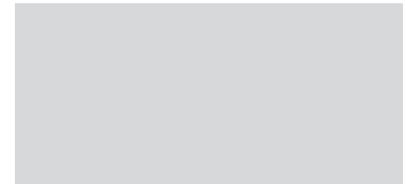
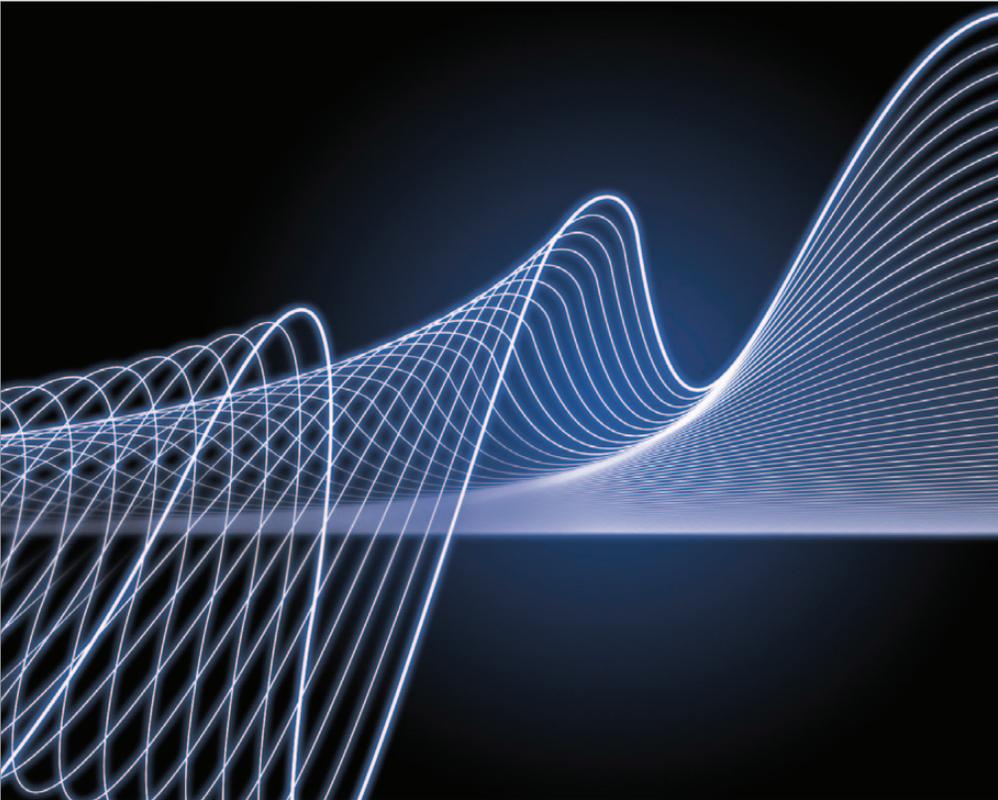


Real-Time PCR: CFX96 System



CFX96™ Real-Time PCR  
Detection System



# The CFX96 System — Designed for the Way You Work

Bio-Rad's 1000-series PCR instruments meet all your PCR needs — whether you are designing your first experiment or running a high-throughput laboratory and analyzing large gene expression studies. The CFX96 real-time PCR detection system builds on the power and flexibility of the C1000™ thermal cycler, adding an easy-to-install interchangeable reaction module to create an exceptional real-time PCR system. The system's unsurpassed thermal performance plus its innovative optical design produce accurate, reliable data, and the powerful, yet intuitive software accelerates every step of your real-time PCR research, shortening the time between getting started and getting great results.



With the CFX96 system, you can:

- **Get great results right away** — quick installation and factory-calibrated optics let you set up the system in seconds
- **Fit experiments into your schedule** — fast thermal cycling produces results in <30 minutes
- **Save research time** — thermal gradient feature lets you optimize reactions in a single experiment
- **Minimize sample and reagent usage** — optimal results obtained with sample volumes as low as 10  $\mu$ l
- **Rely on performance** — innovative technologies with long-lasting LEDs and solid-state components provide maximal reliability and optimal quantitative results
- **Analyze results when and where you want** — software can send email notification with an attached data file when a run is finished
- **Configure the system to fit your laboratory needs** — run a stand-alone system without a computer or up to 4 instruments from 1 computer

Bio-Rad's PCR instruments, reagents, plastics, and software are powerful building blocks for your genomic research, providing the flexibility and reliability you need to accelerate discovery.



