

StepOne[™] and StepOnePlus[™] Real-Time PCR Systems

Remarkably Simple Systems. Simply Remarkable Results.



Step Up to High-Performance Real-Time PCR





Remarkably Simple Systems

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



Figure 1. Software Homepage.

System Features

The StepOne[™] and StepOnePlus[™] Systems bring advanced 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. Highly sensitive, these 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.*

StepOnePlus™ Real-Time PCR System Upgrade Kit

To accommodate your changing real-time PCR needs, Applied Biosystems offers the StepOnePlus™ Real-Time PCR System Upgrade Kit. The upgrade converts a StepOne™ System into a StepOnePlus™ System by upgrading the 48-well block to a 96-well 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 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 simply remarkable features of these systems.

Features at a Glance

	Step0ne™ System	Step0nePlus™ 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®/J0E™, and R0X™ dyes for gene expression analysis, pathogen quantitation, SNP genotyping, and presence/absence assays. Note: 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 touchscreen 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 upgradeable 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 touchscreen 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 of 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

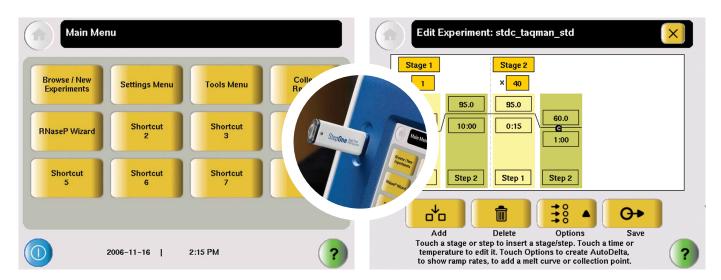


Figure 2. Browse Experiment Page on Touchscreen.

 $Figure \ 3. \ Run \ Monitor \ Screen \ on \ Touch screen.$



Figure 4. Five Distinct Configurations.

Software

The 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, multi-component 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, standard deviation in a replicate group. This feature reduces analysis time and allows even 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 highresolution melt experiments
- Experimental Design Wizards to help you design and set up experiments
- Pipetting protocols and recipes 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 PowerPoint®, Excel®, 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

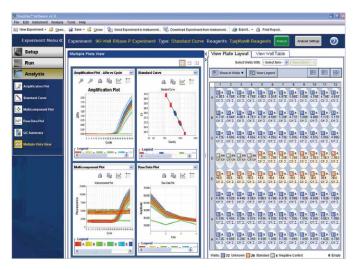


Figure 5. StepOne™ System Multiple Plots View.

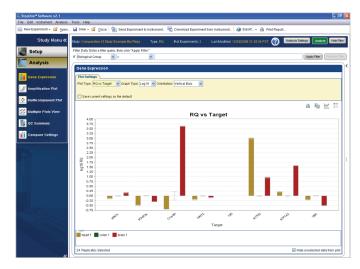
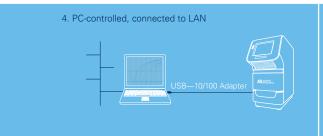
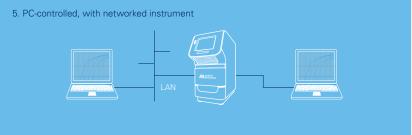


Figure 6. StepOne™ System With the Gene Expression Study Package.

The $StepOne^{\mathbb{M}}$ 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.





Simply Remarkable Applications

Instrument Chemistries

Both StepOne™ Systems are equipped with fluorophore detection chemistries that include FAM™ and VIC® dye-labeled 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 $C_{+}(\Delta\Delta C_{+})$
- 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 Gene Expression, MicroRNA, and Translocation Analysis Assays, please visit www.allgenes.com.

SNP Genotyping Assay information can be found at www.allsnps.com.

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.

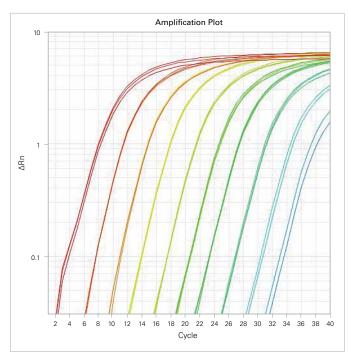


Figure 7. Broad Dynamic Range.

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 a TaqMan® Assay of cDNA containing the 18S target sequence in 10-fold serial dilutions.

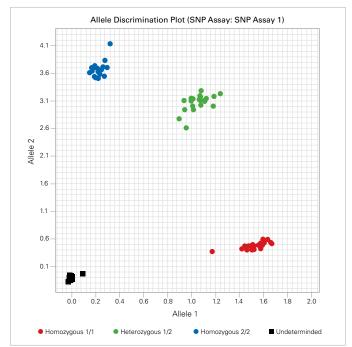


Figure 8. Genotyping Analysis.

