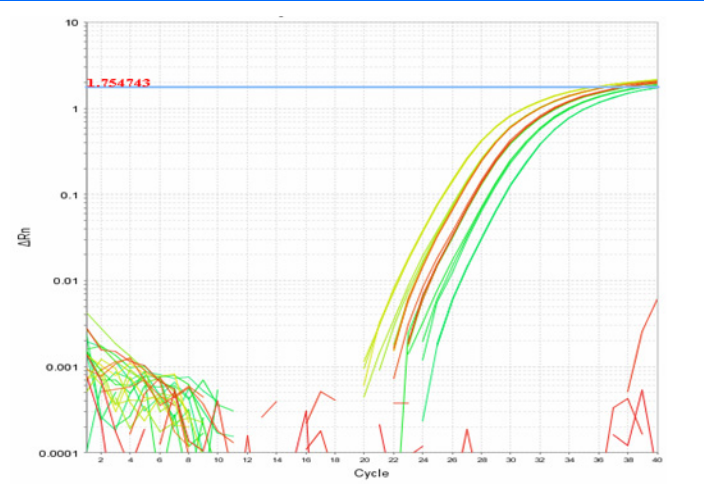
Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity ...	Quantity ...	Comm
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	38.453182						
UNKNOWN	FAM-NFQ...	22.85404	22.761744	0.252	2,314.852	2,472.463	400.435	
UNKNOWN	FAM-NFQ...	22.476973	22.761744	0.252	2,927.722	2,472.463	400.435	
UNKNOWN	FAM-NFQ...	22.954218	22.761744	0.252	2,174.816	2,472.463	400.435	



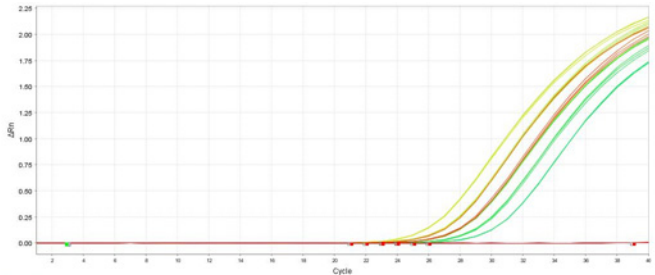
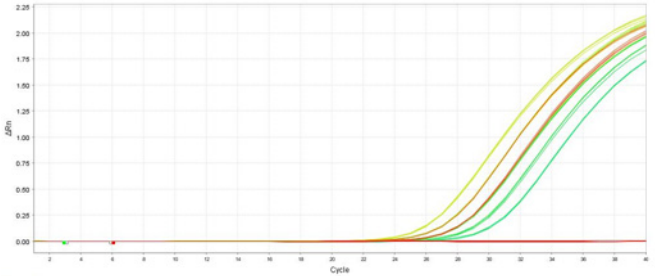
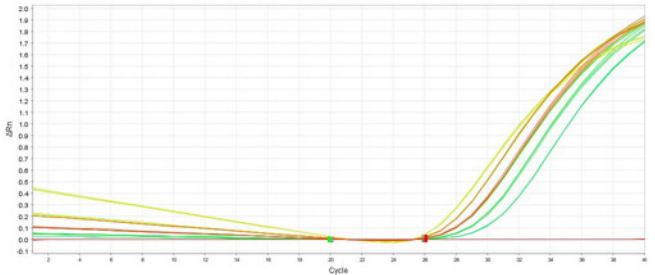
Threshold Set Too High

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.

Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity ...	Quantity ...	Comm
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	Undetermi...						
NTC	FAM-NFQ...	Undetermi...						
UNKNOWN	FAM-NFQ...	37.681107	37.49507	0.202	2,571.177	2,888.429	353.775	
UNKNOWN	FAM-NFQ...	37.27971	37.49507	0.202	3,269.923	2,888.429	353.775	
UNKNOWN	FAM-NFQ...	37.524395	37.49507	0.202	2,824.187	2,888.429	353.775	




Notes

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

If your experiment does not meet the guidelines above, troubleshoot as follows:

- Omit wells (see [“Omit Wells from the Analysis”](#) on page 108).
- or*
- Manually adjust the baseline and/or threshold (see [“View the Analysis Settings”](#) on page 104).

For More Information

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

Notes _____

View the Well Table

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

- The sample name, target name, task, and dyes
- The calculated threshold cycle (C_T), normalized fluorescence (Rn), and quantity values
- Comments
- Flags

About the Example Experiment

In the standard curve example experiment, you review the Well Table for:

- Quantity values
- Flags
- C_T values (including C_T standard deviation)

View the Well Table

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

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

2. Use the Group By dropdown menu to group wells by a specific category. For the example experiment, group the wells by replicate, flag, or C_T value.

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

- a. From the Group By dropdown menu, select **Replicate**. The software groups the replicate wells: negative controls, standards, and samples. In the example experiment, note that the quantity values within each replicate group are similar.

Note: In the example experiment, the Quantity, Quantity Mean, and Quantity SD columns have been moved from their default locations to the beginning of the Well Table. To move a column, click and drag on the column heading.

Notes _____

2a

View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Table Group By Expand All Collapse All

#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity Mean	Quantity SD
1	A1	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
2	A2	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
3	A3	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
RNase P - STANDARD - 10000.0													
4	B2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.874498	26.85865	0.022	10,000		
5	B3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.834158	26.85865	0.022	10,000		
6	B4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.867296	26.85865	0.022	10,000		
RNase P - STANDARD - 1250.0													
7	C3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.93595	29.985449	0.059	1,250		
8	C4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.9701	29.985449	0.059	1,250		
9	C5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	30.050293	29.985449	0.059	1,250		
RNase P - STANDARD - 2500.0													
10	B8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.973732	28.981377	0.021	2,500		
11	C1	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.005375	28.981377	0.021	2,500		
12	C2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.965023	28.981377	0.021	2,500		
RNase P - STANDARD - 5000.0													
13	B5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.843782	27.894386	0.045	5,000		
14	B6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.907658	27.894386	0.045	5,000		
15	B7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.931719	27.894386	0.045	5,000		
RNase P - STANDARD - 625.0													
16	C6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.05255	31.04659	0.01	625		
17	C7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.052055	31.04659	0.01	625		
18	C8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.052055	31.04659	0.01	625		

- b. From the Group By dropdown menu, select **Flag**. The software groups the flagged and unflagged wells. In the example experiment, there are no flagged wells.

2b

View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Table Group By Expand All Collapse All

#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity Mean	Quantity SD
Flagged Wells													
Unflagged Wells													
1	A1	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
2	A2	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
3	A3	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
4	A4	<input type="checkbox"/>	pop1		RNase P	UNKNOWN	FAM-NFQ...	28.96287	28.923796	0.074	2,484.31	2,551.476	126
5	A5	<input type="checkbox"/>	pop1		RNase P	UNKNOWN	FAM-NFQ...	28.838797	28.923796	0.074	2,697.054	2,551.476	126
6	A6	<input type="checkbox"/>	pop1		RNase P	UNKNOWN	FAM-NFQ...	28.96972	28.923796	0.074	2,473.064	2,551.476	126
7	A7	<input type="checkbox"/>	pop2		RNase P	UNKNOWN	FAM-NFQ...	27.976233	27.958857	0.024	4,774.927	4,830.585	76.1
8	A8	<input type="checkbox"/>	pop2		RNase P	UNKNOWN	FAM-NFQ...	27.968481	27.958857	0.024	4,799.501	4,830.585	76.1
9	B1	<input type="checkbox"/>	pop2		RNase P	UNKNOWN	FAM-NFQ...	27.931858	27.958857	0.024	4,917.327	4,830.585	76.1
10	B2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.874498	26.85865	0.022	10,000		
11	B3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.834158	26.85865	0.022	10,000		
12	B4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.867296	26.85865	0.022	10,000		
13	B5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.843782	27.894386	0.045	5,000		
14	B6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.907658	27.894386	0.045	5,000		
15	B7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.931719	27.894386	0.045	5,000		
16	B8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.973732	28.981377	0.021	2,500		
17	C1	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.005375	28.981377	0.021	2,500		
18	C2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.965023	28.981377	0.021	2,500		
19	C3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.93595	29.985449	0.059	1,250		
20	C4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.9701	29.985449	0.059	1,250		
21	C5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	30.050293	29.985449	0.059	1,250		

Notes

- c. From the Group By dropdown menu, select C_T . The software groups the wells by C_T value: low, medium, high, and undetermined. In the example experiment, the C_T values are within the expected range (>8 and <35).