## Reagents That Provide Optimal Performance

Bio-Rad reagents demonstrate best-of-class performance over a wide dynamic range of input RNA, cDNA, and genomic DNA. The broad mix of reverse transcription kits and supermixes for qPCR delivers maximum sensitivity and consistent results every time.



## Don't Worry About Your Consumables

Bio-Rad's plastic consumables have been validated to deliver reliable, reproducible results, leaving you less to worry about.



## Software Solutions

Bio-Rad offers a complete line of software products to accelerate your research. Precision Melt Analysis™ software is a powerful tool to genotype DNA samples based on the thermal denaturation properties of double-stranded DNA. Biogazelle's qbase<sup>PLUS</sup> software makes higher throughput gene expression analysis accessible to every researcher. Identify the role of target genes in specific cellular processes or signaling pathways using a combination of tools from Bio-Rad.

# System Configurations

## Stand-alone



CFX96 or CFX384

## Stand-alone multi-instrument



## Software-controlled



## Software-controlled multi-instrument



CFX Automation System

The CFX96 real-time PCR detection system offers multiple control configurations so it can be easily integrated into your laboratory settings. The CFX96 system can be quickly installed, fitting on any lab bench. Its factory-calibrated optics let you get great results right away and its quiet operation will not add to laboratory noise.

### Stand-Alone

Perform real-time PCR runs on a CFX96 system in stand-alone mode, without the system being attached to a computer. Create a real-time PCR protocol using the C1000 control panel, or transfer a protocol previously created in CFX Manager™ software using a USB flash drive. Choose a data acquisition mode and start an experiment — there is no need to worry about the plate setup because data are acquired from all the wells. When a run is finished, export the data using a USB flash drive, or directly email the data from the C1000 chassis. Data are stored on the instrument during a run, regardless of the setup, so you always have built-in data backup.

### Expanding Your Throughput

The flexibility of the 1000-series thermal cycling platform allows you to adjust your setup as your needs change.

Adding more instruments to increase throughput or expand options is as easy as connecting a USB cable. CFX Manager software can independently run four instruments, including a combination of CFX96 and CFX384™ real-time PCR detection systems and C1000 or S1000™ thermal cyclers. Maximize laboratory throughput by integrating one CFX96 system with a CFX automation system for hands-free loading and unloading of up to 20 96-well plates.

### Integrated for Optimal Results

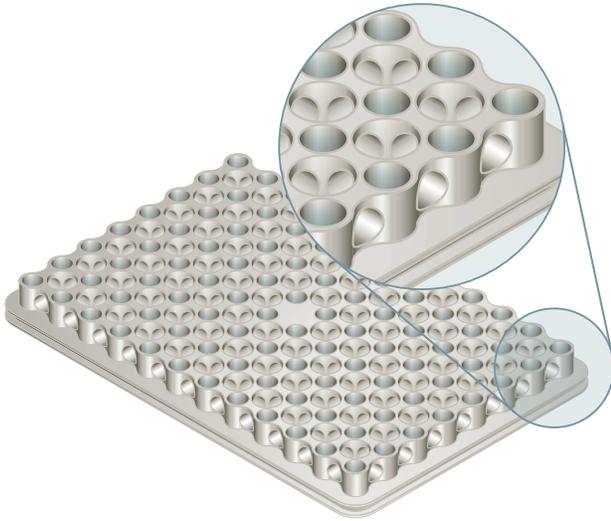
Reliable and reproducible real-time PCR assays require an instrument with validated optical performance. The CFX96 system has unique features which ensure precise assay results, so that you can confidently make comparisons between data sets:

- Internal qualification before every scan to ensure the optics shuttle is homed with micron accuracy
- Internal reference spots, which the system reads to measure the consistency and accuracy of every scan
- System test software to qualify instrument performance

