applied biosystems

StepOne and StepOnePlus Real-Time PCR Systems

Remarkably simple systems.
Simply remarkable results.

ThermoFisher SCIENTIFIC

High-performance, low-throughput real-time PCR

Remarkably simple systems

The Applied Biosystems™ StepOne™ (48-well) and StepOnePlus™ (96-well) Real-Time PCR Systems represent the latest innovations in real-time PCR from a leader in technology solutions for life science research. These remarkably simple real-time PCR systems are specifically designed with a user-friendly yet powerful interface for both new and experienced researchers.

System features

The StepOne and StepOnePlus systems bring low-throughput real-time PCR technology to a new level of accessibility—even for first-time users. Both systems measure amplification as it occurs, cycle by cycle, allowing for precise and quantitative measurements during the exponential phase of PCR. Beginning at the homepage (Figure 1), you can navigate seamlessly through all aspects of the real-time PCR process, including sample and reaction setup, thermal cycling, and fluorescent detection. Focused application software analyzes and interprets experimental results. Depending on the experimental design, the system can even help you select and order real-time PCR reagents online by means of convenient links in the software (optional). The system provides 2-fold discrimination with 99.7% confidence, along with 9 logs of dynamic range. These highly sensitive systems detect 10 copies of RNase P in a 30 µL reaction. Additionally, for laboratories with the StepOne system that require greater throughput, an upgrade kit is available.

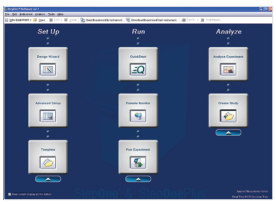

Figure 1. Software homepage.

StepOnePlus Real-Time PCR System Upgrade

The Applied Biosystems™ StepOnePlus™ Real-Time PCR System Upgrade is designed to accommodate your changing real-time PCR needs. The upgrade converts a StepOne system into a StepOnePlus system by upgrading the 48-well block to a 96-well Applied Biosystems™ VeriFlex™ Block and expanding the dye flexibility to four colors. The color of the bar above the block is also updated to indicate that the instrument is now a StepOnePlus system. Just send in your existing StepOne system and you'll receive a StepOnePlus loaner instrument to use until your upgraded system arrives in your lab.

Easy-to-use experimental design wizard

Both systems provide an experimental design wizard to assist you in setting up your first real-time PCR experiment. Just click on the design wizard and start answering questions about the real-time PCR experiment you wish to perform. The design wizard walks you through quantitation methods, detection chemistry, ramp speeds, and nucleic acid template types. From standards to plate layout, the design wizard guides the entire process, including reaction mix and standard dilution calculations. You're now ready to explore the remarkably simple features of these systems.

Features at a glance

	StepOne system	StepOnePlus system
Throughput/wells	48	96
FAM/SYBR green dyes	✓	✓
VIC/JOE dyes	✓	✓
ROX dye	✓	✓
NED/TAMRA dyes		✓
VeriFlex block		✓

System highlights

For both novice and experienced researchers, the StepOne or StepOnePlus systems have what you need.

- Both systems offer the ability to perform high-resolution melt analysis (additional software package required)
- Cost-effective 3-color/48-well (StepOne) or 4-color/96-well (StepOnePlus) systems deliver precise, quantitative real-time PCR results
- Long-life, LED-based optical system records fluorescence from FAM™/SYBR® Green I, VIC®/JOE™, and ROX™ dyes for gene expression analysis, pathogen quantitation, SNP genotyping, and presence/absence assays. The StepOnePlus system also accommodates the TAMRA™ dye
- Both systems perform Fast PCR reactions in less than 40 minutes
- Ultra-compact footprint fits any laboratory setting
- LCD touch screen and USB drive provide configuration flexibility and enable PC-free operation
- Remote monitoring and email notification for convenience and time savings

- The StepOnePlus system features VeriFlex Block technology, which combines six independently controllable Peltier blocks for enhanced PCR functionality and precise temperature control
- The StepOne system is upgradable to the StepOnePlus system to meet your changing research needs