2c

#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity Mean	Quantity SD
Unflagged Wells													
1	A1	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
2	A2	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
3	A3	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
4	A4	<input type="checkbox"/>		pop1	RNase P	UNKNOWN	FAM-NFQ...	28.96287	28.923796	0.074	2,484.31	2,551.476	126
5	A5	<input type="checkbox"/>		pop1	RNase P	UNKNOWN	FAM-NFQ...	28.838797	28.923796	0.074	2,697.054	2,551.476	126
6	A6	<input type="checkbox"/>		pop1	RNase P	UNKNOWN	FAM-NFQ...	28.96972	28.923796	0.074	2,473.064	2,551.476	126
7	A7	<input type="checkbox"/>		pop2	RNase P	UNKNOWN	FAM-NFQ...	27.976233	27.958857	0.024	4,774.927	4,830.585	76.1
8	A8	<input type="checkbox"/>		pop2	RNase P	UNKNOWN	FAM-NFQ...	27.968481	27.958857	0.024	4,799.501	4,830.585	76.1
9	B1	<input type="checkbox"/>		pop2	RNase P	UNKNOWN	FAM-NFQ...	27.931858	27.958857	0.024	4,917.327	4,830.585	76.1
10	B2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.874498	26.85865	0.022	10,000		
11	B3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.834158	26.85865	0.022	10,000		
12	B4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.867296	26.85865	0.022	10,000		
13	B5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.843782	27.894386	0.045	5,000		
14	B6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.907658	27.894386	0.045	5,000		
15	B7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.931719	27.894386	0.045	5,000		
16	B8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.973732	28.981377	0.021	2,500		
17	C1	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.005375	28.981377	0.021	2,500		
18	C2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.965023	28.981377	0.021	2,500		
19	C3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.93595	29.985449	0.059	1,250		
20	C4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.9701	29.985449	0.059	1,250		
21	C5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	30.050293	29.985449	0.059	1,250		

Analysis Guidelines

When you analyze your own standard curve experiment, group the wells by:

- **Replicate** – The software groups the wells by replicate: negative controls, standards, and samples. Look in the Quantity columns to make sure the quantity values for each replicate group are similar. This indicates tight precision.
- **Flag** – The software groups the flagged and unflagged wells. A flag indicates that the software has found an error in the flagged well. For a description of the StepOne software flags, see “View the QC Summary” on page 106.
- **C_T** – The threshold cycle (C_T) is the PCR cycle number at which the fluorescence level meets the threshold. A C_T value >8 and <35 is desirable. A C_T value <8 indicates that there is too much template in the reaction. A C_T value >35 indicates a low amount of target in the reaction; for C_T values >35 , expect a higher standard deviation.

For More Information


For more information on the Well Table, access the StepOne 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 information on performing these procedures, access the StepOne Software Help by clicking  or pressing **F1**.

Notes _____

Section 5.2 Troubleshoot (If Needed)

This section covers:

- View the Analysis Settings 104
- View the QC Summary 106
- Omit Wells from the Analysis 108
- View the Multicomponent Plot 109
- View the Raw Data Plot 112

Notes _____

View the Analysis Settings

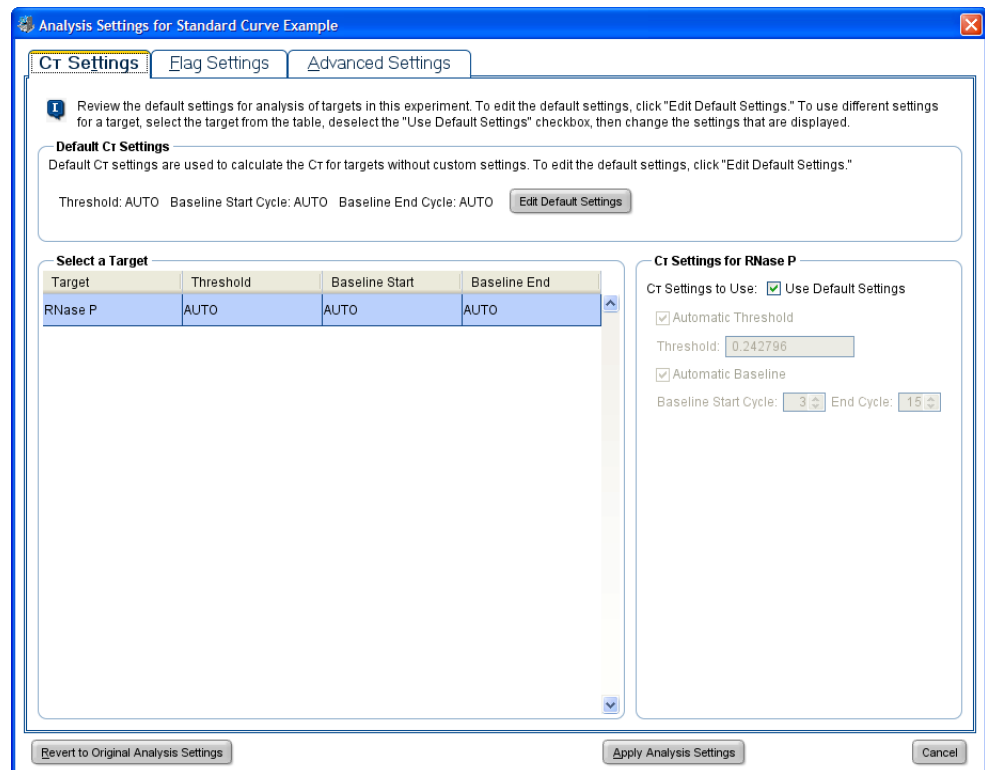
The Analysis Settings dialog box displays the analysis settings for the threshold cycle (C_T), flags, and advanced options. If the default analysis settings in the StepOne software are not suitable for your experiment, you can change the settings in the Analysis Settings dialog box, then reanalyze your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

About the Example Experiment

In the standard curve example experiment, the default analysis settings are used without changes.

View the Analysis Settings

1. From the Experiment Menu pane, select **Analysis**.
2. Click **Analysis Settings** to open the Analysis Settings dialog box.
3. In the example experiment, the default analysis settings are used for each tab:
 - C_T Settings
 - Flag Settings
 - Advanced Settings



Notes _____

Analysis Guidelines

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

- **C_T Settings** – Use this tab to manually set the threshold and baseline. When manually setting the threshold and baseline, Applied Biosystems recommends the following:

Setting	Recommendation
Threshold	Enter a value for the threshold so that the threshold is: <ul style="list-style-type: none"> • Above the background. • Below the plateau and linear regions of the amplification curve. • Within the exponential phase of the amplification curve.
Baseline	Select the Start Cycle and End Cycle values so that the baseline ends before significant fluorescent signal 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 StepOne software.
- **Advanced Settings** – Use this tab to change baseline settings well by well.

For More Information

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

Notes _____


View the QC Summary

The QC Summary screen displays a list of the StepOne software flags, and includes the flag frequency and location for the open experiment.

About the Example Experiment

In the standard curve example experiment, you review the QC Summary screen for any flags triggered by the experiment data. In the example experiment, no flags have been triggered.

View the QC Summary

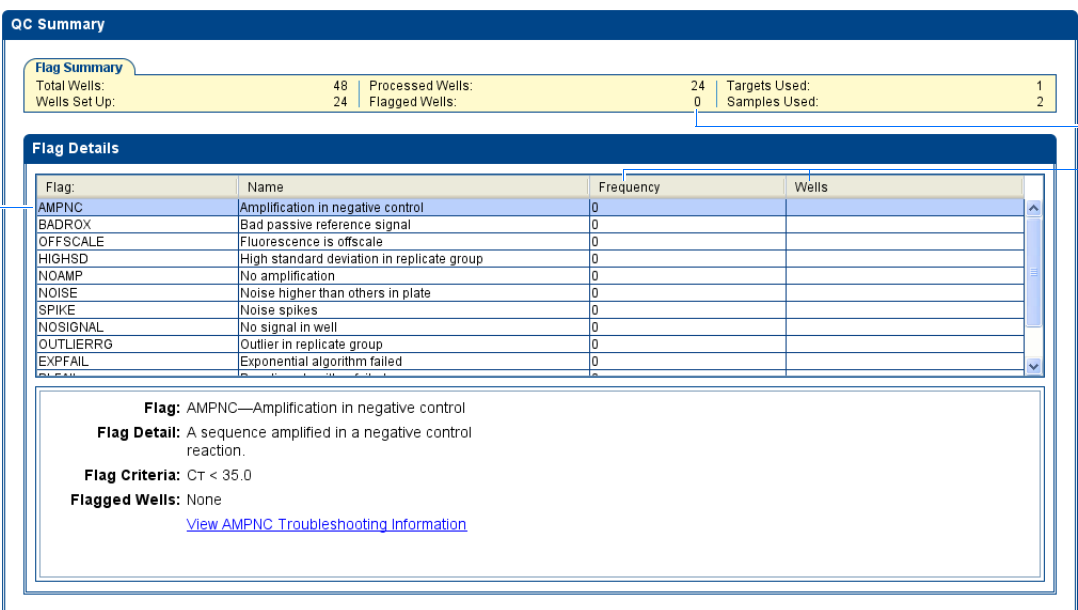
1. From the Experiment Menu pane, select **Analysis** ►  **QC Summary**.