# Fast Thermal Cycling

## Superior Uniformity

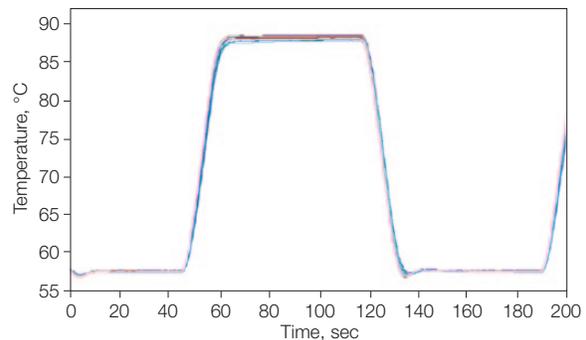
The rate and efficiency of PCR critically depend on the precision of the temperature steps. To obtain reliable, consistent results, all sample wells must maintain proper temperature throughout each incubation step. The CFX96 system uses six independently controlled thermal electric modules (TEs), the heating and cooling elements of the thermal cycler, to maintain tight temperature uniformity at all points during a run — even while ramping.



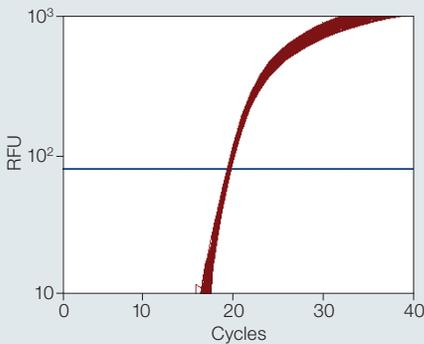
The patent-pending reduced-mass sample block heats and cools more quickly than standard blocks, so average ramp rates are increased and overall run times are reduced.

## Rapid Arrival at Target Temperature

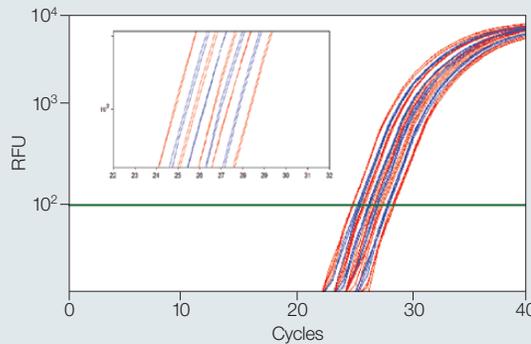
A key component of overall protocol run time is the time to reach target temperature, which is determined by the average ramp rate and the time for the sample block to reach thermal uniformity. Maximum ramp rate is less important, because it can fluctuate significantly during the ramp. The CFX96 system's temperature control produces high average ramp rates and tight uniformity during ramping, to yield fast time to target temperature and faster protocol run times. Run times can be dramatically shortened — to less than 30 minutes — while still producing accurate quantitative results. Now you can tailor your runs around your schedule, instead of tailoring your schedule around your runs.



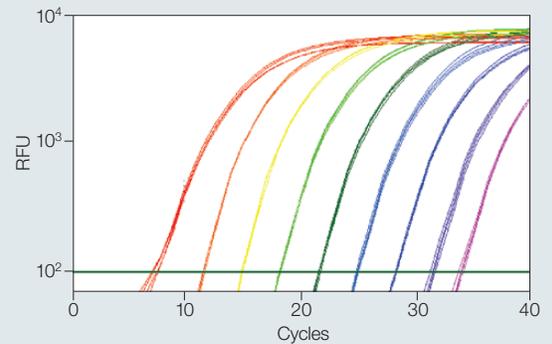
**Rapid arrival at target temperature and superior uniformity.** 1000-series thermal cyclers exhibit high average ramp rates, rapid settling time, and tight thermal uniformity throughout the ramp. This graph shows the temperature measured by probes in 15 wells across a sample block. The traces are nearly indistinguishable due to the tight uniformity. Note the consistent ramp rate throughout heating and cooling.



**Excellent uniformity.** IL-1 $\beta$  plasmid template diluted to  $10^5$  copies/reaction amplified in the presence of a FAM-labeled detection probe with iQ™ supermix. Graph shows 96 replicates of 10  $\mu$ l reactions. Average threshold cycle ( $C_T$ ) =  $19.81 \pm 0.10$ . RFU, relative fluorescence units.