Simply remarkable interface

Flexibility

Both the StepOne and StepOnePlus systems adapt to almost any workflow with flexible instrument control and data management. From the touch screen control panel, you can quick-start your experimental run without PC connectivity. You can also create a new protocol, view the history of your last run, or see protocol details (Figures 2 and 3). When the run is complete, data can be downloaded easily onto a USB flash stick or saved to a PC. Either system can be connected directly to a Local Area Network (LAN), and you can monitor the progress of the experiment, send new instructions to the system, download data, and edit the instrument profile. The software for the two systems also contains a convenient email feature that notifies you when your experiment is complete and ready for analysis.

The StepOne and StepOnePlus systems can be installed in multiple distinct configurations, providing unmatched flexibility and convenience. The unique stand-alone (PC-free) configuration provides an ultra-compact footprint that will fit into any laboratory. A direct connection to a LAN enables remote monitoring of experimental progress and downloading the completed run file to the PC at your desk.

System configurations (Figure 4)

1. PC-controlled
2. PC-free
3. Networked
4. PC-controlled, connected to LAN
5. PC-controlled, with networked instrument

Software

The Applied Biosystems™ StepOne™ and StepOnePlus™ Systems software contains unique features not available in other real-time PCR instruments. For example, data can be analyzed from multiple perspectives in the multiple plots view (Figure 5). The software constructs four-plot, side-by-side views of all data aspects, including the amplification plot, standard curve, multicomponent data plots, and raw data. It also displays this data next to the plate layout for easier analysis. Another novel feature is the software's ability to automatically identify wells that might compromise the success of an experiment. During data analysis, the software generates a quality-control report table that flags wells based on criteria such as amplification in a negative-control well, the absence of a signal in a well, or a high C_t standard deviation in a replicate group. This feature reduces analysis

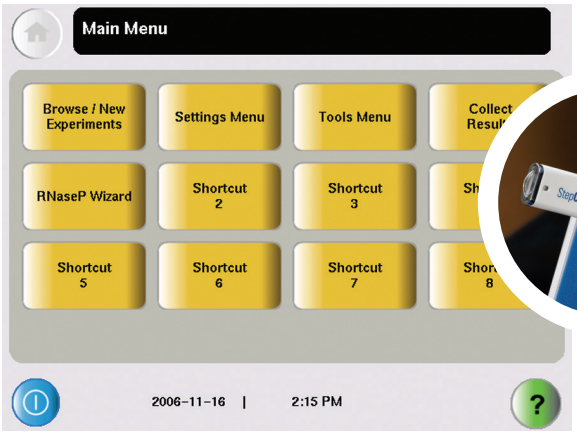

Figure 2. Browse experiment page on the touch screen.

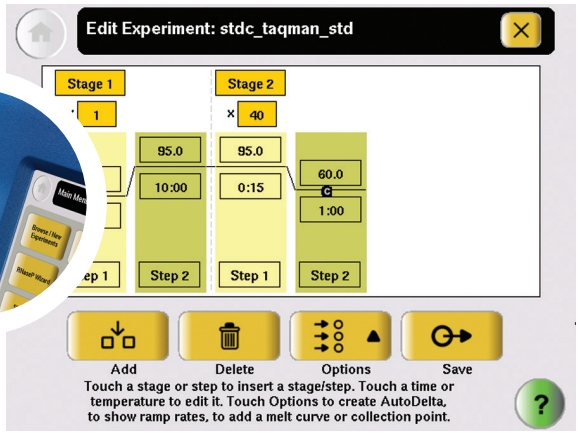

Figure 3. Run monitor screen on the touch screen.

Figure 4. Five distinct configurations.

time and allows researchers new to real-time PCR to have confidence in their results. Furthermore, you can customize this feature by disabling or modifying quality flag settings to suit your experimental needs.