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

2. Review the Flags Summary. In the example experiment, there are 0 flagged wells.
3. In the Flag Details table, look in the Frequency and Wells columns to determine which flags appear in the experiment. In the example experiment, the Frequency column displays 0 for all flags.

Note: A 0 displayed in the Frequency column indicates that the flag does not appear in the experiment.

4. (Optional) Click each flag row to display detailed information about the flag.



QC Summary

Flag Summary

Total Wells:	48	Processed Wells:	24	Targets Used:	1
Wells Set Up:	24	Flagged Wells:	0	Samples Used:	2

Flag Details

Flag	Name	Frequency	Wells
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
OFFSCALE	Fluorescence is offscale	0	
HIGHSD	High standard deviation in replicate group	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
SPIKE	Noise spikes	0	
NOSIGNAL	No signal in well	0	
OUTLIERRG	Outlier in replicate group	0	
EXPFAIL	Exponential algorithm failed	0	

Flag: AMPNC—Amplification in negative control
Flag Detail: A sequence amplified in a negative control reaction.
Flag Criteria: Ct < 35.0
Flagged Wells: None
[View AMPNC Troubleshooting Information](#)

Notes

Possible Flags For standard curve experiments, the flags listed below may be triggered by the experiment data.

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
HIGHSD	High standard deviation in replicate group
MTP	Multiple T _m peaks Note: This flag is only displayed if the experiment includes a melt curve.
NOAMP	No amplification
NOISE	Noise higher than others in plate
NOSIGNAL	No signal in well
OFFSCALE	Fluorescence is offscale
OUTLIERRG	Outlier in replicate group
SPIKE	Noise spikes
THOLDFAIL	Thresholding algorithm failed

Analysis Guidelines

When you analyze your own standard curve 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 StepOne software.

For More Information

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

Notes _____

Omit Wells from the Analysis

Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_T values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outliers can result in erroneous measurements; to ensure precision, omit the outliers from the analysis.

About the Example Experiment

In the standard curve example experiment, there are no outliers; no wells need to be removed from analysis.

Omit Wells

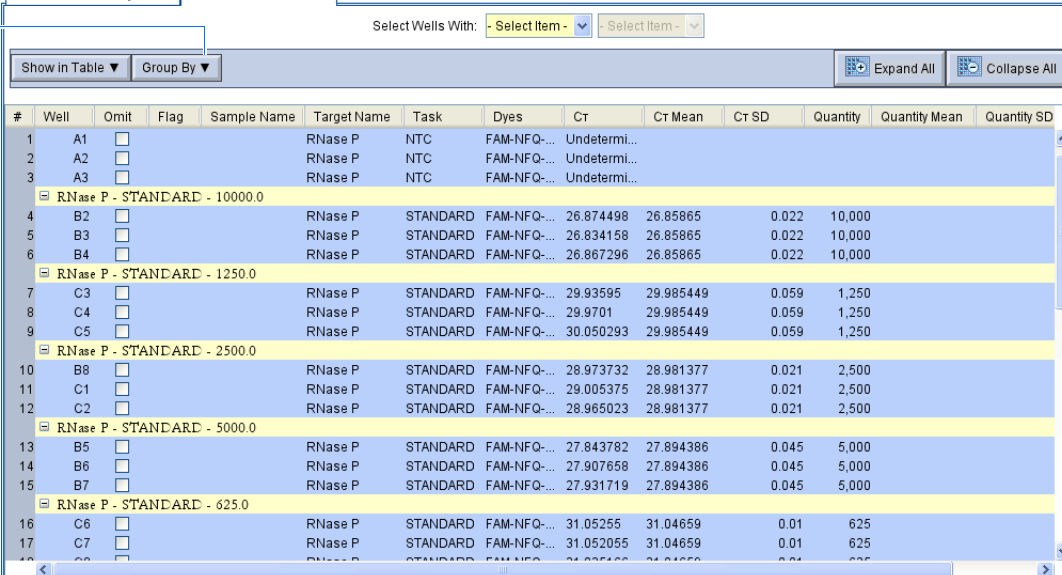
- From the Experiment Menu pane, select **Analysis** ▶  **Amplification Plot**.

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

- In the Amplification Plot screen, select **C_T vs Well** from the Plot Type dropdown menu.
- Select the **View Well Table** tab.
- In the Well Table:
 - From the Group By dropdown menu, select **Replicate**.
 - Look for any outliers in the replicate group (be sure they are flagged). In the example experiment, there are no outliers.

3

4a




#	Well	Omit	Flag	Sample Name	Target Name	Task	Dyes	Ct	Ct Mean	Ct SD	Quantity	Quantity Mean	Quantity SD
1	A1	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
2	A2	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
3	A3	<input type="checkbox"/>			RNase P	NTC	FAM-NFQ...	Undetermi...					
RNase P - STANDARD - 10000.0													
4	B2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.874498	26.85865	0.022	10,000		
5	B3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.834158	26.85865	0.022	10,000		
6	B4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	26.867296	26.85865	0.022	10,000		
RNase P - STANDARD - 1250.0													
7	C3	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.93595	29.985449	0.059	1,250		
8	C4	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.9701	29.985449	0.059	1,250		
9	C5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	30.050293	29.985449	0.059	1,250		
RNase P - STANDARD - 2500.0													
10	B8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.973732	28.981377	0.021	2,500		
11	C1	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	29.005375	28.981377	0.021	2,500		
12	C2	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	28.965023	28.981377	0.021	2,500		
RNase P - STANDARD - 5000.0													
13	B5	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.843782	27.894386	0.045	5,000		
14	B6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.907658	27.894386	0.045	5,000		
15	B7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	27.931719	27.894386	0.045	5,000		
RNase P - STANDARD - 625.0													
16	C6	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.05255	31.04659	0.01	625		
17	C7	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.052055	31.04659	0.01	625		
18	C8	<input type="checkbox"/>			RNase P	STANDARD	FAM-NFQ...	31.052055	31.04659	0.01	625		

Notes

Analysis Guidelines


When you analyze your own standard curve experiment, carefully view the replicate groups for outliers. If needed, remove outliers manually using the Well Table:

1. From the Experiment Menu 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 dropdown menu.
3. Select the **View Well Table** tab.
4. In the Well Table:
 - a. From the Group By dropdown menu, select **Replicate**.
 - b. Look for any outliers in the replicate group (be sure they are flagged).
 - c. Select the **Omit** checkbox 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 StepOne 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.

View the Multicomponent Plot

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


About the Example Experiment

In the standard curve example experiment, you review the Multicomponent Plot screen for:

- ROX™ dye (passive reference)
- FAM™ dye (reporter)
- Spikes, dips, and/or sudden changes
- Amplification in the negative control wells

Notes


View the Multicomponent Plot

- From the Experiment Menu pane, select **Analysis** ▶  **Multicomponent Plot**.