**Exceptional reproducibility can be achieved with SsoFast™ EvaGreen® supermix.** Efficient discrimination and reliable quantification can be obtained from 1.33-fold serial dilutions of input template. The *CBP* gene was amplified from varying amounts of human genomic DNA (5 ng to 500 pg). From left to right: (■) 5 ng, 2.83 ng, 1.60 ng, 903 pg, and 511 pg; (■) 3.76 ng, 2.13 ng, 1.20 ng, and 679 pg. *CBP* efficiency = 96.5%,  $r = 0.996$ . Insert is a magnified view showing robust discrimination and reproducible amplification. RFU, relative fluorescence units.



**The unique fusion polymerase in SsoFast EvaGreen supermix delivers extreme speed and generates exceptional qPCR results in less than 30 min.** Tenfold serial dilutions of 10 ng to 100 ag of cDNA from human spleen were used in each 20  $\mu$ l reaction to detect 18S rRNA. 18S efficiency = 101.8%,  $r = 0.997$ . **Total qPCR run time = 29 min.** RFU, relative fluorescence units.

# Innovative Optical Design

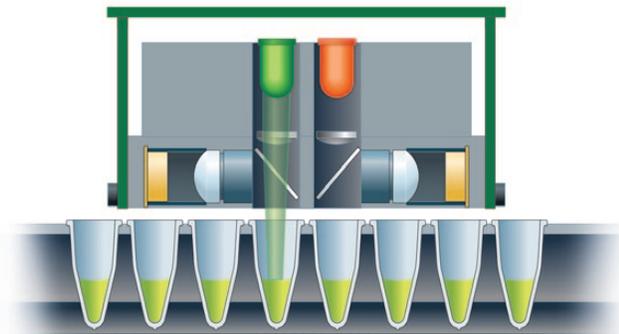
The CFX96 system's solid-state optical technology provides sensitive detection for precise quantitation and target discrimination. Scanning just above the sample plate, the CFX96 optics shuttle individually illuminates and detects fluorescence from each well with high sensitivity and no cross talk. The optical system automatically collects data from all wells during data acquisition, so you can enter or edit well information on your own schedule.

## True Five-Target Multiplexing

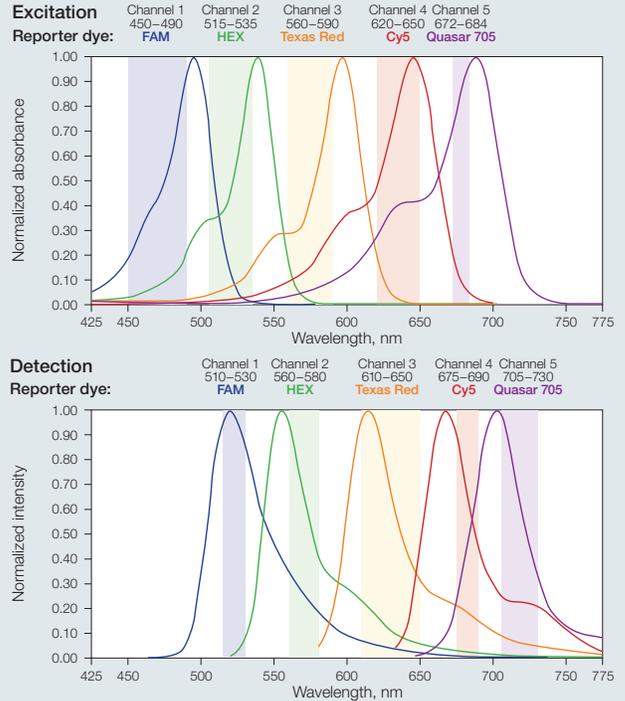
The CFX96 system can discriminate up to five targets in a single reaction well. The optical filter sets are designed to maximize fluorescence detection for specific dyes in specific channels. At every position and with every scan the CFX96 optics shuttle is reproducibly centered above each well, so the light path is always fixed and optimal and there is no need to sacrifice data collection in one of the channels to normalize to a passive reference.