Software highlights

- Optional software package offers the ability to conduct high-resolution melt experiments
- Experimental design wizards help you design and set up experiments
- Pipetting protocols and recipes help to set up experiments quickly
- Advanced setup if you require more flexibility for complex applications such as multiplexing
- Quick-start setup so you can begin a run immediately and enter plate information later
- Real-time monitoring of amplification growth curves enables you to view run progress (can be viewed from a remote PC)
- Multiple plots view for simultaneous data assessment from four perspectives
- Remote monitoring and email notification with run file attached for immediate access to results—even remotely
- Export easily to Microsoft™ PowerPoint™ or Excel™ software, or directly as a .jpeg file

Powerful gene expression study package (Figure 6)

- Import an unlimited number of comparative C_t (relative quantitation) plates to one study
- View data by biological replicate group or technical replicate group
- Normalize to multiple endogenous controls
- Enter known efficiencies to adjust RQ values for each target
- View amplification plot, multicomponent plot, and QC summary within the study to easily identify and eliminate outliers
- Preview the effect of modified analysis settings before permanently applying them to results

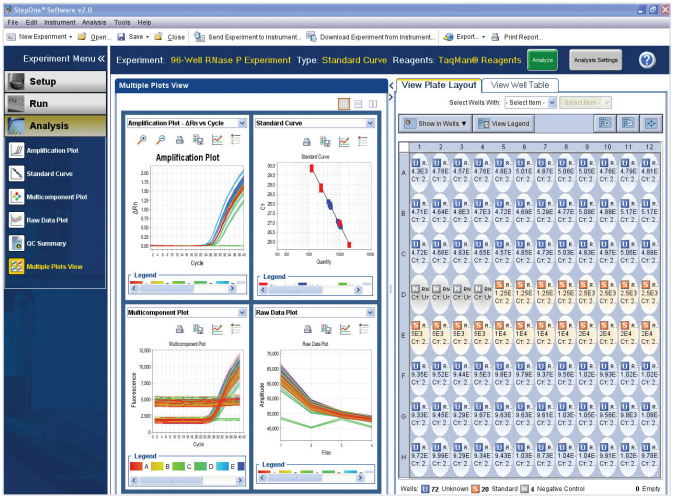

Figure 5. StepOne system multiple plots view.

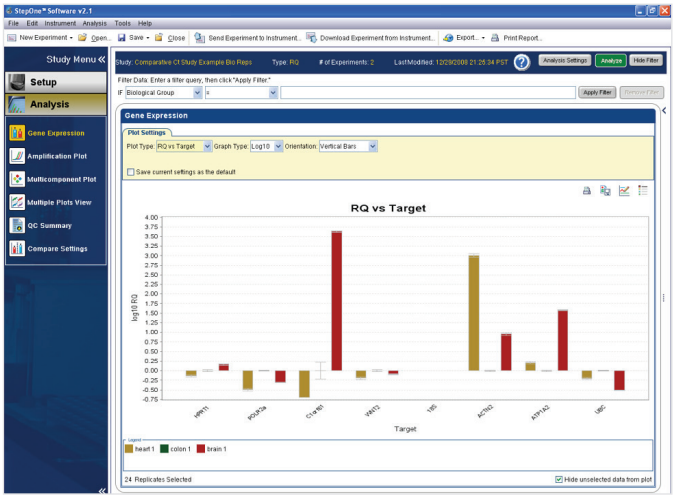

Figure 6. StepOne system with the gene expression study package. The StepOne Software v2.1 includes the gene expression study package to analyze an unlimited number of plates in one study, and displays the results in a publication-ready fashion.

4. PC-controlled, connected to LAN

5. PC-controlled, with networked instrument

Simply remarkable applications

Instrument chemistries

Both StepOne systems are equipped with fluorophore detection chemistries that include FAM and VIC dye–labeled Applied Biosystems™ TaqMan™ MGB probe-based assays, VIC and TAMRA dye–labeled probe-based assays, and SYBR Green I dye chemistry. [note: The TAMRA dye is available only with the StepOnePlus system.] TaqMan probe-based assays provide outstanding specificity and sensitivity, and SYBR Green I dye chemistry is an economical alternative for target identification, initial screening assays, or assays that require only a few reactions.