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

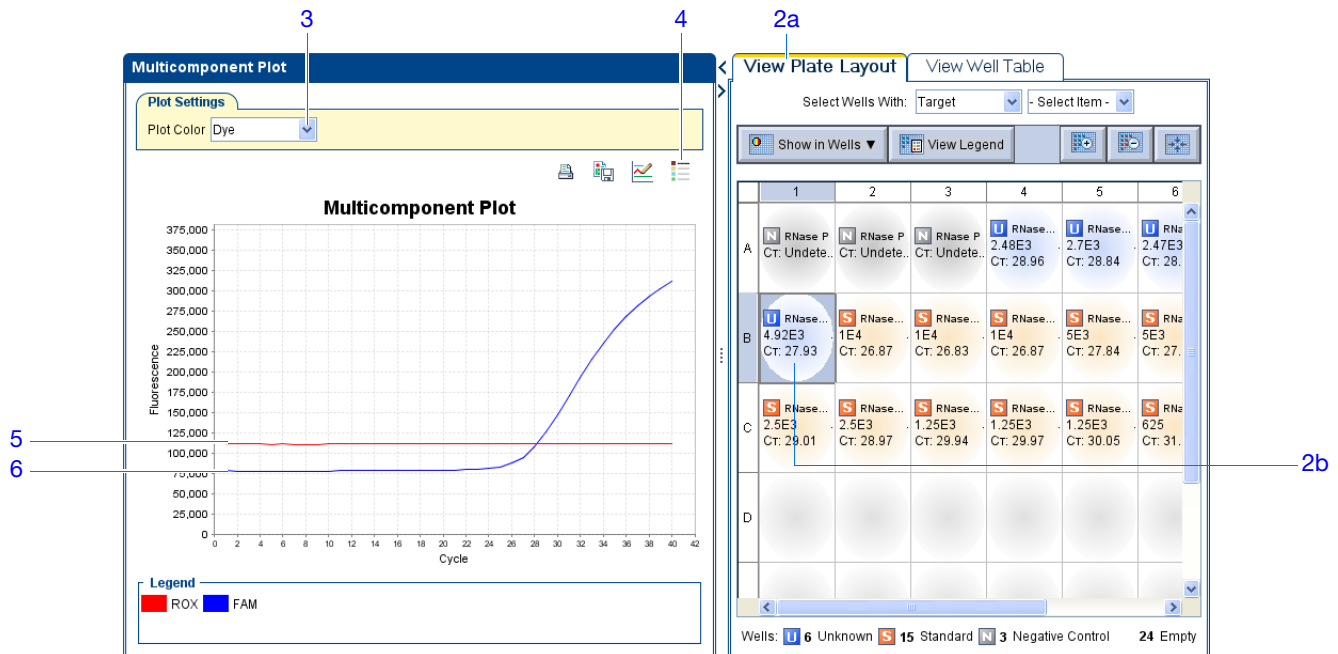
- Display the unknown and standard wells one at a time in the Multicomponent Plot screen:
 - Click the **View Plate Layout** tab.
 - Select one well in the plate layout; the well is shown in the Multicomponent Plot screen.

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

- From the Plot Color dropdown menu, select **Dye**.
- Click  **Show a legend for the plot** (default).

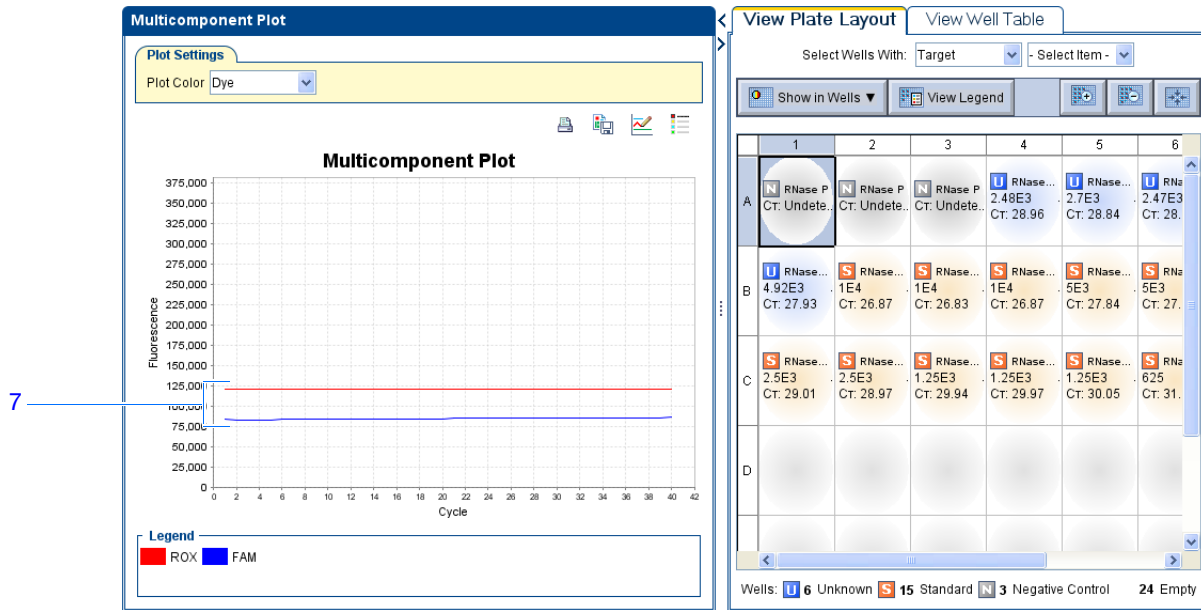
Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

- Check the ROX dye signal. In the example experiment, the ROX dye signal remains constant throughout the PCR process, which indicates typical data.
- Check the FAM dye signal. In the example experiment, the FAM dye signal increases throughout the PCR process, which indicates normal amplification.



Notes

7. Select the negative control wells one at a time and check for amplification. In the example experiment, there is no amplification in the negative control wells.



Analysis Guidelines

When you analyze your own standard curve experiment, look for:

- Passive reference – The passive reference dye fluorescence level should remain relatively constant throughout the PCR process.
- Reporter dye – The reporter dye fluorescence level should display a flat region corresponding to the baseline, 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 fluorescent signal.
- 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 StepOne Software Help by clicking  or pressing **F1**.

Notes


View the Raw Data Plot

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


About the Example Experiment

In the standard curve 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 Experiment Menu pane, select **Analysis** ▶  **Raw Data Plot**.

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

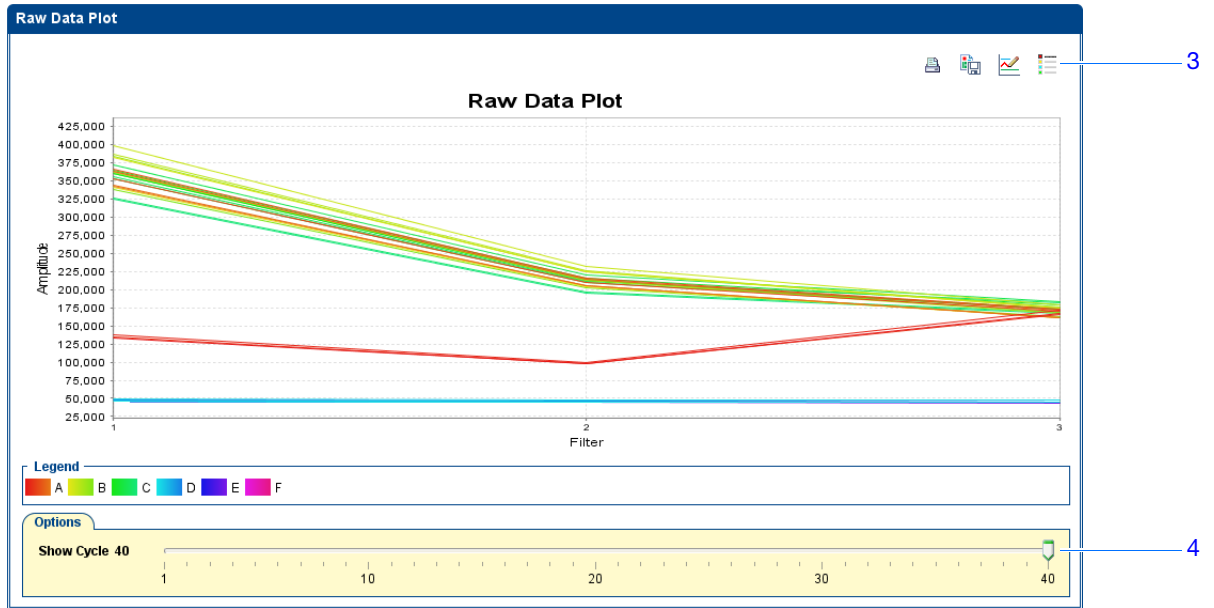
2. Display all 48 wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the View Plate Layout tab.
3. Click  **Show a legend for the plot** (default).

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. In the example shown below, Row A is red, Row B is yellow/green, Row C is green, and so on.

4. Click and drag the Show Cycle pointer from cycle 1 to cycle 40. In the example experiment, there is a stable increase in signal from filter 1, which corresponds to the FAM™ dye filter.

Notes _____



The filters are:

StepOne system		StepOnePlus system	
Filter	Dye	Filter	Dye
1	FAM™ dye	1	FAM™ dye
	SYBR® Green dye		SYBR® Green dye
2	JOE™ dye	2	JOE™ dye
	VIC® dye		VIC® dye
3	ROX™ dye	3	TAMRA™ dye
			NED™ dye
		4	ROX™ dye

Analysis Guidelines

When you analyze your own standard curve 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 StepOne Software Help by clicking or pressing **F1**.

Notes

Notes _____




Alternate Experiment Workflows

A

This appendix covers:



- [Advanced Setup Workflow](#) 116
- [QuickStart Workflow](#) 117
- [Template Workflow](#) 119
- [Export/Import Workflow](#) 121

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



Notes

Advanced Setup Workflow




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

1. Double-click  (StepOne software shortcut) or select **Start ▶ All Programs ▶ Applied Biosystems ▶ StepOne Software ▶ <software name>** where <software name> is the current version of the StepOne software.
2. From 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
<i>Genotyping</i>	Define the SNP assays, then assign them to wells in the reaction plate.
<i>All other experiments</i>	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	Action
<i>Relative standard curve</i>	a. Prepare the template.
Standard curve	b. Prepare the sample dilutions.
	c. Prepare the standard dilution series.
	d. Prepare the reaction mix.
	e. Prepare the reaction plate.