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 TaqMan® SNP Genotyping Assay (using the StepOnePlus™ System).

VeriFlex™ Blocks

The 96-well StepOnePlus™ System features 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, simply remarkable real-time PCR results are available right out of the box (Figures 9 and 10). Both systems can discriminate between two populations of 5,000 and 10,000 template copies of a TaqMan® Assay 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).

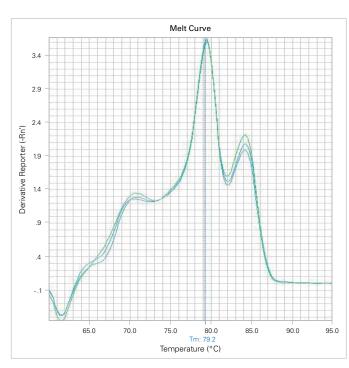


Figure 9. Melt Curve Analysis.

In this experiment, 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.



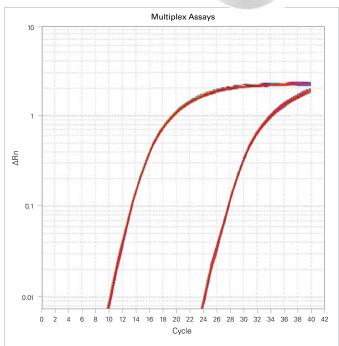
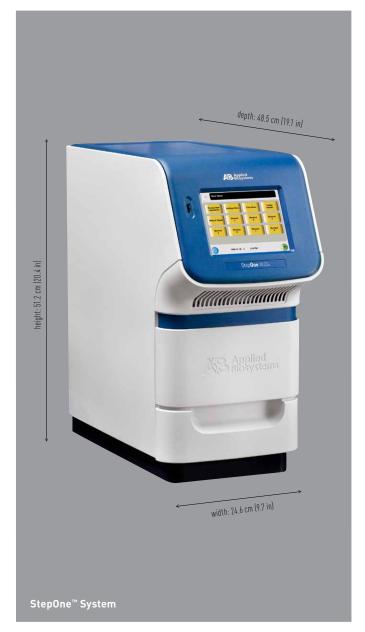


Figure 10. Multiplex Assay.

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-ß target sequences respectively.

Systems at a Glance



- 48 wells for lower throughput
- 3 colors for basic applications research
- Easily upgradeable to StepOnePlus™ System



- 96 wells for higher throughput
- 4 colors for more flexibility
- $\bullet \ \ \mathsf{VeriFlex}^{\scriptscriptstyle\mathsf{M}} \ \mathsf{Block} \ \mathsf{technology} \ \mathsf{for} \ \mathsf{thermal} \ \mathsf{cycling} \ \mathsf{flexibility}$

The Remarkable Applied Biosystems Solution

Applied Biosystems offers 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 the Applied Biosystems Store for convenient ordering of real-time PCR master mixes and consumable plasticware (optional).

Service and Support

Purchase of either of these systems includes a limited warranty on parts and labor.* Depot repair and update services are available for the StepOne™ and StepOnePlus™ Systems through Applied Biosystems. Technical support and service are provided worldwide. Additionally, an upgrade is available from the StepOne™ to the StepOnePlus™ System.

SMART Monitoring Service

The StepOne™ and StepOnePlus™ Systems include SMART Monitoring and remote diagnostic capabilities. In the event your instrument experiences a technical issue, your networked system can be diagnosed remotely by the LifeTechnologies Technical Assistance Center, maximizing instrument uptime. Contact the Applied Biosystems support team at abcc@appliedbiosystems.com for information on how to download and enable this feature.

Compliance Services

If you operate in a regulated environment, global standards (e.g., GLP, GCP, GMP, ISO17025, ISO15189) and country-specific regulations (e.g., US FDA 21 CFR Part 11, CLIA, CAP) require validation and verification of computerized laboratory systems to ensure the data is accurate, reliable, and the system consistently performs over time. In addition to our service plans, we offer a range of Compliance Services to help you meet these requirements and ease that burden.

Our services include instrument qualification (IQ/OQ) to help you commission your StepOne™ or StepOnePlus™ System for lab testing. Our re-qualification services (OQ/IPV), when added to a service plan, support proactive re-testing and as-needed testing after repairs. This helps ensure consistent instrument performance. Our professionals, working closely with your staff, can help you maintain your validated state while controlling costs, assisting with your change control, and managing your compliance risks.

Applied Biosystems Training Programs

Theoretical and practical hands-on laboratory sessions provide in-depth instruction for customers seeking to gain proficiency and streamline workflows with Applied Biosystems solutions. Applied Biosystems Application Support Centers offer interactive, computer-based training, fully staffed working laboratories, and the unique opportunity to collaborate with Applied Biosystems scientists and product development teams. Many hands-on courses can also be conducted at the customer site. Regional training and live and self-paced web-based courses are also available.