Software analysis (Figures 7 and 8)

The software for the StepOne systems supports a variety of analysis methods, including:

- Absolute quantitation
  - standard curve
- Relative quantitation
  - relative standard curve
  - comparative C_t ($\Delta\Delta C_t$)
- Presence/absence (plus/minus) assays with an internal positive control
- Melt curve analysis
- Genotyping (including real-time amplification)
- High-resolution melt analysis (additional software package required)

Applications

The StepOne systems support any real-time PCR application. Predesigned or custom assays exist for the following applications:

- SNP genotyping
- Gene expression profiling
- MicroRNA expression
- Methylation
- Translocation analysis
- Gene detection
- Viral load analysis
- Mutation scanning

For information about existing Applied Biosystems™ gene expression, microRNA, and translocation analysis assays, go to **thermofisher.com/allgenes**

SNP genotyping assay information can be found at **thermofisher.com/taqmansnp**

Speed

The StepOne and StepOnePlus systems perform both standard and Fast thermal cycling on the same block with no modification. Standard thermal cycling requires less than 2 hours, and Fast thermal cycling significantly reduces the run times of quantitative real-time PCR applications by delivering results in 40 minutes. Fast cycling is ideal for maximizing the number of runs on an instrument in any given workday.

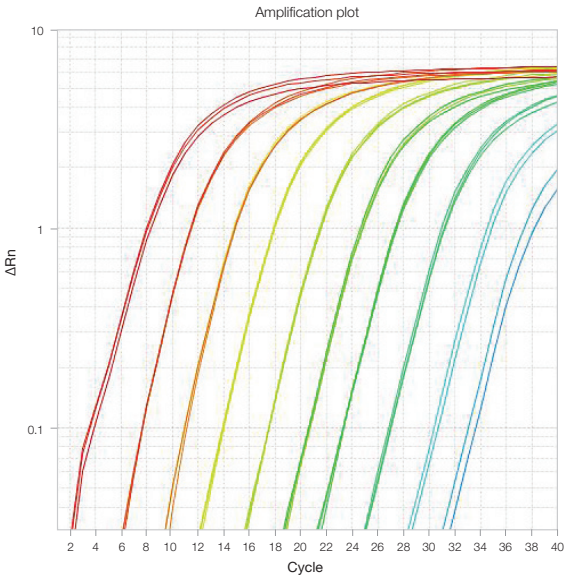

Figure 7. Broad dynamic range. Amplification plot shows log of change in normalized reporter fluorescence plotted vs. PCR cycle number. This plot from the StepOne system illustrates 9 logs of linear dynamic range for an Applied Biosystems™ TaqMan™ Assay of cDNA containing the 18S target sequence in 10-fold serial dilutions.

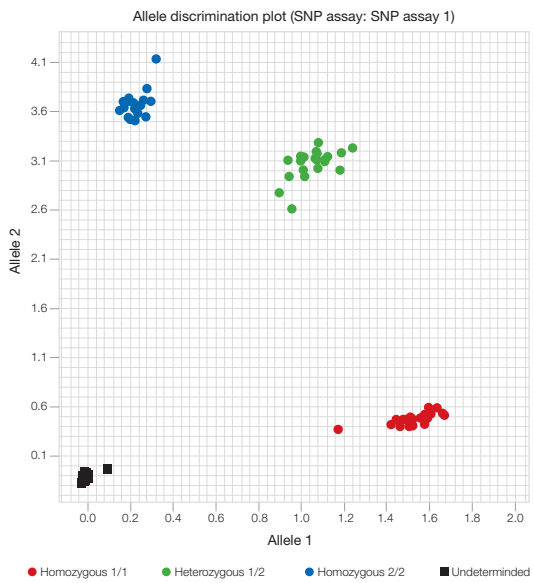

Figure 8. Genotyping analysis. StepOne and StepOnePlus software automatically determines genotypes and generates an intuitive graphic representation of results in a cluster plot report that helps you view data across populations or samples. Results are from human CYP2C19*2 Applied Biosystems™ TaqMan™ SNP Genotyping Assay (using the StepOnePlus system).