Notes _____

Experiment Type	Action
Comparative C_T	a. Prepare the template.
Genotyping	b. Prepare the sample dilutions.
Presence/absence	c. Prepare the reaction mix.
	d. Prepare the reaction plate.

5. Run the experiment:

- 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 StepOne software.
- b. From the Experiment Menu, 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	Action
Relative standard curve	a. Prepare the template.
Standard curve	b. Prepare the sample dilutions.
	c. Prepare the standard dilution series.
	d. Prepare the reaction mix.
	e. Prepare the reaction plate.
Comparative C_T	a. Prepare the template.
Genotyping	b. Prepare the sample dilutions.
Presence/absence	c. Prepare the reaction mix.
	d. Prepare the reaction plate.

Notes _____

2. QuickStart the experiment:
 - a. Double-click  (StepOne software shortcut) or select **Start ▶ All Programs ▶ Applied Biosystems ▶ StepOne Software ▶ <software name>** where <software name> is the current version of the StepOne software.
 - b. From 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:
 - 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 StepOne software, complete the plate setup:

Experiment Type	Action
<i>Genotyping</i>	<ol style="list-style-type: none"> a. Select and complete the Define SNP Assays and Samples tab. b. Select and complete the Assign SNP Assays and Samples tab.
<i>All other experiments</i>	<ol style="list-style-type: none"> a. Select and complete the Define Targets and Samples tab. b. Select and complete the Assign Targets and Samples tab.


5. Analyze the data:
 - a. Open the experiment in the StepOne software.
 - b. From the Experiment Menu, 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 are useful when you want to create many experiments with the same setup information.


Create a Template

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


where <software name> is the current version of the StepOne 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 116](#)).

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. From 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 in [step d](#), 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 116](#)).
4. Click  **Save**, enter a file name, then click **Save** to save the experiment.
5. Prepare the PCR reactions:

Experiment Type	Action
Relative standard curve	a. Prepare the template.
Standard curve	b. Prepare the sample dilutions. c. Prepare the standard dilution series. d. Prepare the reaction mix. e. Prepare the reaction plate.

Notes

Experiment Type	Action
Comparative C_T	a. Prepare the template.
Genotyping	b. Prepare the sample dilutions.
Presence/absence	c. Prepare the reaction mix.
	d. Prepare the reaction plate.

6. Run the experiment:

- 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 StepOne software.
- b. From the Experiment Menu, 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).


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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.

Note: Setup data exported from experiments in StepOne Software v1.0 can be imported into experiments in StepOne Software v2.0 or later. However, setup data exported from experiments in StepOne Software v2.0 or later cannot be imported into experiments in StepOne Software v1.0.


Export Setup Data

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

where <software name> is the current version of the StepOne 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 116](#)).

3. Select **File ▶ Export**.
4. Select the **Export Properties** tab (default), then:
 - a. Select **Setup**.
 - b. Select **One File** from the dropdown menu.
 - c. Enter a name, then select a location for the export file.
 - d. Select  (*.txt) from the File Type dropdown menu.

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.

Notes

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. From 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**.
- 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 116](#)).
3. Prepare the PCR reactions:

Experiment Type	Action
<i>Relative standard curve</i>	a. Prepare the template.
Standard curve	b. Prepare the sample dilutions. c. Prepare the standard dilution series. d. Prepare the reaction mix. e. Prepare the reaction plate.
<i>Comparative C_T</i>	a. Prepare the template.
Genotyping	b. Prepare the sample dilutions. c. Prepare the reaction mix.
Presence/absence	d. Prepare the reaction plate.

4. Run the experiment:
 - 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.

Notes _____

5. Analyze the data:
 - a. Open the experiment in the StepOne software.
 - b. From the Experiment Menu, 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



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Bibliography



Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

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.

Advanced Setup	In the StepOne™ software, a feature that allows you to set up your experiment according to your experiment design. Advanced Setup provides you with maximum flexibility in the design and setup of your experiment.
AIF	See assay information file (AIF) .
allele	For a given target, any of the different sequences that occurs in the population.
allelic discrimination plot	Display of data collected during the post-PCR read. The allelic discrimination plot is a graph of the normalized reporter signal from the allele 1 probe plotted against the normalized reporter signal from the allele 2 probe.
amplicon	A segment of DNA amplified during PCR.
amplification	Part of the instrument run in which PCR produces amplification of the target. For quantitation experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data are used to calculate results. For genotyping or presence/absence experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data can be used for troubleshooting.
amplification efficiency (EFF%)	<p>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:</p> <ul style="list-style-type: none">• 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	<p>Display of data collected during the cycling stage of PCR amplification. Can be viewed as:</p> <ul style="list-style-type: none">• Baseline-corrected normalized reporter (ΔR_n) vs. cycle• Normalized reporter (R_n) vs. cycle• Threshold cycle (C_T) vs. well

amplification stage	<p>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.</p> <p>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.</p>
assay	<p>In the StepOne™ and StepOnePlus™ systems, a PCR reaction mix that contains primers to amplify a target and a reagent to detect the amplified target.</p>
Assay ID	<p>Identifier assigned by Applied Biosystems to TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays.</p>
assay information file (AIF)	<p>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.</p>
assay mix	<p>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.</p>
AutoDelta	<p>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:</p> <ul style="list-style-type: none">• AutoDelta on: ▲• AutoDelta off: ▲
automatic baseline	<p>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.</p>
automatic C_T	<p>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 (C_T).</p>
baseline	<p>In the amplification plot, a line fit to the fluorescence levels during the initial stages of PCR, when there is little change in fluorescence signal.</p>

baseline-corrected normalized reporter (ΔR_n)	<p>The magnitude of normalized fluorescence signal generated by the reporter:</p> <ol style="list-style-type: none"> 1. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. In the ΔR_n vs. Cycle amplification plot, ΔR_n is calculated at each cycle as: $\Delta R_n (\text{cycle}) = R_n (\text{cycle}) - R_n (\text{baseline}), \text{ where } R_n = \text{normalized reporter}$ 2. In genotyping experiments and presence/absence experiments, the difference in normalized fluorescence signal generated by the reporter between the pre-PCR read and the post-PCR read. In the allelic discrimination plot (genotyping experiments) and the presence/absence plot (presence/absence experiments), ΔR_n is calculated as: $\Delta R_n = R_n (\text{post-PCR read}) - R_n (\text{pre-PCR read}), \text{ where } R_n = \text{normalized reporter}$ <p>See also normalized reporter (R_n).</p>
blocked IPC	In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the StepOne™ software, the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells .
calibrator	See reference sample .
chemistry	See reagents .
colocated layout	A system layout in which the StepOne™ or StepOnePlus™ instrument is directly connected to a colocated computer by the yellow cable. In this layout, you can control the instrument with the StepOne™ software on the colocated computer or with the instrument touchscreen.
comparative C_T ($\Delta\Delta C_T$) method	Method for determining relative target quantity in samples. With the comparative C_T ($\Delta\Delta C_T$) method, the StepOne™ software measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. The software determines the relative quantity of target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample.
C_T	See threshold cycle (C_T) .
custom dye	Dye that is not supplied by Applied Biosystems. Custom dyes may be adapted for use in experiments on the StepOne™ and StepOnePlus™ systems. When using custom dyes, the custom dye should be added to the Dye Library and a custom dye calibration performed.
	<hr/> IMPORTANT! Applied Biosystems does not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus™ system. <hr/>
cycle threshold	See threshold cycle (C_T) .