TaqMan® SNP Genotyping Assays

The TaqMan® SNP Genotyping Assays collection includes over 4.5 million pre-designed human and mouse genome-wide assays of which 2.5 million are HapMap SNPs, 70,000 cSNPs, 160,000

validated assays, and over 2,500 Drug Metabolism Genotyping Assays. Additionally, our 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 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.

Ambion® and Applied Biosystems® RNA Isolation Kits

Ambion and Applied Biosystems offer a range of RNA isolation kits that are suitable for a wide 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 Ambion's standard RNA isolation kits.

TaqMan® Gene Expression Master Mix

Tailored for quantitative real-time PCR experiments, the 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 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 offers TaqMan® MicroRNA Assays to quantitate microRNAs with the sensitivity and specificity of TaqMan® Assay chemistry. MicroRNA Assays are available for human, mouse, rat, *Arabidopsis*, *Drosophila*, and *C. elegans*.

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 Primer Express Software ships with the StepOne $^{\mathbb{M}}$ and StepOnePlus $^{\mathbb{M}}$ Systems.

For more information about the products, reagents, consumables, assays, and kits listed on this page, please visit info.appliedbiosystems.com/steponeplus.

^{*} Warranty length varies by region.

TaqMan® Assays

Applied Biosystems offers the most comprehensive set of inventoried TagMan® Gene Expression and SNP Genotyping Assays available. More than 700,000 Gene Expression Assays and over 4.5 million pre-designed 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. Applied Biosystems also offers TagMan® MicroRNA Assays to quantitate miRNA with the sensitivity and specificity of TagMan® Assay chemistry. For more information on gene expression assays, visit www.allgenes. com; for information on SNP Genotyping Assays, visit www.allsnps.com.

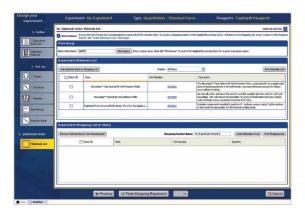


Figure 11. The Reagents Ordering Page in the Experimental Design Wizard.

TanMan® Gene Expression SNP Genotyping and MicroRNA Assays

TaqMan® Assays Selection Guide		Application	ation	
	Gene Expression*	SNP Genotyping [†]	MicroRNA [‡]	
TaqMan® Pre-designed Assays (Inventoried and Made-to-Order)	Yes	Yes	Yes	
Custom TaqMan® Assays	Yes	Yes	No	
Species	ies Number of Inventoried and Made-to-Order Assays			
Human	>2,024,000	>4,000,000	>300	
Mouse	>179,000	>10,000	>240	
Rat	>128,000	§	>180	
Drosophila melanogaster	>38,000	§	>50	
Arabidopsis thaliana	>95,000	§	>40	
Caenorhabditis elegans	>90,000	§	>60	
Canine	>6,000	§	N/A	
Rhesus macaque	>70	§	N/A	
* Includes miRNA, gene copy number, and mit † Includes HapMap and drug metabolism gene ‡ 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).

Select Reagents, Consumables, and Service Contract Offerings for Your Applied Biosystems® StepOne™ and StepOnePlus™ Real-Time PCR Systems

Category/Product Description	Quantity	P/N
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 ™ <i>1-Step</i> Kit	5 mL	4392938
MeltDoctor™ HRM Master Mix	5 mL	4415440

Ordering Information

Description	Part Number
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 v 3.0 for 1 license	4461357
High Resolution Melt Software v 3.0 for 10 licenses	4461456
StepOne™ Pure Dye Calibration Service	
PDC StepOne [™]	4460599
PDC StepOnePlus™	4460600
Calibration Services—Installation Qualification/Operational, Qualificational Qualification/Instrument, and Performance Verification (
StepOne™ IQOQ	4413678
Step0nePlus™ IQ0Q	4415138
C. C. N. C. (17)	4415178
StepOne [™] OQ/IPV	
StepOne™ OQ/IPV StepOnePlus™ OQ/IV	4415318

Service Plan

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FOI	more information.	. contact vour to	tat sales representative	. SUSTEPUNE

To learn more about the new StepOne[™] and StepOnePlus[™] Real-Time PCR Systems from Applied Biosystems, contact your local sales representative, or visit us on the web at **info.appliedbiosystems.com/steponeplus**.

Life Technologies offers a breadth of products $\,$ DNA $\,$ $\,$ RNA $\,$ $\,$ $\,$ protein $\,$ $\,$ cell culture $\,$ $\,$ instruments

For Research Use Only. Not intended for human or animal therapeutic or diagnostic use.

NOTICE TO PURCHASER:

The StepOne™ and StepOnePlus™ Real-Time PCR Systems are covered by US patents, and claims in their non-US counterparts, owned by Life Technologies Corporation. No right is conveyed expressly, by implication, or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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