VeriFlex Blocks

The 96-well StepOnePlus system features Applied Biosystems™ VeriFlex™ Block technology, which brings six independently controllable peltier blocks together for precise temperature control and enhanced PCR functionality. VeriFlex Blocks deliver flexibility for those who have probes and primers that are optimized at different annealing temperatures.

VeriFlex Block (96-well)

Results

Because the StepOne and StepOnePlus systems are factory calibrated for optical and thermal accuracy, remarkably simple real-time PCR results are available right out of the box (Figures 9 and 10). Both systems can discriminate between two TaqMan Assays containing 5,000 and 10,000 copies of template with 99.7% confidence. Both systems also demonstrate a linear dynamic range of 9 log units or more, as shown by the amplification plot (Figure 7).

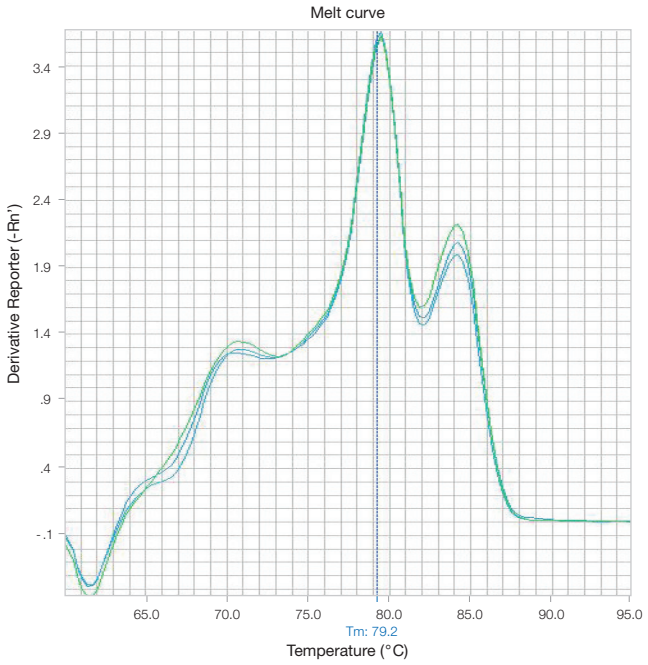

Figure 9. Melt curve analysis. Standard PCR was run to generate amplicons for 18S, β-actin, and GAPDH. Aliquots of each amplicon were combined into one reaction tube and a melt curve analysis was performed.

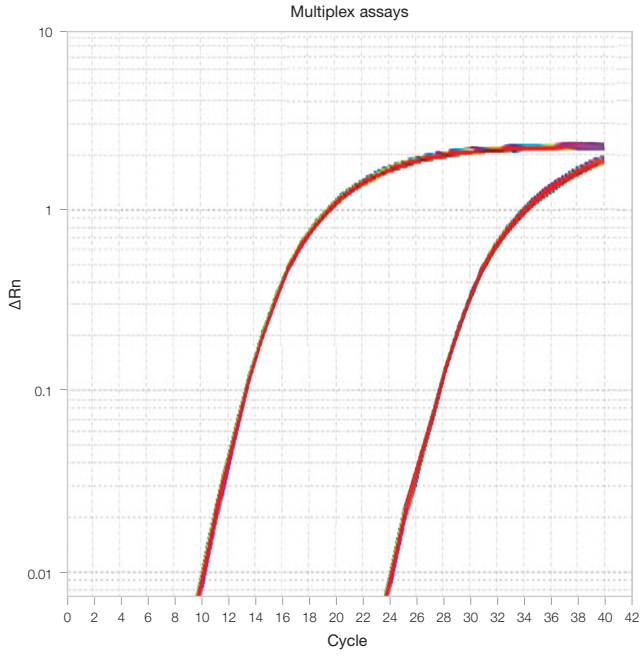

Figure 10. Multiplex assay. Multiplex TaqMan Assays on the StepOnePlus system showing amplification of cDNA (96 samples) using probes labeled with VIC and FAM reporters for 18S and TGF-β target sequences, respectively.