cycling stage	In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. For cycling stages, you can enable AutoDelta settings. See also amplification stage .
data collection	A process during the instrument run in which an instrument component detects fluorescence data from each well of the reaction plate. The instrument transforms the signal to electronic data, and the data are saved in the experiment file. In the StepOne™ software, a data collection point is indicated by an icon in the thermal profile: <ul style="list-style-type: none">• Data collection on: • Data collection off: 
delta Rn (ΔR_n)	See baseline-corrected normalized reporter (ΔR_n) .
derivative reporter ($-R_n'$)	The negative first-derivative of the normalized fluorescence generated by the reporter during PCR amplification. In the derivative reporter ($-R_n'$) vs. temperature melt curve, the derivative reporter signal is displayed in the y-axis.
Design Wizard	A feature in the StepOne™ software that helps you set up your experiment by guiding you through best practices as you enter your experiment design.
diluent	A reagent used to dilute a sample or standard before adding it to the PCR reaction. The diluent can be water or buffer.
Diluted Sample Concentration (10X for Reaction Mix)	In the StepOne™ software, a field displayed on the Sample Dilution Calculations tab of the Reaction Setup screen. For this field, enter the sample concentration you want to use to add to the reaction mix for all samples in the experiment. “10X for Reaction Mix” indicates that the software assumes the sample or standard component of the reaction mix is at a 10X concentration. For example, if the diluted sample concentration is 50.0 ng/ μ L (10X), the final sample concentration in the reaction is 5 ng/ μ L (1X).
dilution factor	See serial factor .
dissociation curve	See melt curve .
EFF%	See amplification efficiency (EFF%) .
endogenous control	A target or gene that should be expressed at similar levels in all samples you are testing. Endogenous controls are used in relative standard curve and comparative C_T ($\Delta\Delta C_T$) experiments to normalize fluorescence signals for the target you are quantifying. Housekeeping genes can be used as endogenous controls. See also housekeeping gene .
endpoint read	See post-PCR read .

experiment	Refers to the entire process of performing a run using the StepOne™ or StepOnePlus™ systems, including setup, run, and analysis. The types of experiments you can perform using the StepOne and StepOnePlus systems: <ul style="list-style-type: none"> • 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 StepOne™ or StepOnePlus™ system: <ul style="list-style-type: none"> • Standard curve • Comparative C_T ($\Delta\Delta C_T$) • Relative standard curve • Melt curve (not available in the Design Wizard) • Genotyping • Presence/absence <p>The experiment type you select affects the setup, run, and analysis.</p>
forward primer	Oligonucleotide that flanks the 5' end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.
holding stage	In the thermal profile, a stage that includes one or more steps. You can add a holding stage to the thermal profile to activate enzymes, to inactivate enzymes, or to incubate a reaction.
housekeeping gene	A gene that is involved in basic cellular functions and is constitutively expressed. Housekeeping genes can be used as endogenous controls. See also endogenous control .
internal positive control (IPC)	In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. You can use the IPC to distinguish between true negative results (that is, the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.
inventoried assays	TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays that have been previously manufactured, passed quality control specifications, and stored in inventory.
IPC	In presence/absence experiments, abbreviation for internal positive control (IPC). In the StepOne™ software, the task for the IPC target in wells that contain the IPC and do not contain IPC blocking agent. See also internal positive control (IPC) .
IPC blocking agent	Reagent added to PCR reactions to block amplification of the internal positive control (IPC).

IPC+	See negative control-IPC wells .
made-to-order assays	TaqMan® Gene Expression Assays or TaqMan® SNP Genotyping Assays that are manufactured at the time of order. Only assays that pass manufacturing quality control specifications are shipped.
manual baseline	An analysis setting in which you enter the baseline start and end values for the amplification plot. You can apply the manual baseline setting to specific wells in the reaction plate.
manual C_T	An analysis setting in which you enter the threshold value and select whether to use automatic baseline or manual baseline values. The software uses the baseline and the threshold values to calculate the threshold cycle (C _T).
melt curve	A plot of data collected during the melt curve stage. Peaks in the melt curve can indicate the melting temperature (T _m) of the target or can identify nonspecific PCR amplification. In the StepOne™ software, you can view the melt curve as normalized reporter (R _n) vs. temperature or as derivative reporter (–R _n ') 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 (T_m)	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 T _m is displayed in the melt curve.
multicomponent plot	A plot of the complete spectral contribution of each dye for the selected well(s) over the duration of the PCR run.
negative control (NC)	In the StepOne™ software, the task for targets or SNP assays in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Previously called no template control (NTC).
negative control-blocked IPC wells	In presence/absence experiments, wells that contain IPC blocking agent instead of sample in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample and amplification of the IPC is blocked. Previously called no amplification control (NAC).
negative control-IPC wells	In presence/absence experiments, wells that contain IPC template and buffer or water instead of sample. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample. Previously called IPC+.
no amplification control (NAC)	See negative control-blocked IPC wells.
no template control (NTC)	See negative control (NC) .

nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3' end of TaqMan [®] probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence signal. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantitation. The minor groove binder (MGB) increases the melting temperature (T _m) 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 (R_n)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.
omit well	An action that you perform before reanalysis to omit one or more wells from analysis. Because no algorithms are applied to omitted wells, omitted wells contain no results.
outlier	For a set of data, a datapoint that is significantly smaller or larger than the others.
passive reference	A dye that produces fluorescence signal. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in concentrations or volume. Normalization to the passive reference signal allows for high data precision.
plate layout	<p>An illustration of the grid of wells and assigned content in the reaction plate. In StepOne[™] systems, the grid contains 6 rows and 8 columns. In StepOnePlus[™] systems, the grid contains 8 rows and 12 columns.</p> <p>In the StepOne[™] software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.</p>
point	One standard in a standard curve. The standard quantity for each point in the standard curve is calculated based on the starting quantity and serial factor.
positive control	In genotyping experiments, a DNA sample with a known genotype, homozygous or heterozygous. In the StepOne [™] software, the task for the SNP assay in wells that contain a sample with a known genotype.
post-PCR read	Used in genotyping and presence/absence experiments, the part of the instrument run that occurs after amplification. In genotyping experiments, fluorescence data collected during the post-PCR read are displayed in the allelic discrimination plot and used to make allele calls. In presence/absence experiments, fluorescence data collected during the post-PCR read are displayed in the presence/absence plot and used to make detection calls. Also called endpoint read.
pre-PCR read	Used in genotyping and presence/absence experiments, the part of the instrument run that occurs before amplification. The pre-PCR read is optional but recommended. Fluorescence data collected during the pre-PCR read can be used to normalize fluorescence data collected during the post-PCR read.
primer mix	PCR reaction component that contains the forward primer and reverse primer designed to amplify the target.

primer/probe mix	PCR reaction component that contains the primers designed to amplify the target and a TaqMan [®] probe designed to detect amplification of the target.
pure dye	See custom dye and system dye .
quantitation method	In quantitation experiments, the method used to determine the quantity of target in the samples. In StepOne [™] and StepOnePlus [™] systems, there are three types of quantitation methods: standard curve, relative standard curve, and comparative C _T ($\Delta\Delta C_T$).
quantity	In quantitation experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.
quencher	A molecule attached to the 3' end of TaqMan [®] probes to prevent the reporter from emitting fluorescence signal while the probe is intact. With TaqMan [®] reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher. With SYBR [®] Green reagents, no quencher is used. <hr/> IMPORTANT! Applied Biosystems does not recommend the use of TAMRA [™] dye as reporter or quencher with the StepOne [™] system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus [™] system. <hr/>
QuickStart	A feature in StepOne [™] and StepOnePlus [™] systems that allows you to run an experiment without entering plate setup information. QuickStart requires a colocated layout with the instrument powered on and an intact instrument-computer connection.
R² value	Regression coefficient calculated from the regression line in the standard curve. The R ² value indicates the closeness of fit between the standard curve regression line and the individual C _T data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.
ramp	The rate at which the temperature changes during the instrument run. Except for the melt curve step, the ramp is defined as a percentage. For the melt curve step, the ramp is defined as a temperature increment. In the graphical view of the thermal profile, the ramp is indicated by a diagonal line.
ramp speed	Speed at which the temperature ramp occurs during the instrument run. Available ramp speeds include fast and standard. <ul style="list-style-type: none">• 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. <hr/> IMPORTANT! TaqMan Fast reagents are not supported for genotyping or presence/absence experiments. <hr/>
raw data plot	A plot of raw fluorescence signal (not normalized) for each optical filter.

reaction mix	A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control).
reagents	The PCR reaction components you are using to amplify the target and to detect amplification. Types of reagents used on the StepOne™ and StepOnePlus™ systems: <ul style="list-style-type: none"> • 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 (ΔΔC _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 StepOne™ software measures amplification of the target and of the endogenous control in samples, in a reference sample, and in a standard dilution series. Measurements are normalized using the endogenous control. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates target quantity in the samples and in the reference sample. The software determines the relative quantity of target in each sample by comparing target quantity in each sample to target quantity in the reference sample.
Remote Monitor	A feature in the StepOne™ software that allows you to monitor a StepOne™ or StepOnePlus™ instrument over the network. With the Remote Monitor, you can monitor the instrument status, send an experiment to the instrument, monitor amplification plots and temperature plots in real time, and download the results to your computer. You cannot operate the StepOne or StepOnePlus instrument using the Remote Monitor.

replicate group	A set of identical reactions in an experiment.
replicates	Total number of identical reactions containing identical components and identical volumes.
reporter	Fluorescent dye used to detect amplification. If you are using TaqMan [®] reagents, the reporter dye is attached to the 5' end. If you are using SYBR [®] Green reagents, the reporter dye is SYBR [®] Green dye.
reverse primer	An oligonucleotide that flanks the 3' end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.
reverse transcriptase	An enzyme that converts RNA to cDNA. Reverse transcriptase is added to the PCR reaction to perform 1-step RT-PCR.
Rn	See normalized reporter (Rn) .
ROX[™] dye	A dye supplied by Applied Biosystems and precalibrated on the StepOne [™] and StepOnePlus [™] systems. ROX dye is used as the passive reference.
rs number	See refSNP ID .
run method	Definition of the reaction volume and the thermal profile for the StepOne [™] or StepOnePlus [™] instrument run.
sample	The template that you are testing.
Sample DNA (10X)	In the StepOne [™] software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample DNA is added to the reaction mix at a 10X concentration. For example, if the reaction volume is 20 μ L, the calculated volume of sample for 1 reaction is 2 μ L.
Sample Library	In the StepOne [™] software, a collection of samples. The Sample Library contains the sample name and the sample color.
Sample or Standard (10X)	In the StepOne [™] software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample or standard is added to the reaction mix at a 10X concentration. For example, if the reaction volume is 20 μ L, the calculated volume of sample or standard for 1 reaction is 2 μ L.
sample/SNP assay reaction	In genotyping experiments, the combination of which sample to test and which SNP assay to perform in one PCR reaction. Each PCR reaction can contain only one sample and one SNP assay.
sample/target reaction	In quantitation experiments, the combination of which sample to test and which target to detect and quantify in one PCR reaction. In the Design Wizard, you can detect and quantify only one target in one PCR reaction. Use Advanced Setup to detect and quantify more than one target in one PCR reaction.

serial factor	In the StepOne™ software, a numerical value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10X, the difference between any 2 adjacent points in the curve is 10-fold.
series	See standard dilution series .
slope	Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of -3.32 indicates 100% amplification efficiency. See also amplification efficiency (EFF%) and regression line .
SNP	Abbreviation for single nucleotide polymorphism. The SNP can consist of a base difference or an insertion or deletion of one base.
SNP assay	Used in genotyping experiments, a PCR reaction that contains primers to amplify the SNP and two probes to detect different alleles.
SNP Assay Library	In the StepOne™ software, a collection of SNP assays to add to genotyping experiments. The SNP assays in the library contain the SNP assay name, SNP assay color, and for each allele, the allele name or base(s), reporter, quencher, and allele colors. The SNP assays in the library may also contain the assay ID and comments about the SNP assay.
spatial calibration	Type of StepOne™ and StepOnePlus™ system calibration in which the system maps the positions of the wells in the sample block(s). Spatial calibration data are used so that the software can associate increases in fluorescence during a run with specific wells in the reaction plate.
stage	In the thermal profile, a group of one or more steps. There are three types of stages: holding stage (including pre-PCR read and post-PCR read), cycling stage (also called amplification stage), and melt curve stage.
standalone layout	A system layout in which the StepOne™ or StepOnePlus™ instrument is <i>not</i> connected to a computer by the yellow cable. In this layout, you control the instrument only with the instrument touchscreen, and you use a USB drive or network connection to transfer data between the instrument and computer.
standard	Sample that contains known standard quantities. Standard reactions are used in quantitation experiments to generate standard curves. See also standard curve and standard dilution series .
standard curve	In standard curve and relative standard curve experiments: <ul style="list-style-type: none">• The best-fit line in a plot of the C_T values from the standard reactions plotted against standard quantities. See also regression line.• A set of standards containing a range of known quantities. Results from the standard curve reactions are used to generate the standard curve. The standard curve is defined by the number of points in the dilution series, the number of standard replicates, the starting quantity, and the serial factor. See also standard dilution series.

standard curve method	Method for determining absolute target quantity in samples. With the standard curve method, the StepOne™ software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples. See also standard and standard curve .
standard dilution series	In standard curve and relative standard curve experiments, a set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards. For example, the standard stock is used to prepare the first dilution point, the first dilution point is used to prepare the second dilution point, and so on. In the StepOne™ software, the volumes needed to prepare a standard dilution series are calculated by the number of dilution points, the number of standard replicates, the starting quantity, the serial factor, and the standard concentration in the stock. See also standard curve .
standard quantity	A known quantity in the PCR reaction. <ul style="list-style-type: none">• In standard curve experiments, the quantity of target in the standard. In the StepOne™ software, the units for standard quantity can be for mass, copy number, viral load, or other units for measuring the quantity of target.• In relative standard curve experiments, a known quantity in the standard. Standard quantity can refer to the quantity of cDNA or the quantity of standard stock in the PCR reaction. The units are not relevant for relative standard curve experiments because they cancel out in the calculations.
starting quantity	When defining a standard curve in the StepOne™ software, corresponds to the highest or lowest quantity.
step	A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt curve steps), hold temperature, hold time (duration), and you can turn data collection on or off for the ramp or the hold parts of the step. For cycling stages, a step is also defined by the AutoDelta status. With StepOnePlus™ systems, which contain the VeriFlex™ blocks, each step contains 6 temperatures (1 for each VeriFlex block).
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	<p>Dye supplied by Applied Biosystems and precalibrated on the StepOne™ or StepOnePlus™ system. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Maintenance Manager.</p> <p>System dyes on the StepOne system:</p> <ul style="list-style-type: none"> • FAM™ dye • JOE™ dye • ROX™ dye • SYBR® Green dye • VIC® dye <p>System dyes on the StepOnePlus system:</p> <ul style="list-style-type: none"> • FAM™ dye • JOE™ dye • NED™ dye • ROX™ dye • SYBR® Green dye • TAMRA™ dye • VIC® dye <hr/> <p>IMPORTANT! Applied Biosystems does not recommend the use of TAMRA™ dye as reporter or quencher with the StepOne™ system. TAMRA dye may be used as a reporter or quencher with the StepOnePlus™ system.</p> <hr/>
TaqMan® reagents	PCR reaction components that consist of primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.
target	The nucleic acid sequence that you want to amplify and detect.
target color	In the StepOne™ software, a color assigned to a target to identify the target in the plate layout and analysis plots.
Target Library	In the StepOne™ software, a collection of targets to add to experiments. The targets in the library contain the target name, reporter, quencher, and target color. The target in the library may also contain comments about the target.
task	<p>In the StepOne™ software, the type of reaction performed in the well for the target or SNP assay. Available tasks:</p> <ul style="list-style-type: none"> • Unknown • Negative Control • Standard (standard curve and relative standard curve experiments) • Positive control (genotyping experiments) • IPC (presence/absence experiments) • Blocked IPC (presence/absence experiments)

temperature plot	In the StepOne™ software, a display of temperatures for the sample, instrument cover, and instrument block during the StepOne™ or StepOnePlus™ instrument run.
template	<p>In the Design Wizard of the StepOne™ software (and in QuickStart for quantitation experiments), the type of nucleic acid to add to the PCR reaction. The recommended template varies according to experiment type:</p> <ul style="list-style-type: none"> • 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 StepOne™ or StepOnePlus™ instrument run.
threshold	<ol style="list-style-type: none"> 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 C_T) or can be set manually (see manual C_T). 2. In presence/absence experiments, the level of fluorescence above which the StepOne™ software assigns a presence call.
threshold cycle (C_T)	The PCR cycle number at which the fluorescence meets the threshold in the amplification plot.
T_m	See melting temperature (T_m) .
touchscreen	Instrument display that you touch to control the StepOne™ or StepOnePlus™ instrument.
unknown	<p>In the StepOne™ software, the task for the target or SNP assay in wells that contain the sample you are testing:</p> <ul style="list-style-type: none"> • In quantitation experiments, the task for the target in wells that contain a sample with unknown target quantities. • In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype. • In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known.
unknown-IPC wells	In presence/absence experiments, wells that contain a sample and internal positive control (IPC).

VeriFlex™ Technology	The StepOnePlus™ instrument contains six independently thermally regulated VeriFlex™ blocks, creating up to six different zones for the 96 sample wells. After you enable the VeriFlex blocks in the StepOne™ software, you can set a different temperature for one or more of the VeriFlex blocks.
y-intercept	In the standard curve, the value of y where the regression line crosses the y-axis. The y-intercept indicates the expected threshold cycle (C_T) for a sample with quantity equal to 1.
zone	One of up to six sample temperatures among the 96 wells formed by independently thermally regulated VeriFlex™ blocks during the StepOnePlus™ instrument run. You can set a different temperature for one or more of the VeriFlex blocks, or you can set the same temperature for each of the VeriFlex blocks.
	<hr/> Note: For melt curve steps, you need to set the same temperature for each of the VeriFlex blocks. <hr/>
zone boundary	The edge of a zone for samples formed by the six independently thermally regulated VeriFlex™ blocks. In the StepOne™ software, the zone boundaries are displayed in the plate layout as thick red lines.