Systems at a glance

StepOne system

- 48 wells for lower throughput
- 3 colors for basic applications research
- Easily upgradable to StepOnePlus system

StepOnePlus system

- 96 wells for higher throughput
- 4 colors for more flexibility
- VeriFlex Block technology for thermal cycling flexibility

Remarkable Applied Biosystems solutions

We offer a complete range of real-time PCR reagents and design/analysis software to assist both experienced and novice users of the StepOne and StepOnePlus Real-Time PCR Systems. During experimental setup, the design wizard automatically creates a material list that links directly to our online store for convenient ordering of Applied Biosystems™ real-time PCR master mixes and consumable plasticware (optional).

Service and support at every step

Online instrument management

Sign in to your thermofisher.com account to access the award-winning, free online Instrument Management Tool* that enables faster responses to requests for service or service quotes, plus get instant connection to key instrument and service information.

Comprehensive instrument warranty

Our factory-trained and certified field service engineers (FSEs) are focused on delivering the highest-quality workmanship. During the warranty period, all repairs, including engineer time and parts, are covered.

Flexible service plans

Our rapid exchange service provides a new or refurbished instrument shipped to your lab whenever your instrument requires repair. Simply use our prepaid shipping box to return the damaged instrument back to us.

The AB repair center plan is the cost-effective choice for labs that send their instrument away for repair—this plan provides an optional loaner instrument so that your lab can maintain productivity while the instrument is being repaired. The instrument is sent back upon repair.**

Compliance and validation services

Our compliance and validation services are designed to help you balance business and regulatory requirements. From risk assessment to hardware and software qualification to full-system validation, we partner with you to help mitigate regulatory risks and get your processes up and running.

Training courses

Our application and instrument training programs are led by scientists who aim to enhance your workday through experimental design best practices, workflow training, and instrument troubleshooting. Hands-on classes are available at scientific training centers, in your lab, or through our Applied Biosystems™ LabCoat Live™ virtual training.

Technical support

Contact our team of technical support scientists with questions about product selection or use, assay or experiment design, and data analysis or troubleshooting. Online support centers are available 24 hours.

How to reach us

Go to **thermofisher.com/contactus** to find your local support or technical support team. For product FAQs, protocols, training courses, and webinars, go to **thermofisher.com/technicalresources**

Reagents and consumables

TaqMan SNP Genotyping Assays

The TaqMan SNP Genotyping Assays collection includes over 7 million predesigned human and mouse genome-wide assays of which 3.5 million are HapMap SNPs, 70,000 cSNPs, 160,000 validated assays, and over 2,700 drug metabolism genotyping assays. Additionally, our Applied Biosystems™ Custom TaqMan™ SNP Genotyping Assays service lets you create your own custom assays by submitting target SNP sequences for any genome.

MeltDoctor HRM Master Mix

The Applied Biosystems™ MeltDoctor™ HRM Master Mix contains all components needed for HRM-PCR (excluding template and primers). It is formulated for superior HRM performance across a wide range of genomic targets. Unlike some mixes available from other providers, the MeltDoctor HRM Master Mix does not require additional mixing prior to use, and was developed and optimized solely for HRM applications.

RNA isolation kits

A wide range Invitrogen™ and Applied Biosystems™ RNA isolation kits are suitable for a variety of sample types, including animal and plant tissue, cultured cells, blood, bacteria, and yeast. Blood, bacteria, and formalin-fixed material require RNA isolation kits designed specifically for these sample types, whereas most eukaryotic samples can be processed with excellent results using standard RNA isolation kits.

* Instrument Management Tool not available in all regions.
** Not available in all regions.

TaqMan Gene Expression Master Mix

Tailored for quantitative real-time PCR experiments, the Applied Biosystems™ TaqMan™ Gene Expression Master Mix delivers robust performance for both routine and challenging quantitative applications. It enables specific target detection across a large dynamic range with high sensitivity down to a single copy number. Furthermore, it offers duplex capability and reproducibility at less than 2-fold discrimination.

TaqMan Genotyping Master Mix

Specifically formulated for reliable, cost-effective detection of single nucleotide polymorphism (SNP) detection, the Applied Biosystems™ TaqMan™ Genotyping Master Mix provides accurate and reproducible allelic discrimination with well-separated clusters for exceptional call rates, premium performance in challenging genotyping assays, and excellent benchtop stability for superior flexibility to meet various throughput needs.

TaqMan MicroRNA Assays

Applied Biosystems™ TaqMan™ MicroRNA Assays can quantitate microRNAs with the sensitivity and specificity of TaqMan Assay chemistry. Also, the new Applied Biosystems™ TaqMan™ Advanced miRNA Assays use ligation-based universal reverse transcription for a streamlined and highly sensitive workflow.

Applied Biosystems Primer Express Software

Applied Biosystems™ Primer Express Software v3.0 facilitates primer design with TaqMan probes for real-time PCR or endpoint PCR analyses. It is also ideal for primer design using SYBR Green I dye chemistries. The StepOne and StepOnePlus systems ship with Primer Express Software.

For more information about the products, reagents, consumables, assays, and kits listed on this page, go to **thermofisher.com/steponeplus**

TaqMan Assays

We offer the most comprehensive set of inventoried TaqMan Gene Expression and SNP Genotyping Assays. More than 1.5 million gene expression assays and over 7 million predesigned human, and 10,000 predesigned mouse SNP genotyping assays are available at your fingertips. Alternatively, you can submit your target DNA sequence from any organism, and we'll custom-build an assay for you. TaqMan MicroRNA Assays can quantitate miRNA with the sensitivity and specificity of Applied Biosystems™ TaqMan™ Assay chemistry. For more information on gene expression assays, go to **thermofisher.com/allgenes** or for information on SNP Genotyping Assays, go to **thermofisher.com/taqmansnp**

Taqman Gene Expression, SNP Genotyping, and MicroRNA Assays

TaqMan Assays selection guide	Application		
	Gene expression*	SNP genotyping†	MicroRNA‡
TaqMan predesigned Assays (inventoried and made-to-order)	Yes	Yes	Yes
Custom TaqMan Assays	Yes	Yes	No
Species	**Number of inventoried and made-to-order assays**		
Human	205,000	7,000,000	5,000
Mouse	176,000	>10,000	2,300
Rat	146,000	§	1,000
Drosophila melanogaster	41,000	§	460
Arabidopsis thaliana	97,000	§	340
Caenorhabditis elegans	92,000	§	460
Canine	55,000	§	N/A
Rhesus macaque	69,000	§	N/A

* Includes miRNA, gene copy number, and mitochondrial assays.
† Includes HapMap and drug metabolism genotyping assays.
‡ Gene expression only.
§ Custom TaqMan Assays are available for any SNP, transcript, and genome.

Reagents and consumables

A complete line of reagents, including TaqMan master mixes, SYBR Green master mixes, and consumables, including 96-well plates, is available for use with the StepOne and StepOnePlus Real-Time PCR Systems. These products can easily be added to a shopping list for future reference or for ordering through the materials list link in the experimental design wizard (Figure 11).

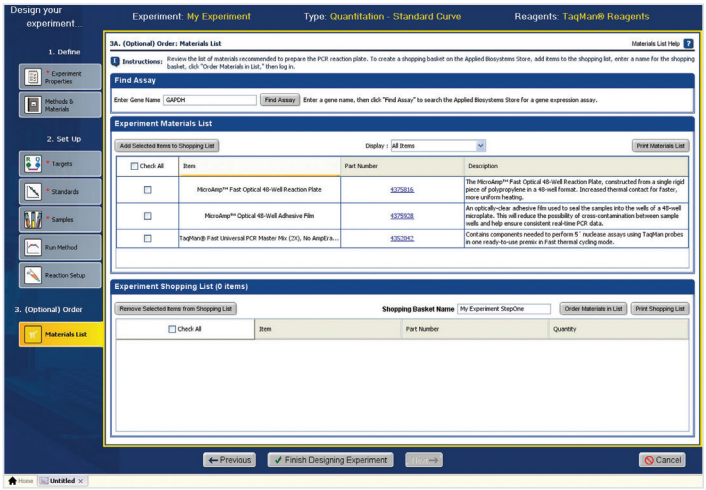

Figure 11. The reagents ordering page in the experimental design wizard.