Numerics

1-step RT-PCR 7, 24, 32, 37

2-step RT-PCR 7, 11, 24

A

Advanced Setup 9, 10, 24, 30, 56, 116

alternate experiment workflows. See workflows.

amplification efficiency 28, 92

Amplification Plot screen
 monitor during a run 70
 view after a run 92

amplification plot, typical 96

AMPNC flag 107

analysis screens

Amplification Plot screen 92
 Multicomponent Plot screen 109
 Multiple Plots screen 89
 navigation tips 88
 QC Summary screen 106
 Raw Data Plot screen 112
 software elements 85
 Standard Curve screen 90
 Well Table 99

analysis settings

advanced 105
 baseline 105
 CT 105
 flag 105
 threshold 105
 view 104

analyze experiment

analyze 84
 for more information 92, 98, 101, 105, 107, 109,
 111, 113
 guidelines 85, 92, 96, 101, 105, 107, 109, 111, 113
 omit wells 108
 publish the data 102
 view Amplification Plot screen 92
 view analysis settings 104
 view Multicomponent Plot screen 109
 view Multiple Plots screen 89
 view QC Summary screen 106
 view Raw Data Plot screen 112
 view Standard Curve screen 90
 view Well Table 99
 workflow 82

Applied Biosystems

contacting xii
 customer feedback on documentation xii

Technical Support xii

Applied Biosystems StepOne Real-Time PCR System.
 See StepOne system.

assumptions for using this guide viii

B

BADROX flag 107

baseline

correct values 96
 examples 98
 manually adjust 105

biohazardous waste, handling xx

BLFAIL flag 107

C

CAUTION, description xiii

chemical safety xviii

chemical waste safety xix

colocated layout

data transfer 77
 monitoring 69
 startup 65

consumables

Also see materials required 4
 supported 4

conventions used in this guide viii

create new experiment 17

CT 101

CTFAIL flag 107

Custom assays 37

customer feedback, on Applied Biosystems
 documents xii

D

DANGER, description xiii

data

about data collection 2
 example experiment 9, 84
 publish 102
 transfer 76

design experiment

create new 17
 define experiment properties 20
 define methods and materials 22
 finish Design Wizard 42
 for more information 22, 24, 26, 28, 31, 33, 38, 41,

44
 guidelines 21, 23, 26, 28, 30, 32, 37, 41, 44
 order materials 38
 review reaction setup 33
 set up run method 31
 set up samples 29
 set up standards 27
 set up targets 25
 software elements 18
 workflow 16

Design Wizard
 Experiment Properties screen 20
 finish 42
 Materials List screen 38
 Methods & Materials screen 22
 Reaction Setup screen 33
 Run Method screen 31
 Samples screen 29
 software elements 18
 Standards screen 27
 Targets screen 25

deviation, standard 97
 documentation, related ix

E

electrical safety xx
 electromagnetic compatibility standards. *See* EMC standards
 EMC standards xxii
 ergonomics, safety xxii
 example experiment
 analyze 82
 data 12
 data location 12
 description 10
 design 16
 name 20
 prepare 46
 run 60
 workflow 13

Experiment Properties screen 20
 EXPFAIL flag 107
 Export/Import 10, 121

F

flag 101
 flags
 analysis settings 105
 in standard curve experiments 107

G

General xxi
 guidelines
 analysis 85, 92, 96, 101, 105, 107, 109, 111, 113
 chemical safety xviii

chemical waste disposal xix
 chemical waste safety xix
 design 21, 23, 26, 28, 30, 32, 37, 41, 44
 preparation 48, 50, 52, 56
 run 64

H

hazard icons. *See* safety symbols, on instruments
 hazard symbols. *See* safety symbols, on instruments
 hazards. *See* safety
 Help system, accessing xi
 HIGHSD flag 107

I

installation category xxi
 instrument operation, safety xvii
 Inventoried assays 37

L

library 32
 load reaction plate 62

M

Made to Order assays 37
 Materials List screen 38
 materials required 47, 49, 51, 54
 Methods & Materials screen 22
 monitor run
 Amplification Plot screen 70
 colocated layout 69
 Run Method screen 72
 standalone layout 75
 standalone layout (remote) 73
 Temperature Plot screen 71

moving and lifting, safety xvii

MSDSs
 description xviii
 obtaining xviii

MSDSs, obtaining xii
 MTP flag 107
 Multicomponent Plot screen 109
 Multiple Plots screen 89
 multiplex PCR 6, 24

N

navigation tips
 display multiple plots 89
 select wells 88

negative control, component of experiment 6
 NOAMP flag 107
 NOISE flag 107

NOSIGNAL flag 107
notification settings 63

O

OFFSCALE flag 107
omit wells 108
online Help. *See* Help system
order materials 38
other fluorescent-based reagents 9
OUTLIERRG flag 107
outliers. *See* omit wells.
overvoltage category (rating) xxi

P

prepare experiment
 for more information 48, 53, 57
 guidelines 48, 50, 52, 56
 reaction mix
 prepare 51
 reaction plate 53
 sample dilutions 47
 standard dilution series 49
 workflow 46
prepare for run 61
print reaction setup instructions 36
publish data 102

Q

QC Summary screen 106
QuickStart 10, 117

R

radioactive waste, handling xx
ramp speed 22, 24
Raw Data Plot screen 112
reaction mix
 calculated volumes 34
 volumes 51
reaction plate
 layout 42
 load 62
 prepare 53
 unload from the instrument 76
Reaction Setup screen 33
reagents
 other fluorescent-based 9
 SYBR Green 8
 TaqMan 8
repetitive motion, safety xxii
replicate, component of experiment 6
results, interpreting 83
run experiment

alerts 72
 enable notification settings 63
 for more information 63, 72, 73
 guidelines 64
 monitor 69
 prepare for 61
 start 65
 transfer data 76
 workflow 60
Run Method library 32
Run Method screen 31
 monitor during a run 72

S

safety
 before operating the instrument xvii
 biological hazards xxi
 chemical xviii
 chemical waste xix
 conventions xiii
 electrical xx
 ergonomic xxii
 guidelines xviii, xix, xx
 instrument operation xvii
 moving and lifting instrument xvii
 moving/lifting xvii
 repetitive motion xxii
 standards xxii
 workstation xxii
safety labels, on instruments xvi
safety standards xxii
safety symbols, on instruments xv
sample dilutions
 calculated volumes 36, 47
 prepare 47
Sample screen 29
samples
 design guidelines 30
 dilutions 47
 sample reactions (unknowns) 55
 set up 29
select wells 88
singleplex PCR 6, 24
software elements
 analysis screens 85
 Design Wizard 18
SPIKE flag 107
standalone layout
 data transfer 78
 monitoring 75
 remote data transfer 77
 Remote Monitor 73
 startup 66
standard curve experiments
 about 6
standard deviation, effect of threshold on 97

standard dilution series
 calculated volumes 35
 component of experiment 6
 prepare 49

standards
 component of experiment 6
 design guidelines 28
 diluting 49
 EMC xxii
 safety xxii
 set up 27
 Set Up Standards checkbox 25, 26
 standard reactions 55

Standards screen 27

start run
 colocated layout 65
 standalone layout 66

StepOne system
 consumables 4
 data collection 2
 filters 3, 113
 layouts 65, 69, 76
 reagents 8

SYBR Green reagents 3, 8, 24, 26, 37, 113

symbols, safety xv

T

TaqMan reagents 3, 8, 23, 24, 26, 37, 113

targets
 design guidelines 26
 set up 25

Targets screen 25

Technical Support, contacting xii

Temperature Plot screen 71

Template 10, 119

template. See samples.

text conventions viii

THOLDFAIL flag 107

threshold
 correct values 96
 examples 97
 manually adjust 105

training, information on xii

transfer data 76

troubleshooting
 adjust baseline 105
 adjust threshold 105
 flags 107
 omit wells 108
 view analysis settings 104
 view Multicomponent Plot screen 109
 view QC Summary screen 106
 view Raw Data Plot screen 112

U

unload instrument 76

user attention words, described viii

using this guide
 as a tutorial 9
 with your own experiments 9

W

WARNING, description xiii

waste disposal, guidelines xx

waste profiles, description xx

Well Table 99

wells
 negative control 29, 42, 53
 omit 108
 selecting 88
 standard 29, 42, 53
 unknown 29, 42, 53

workflows
 Advanced Setup 116
 example experiment 13
 Export/Import 10, 121
 QuickStart 10, 117
 Template 10, 119

workstation safety xxii

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