Select reagents, consumables, and service contract offerings for your StepOne and StepOnePlus Real-Time PCR Systems

Category/product description	Quantity	Cat. No.
Seals and covers		
MicroAmp 48-Well Optical Adhesive Film	25 films	4375928
MicroAmp 48-Well Optical Adhesive Film	100 films	4375323
MicroAmp 96-Well Optical Adhesive Film	25 films	4360954
MicroAmp 96-Well Optical Adhesive Film	100 films	4311971
Reaction plates		
MicroAmp Fast Optical 48-Well Reaction Plate	20 plates	4375816
MicroAmp Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL)	20 plates	4346906
MicroAmp Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL)	200 plates	4366932
8-Well strips		
MicroAmp Fast 8-Tube Strip, 0.1 mL	125 strips	4358293
MicroAmp Optical 8-Cap Strip	300 strips	4323032
Accessories		
MicroAmp Fast 48-Well Tray	10 trays	4375282
MicroAmp 96-Well Tray/Retainer Set for VeriFlex Block Systems	10 trays	4379983
MicroAmp 48-Well Base Adaptor	5 adaptors	4375284
Reagents		
Fast SYBR Green Master Mix	5 mL	4385612
Power SYBR Green PCR Master Mix	5 mL	4367659
TaqMan Gene Expression Master Mix	5 mL	4369016
TaqMan Genotyping Master Mix	10 mL	4371355
TaqMan Fast Universal PCR Master Mix	2 x 1.25 mL	4352042
TaqMan RNA-to-C_T *1-Step* Kit	5 mL	4392938
MeltDoctor HRM Master Mix	5 mL	4415440

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Ordering information

Description	Cat. No.
StepOne Real-Time PCR System	4376357
StepOne Real-Time PCR System with Laptop Computer	4376373
StepOne Real-Time PCR System with Tower Computer	4376374
StepOnePlus Real-Time PCR System	4376600
StepOnePlus Real-Time PCR System with Laptop Computer	4376598
StepOnePlus Real-Time PCR System with Tower Computer	4376599
StepOnePlus Real-Time PCR System Upgrade Kit*	4379216
High Resolution Melt Software v3.0.1 for 1 license	4461357
High Resolution Melt Software v3.0.1 for 10 licenses	4461456
StepOne pure dye calibration service	
PDC StepOne	4460599
PDC StepOnePlus	4460600
Installation qualification and operation qualification (IQ/OQ), operation qualification and instrument performance verification (OQ/IPV)	
StepOne IQ/OQ	4413678
StepOnePlus IQ/OQ	4415138
StepOne OQ/IPV	4415178
StepOnePlus OQ/IPV	4415318
Service plans	
StepOne AB Repair Center Support 1 year plan	ZG03SCSTEPONE
StepOnePlus AB Repair Center Support 1 year plan	ZG03SCSTEPONEP
StepOne Rapid Exchange 1 year plan	ZGEXSCSTEPONE
StepOnePlus Rapid Exchange 1 year plan	ZGEXSCSTEPONEP
StepOne AB Repair Center Support 3 Year Plan	ZG03SCSTEPONE3Y
StepOnePlus AB Repair Center Support 3 Year Plan	ZG03CSSTEPONEP3Y
StepOne Rapid Exchange 3 Year Plan	ZGEXSCSTEPONE3Y
StepOnePlus Rapid Exchange 3 Year Plan	ZGEXCSTEPONEP3Y

* For users of the StepOne Real-Time PCR System.