## Multiple Data Acquisition Modes

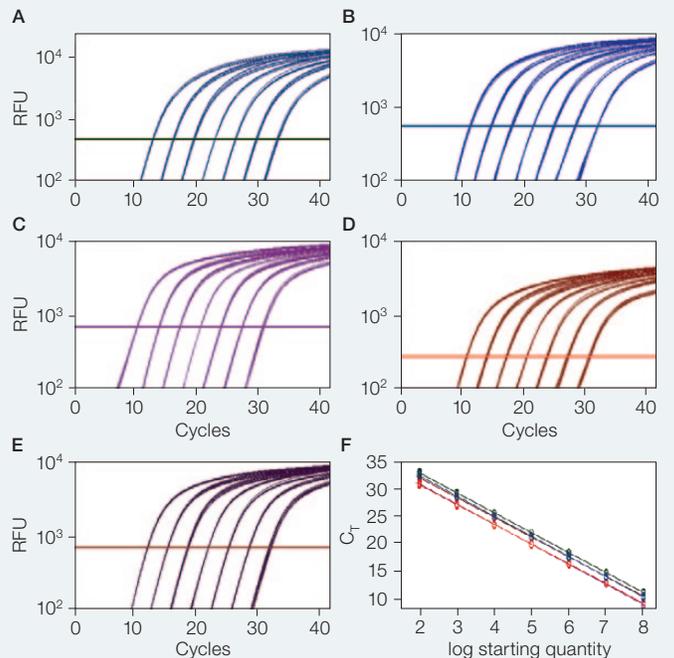
The CFX96 system can acquire data using several modes. Choose to acquire data for SYBR® Green I and single-color FAM protocols using the fast scan mode, or choose to acquire data from all channels when performing multiplex protocols. The CFX96 system's optics shuttle includes one channel with an LED-filter photodiode combination designated for single-color fluorescence resonance energy transfer (FRET) experiments, further expanding your experimental options.



As the CFX96 optics shuttle travels across the plate, light is focused directly into the center of each sample well. Side view of the optics shuttle shows the green LED firing over a well.



Excitation and detection wavelength ranges for the CFX96 system.



Linearity of five-target multiplex detection. A–E, fluorescence data from a series of 10-fold dilutions of plasmid DNA ( $10^8$ – $10^2$  copies) amplified using reporter dyes to monitor five targets: ■, FAM/actin; ■, HEX/GAPDH; ■, Texas Red/cyclophilin; ■, Cy5/tubulin; ■, Quasar 705/IL-1 $\beta$ ; F, standard curves generated from data in A–E, reaction efficiencies range from 97 to 103%.  $C_T$ , threshold cycle; RFU, relative fluorescence units.

# Powerful Software

CFX Manager software is powerful software for a powerful instrument. It accommodates user needs and different types of experiments with intuitive navigation and customizable settings.

## Getting Started

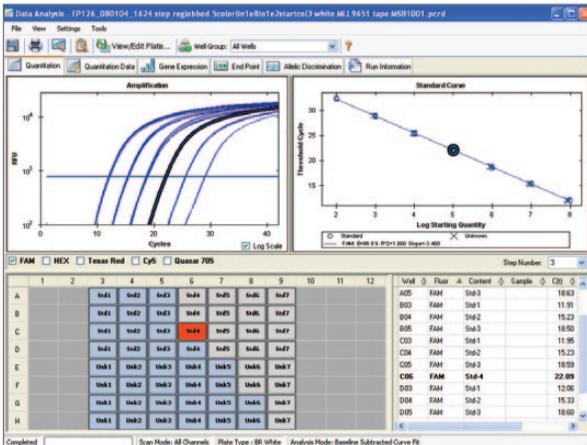
CFX Manager software's convenient wizards and intuitive navigation make it easy to get started and to get great real-time PCR results. Get an experiment started with the Quick Plate feature, then enter or edit well information on your own schedule — before, during, or after a run has finished.

## Getting Answers

CFX Manager software can send email notification with an attached data file when a run is finished, so you can analyze your results when and where you want.

The software has built-in data analysis modules and you can easily customize how your data are analyzed and displayed. A well-grouping feature is available to analyze multiple experiments from a single plate.

Perform gene expression analysis by relative quantity ( $\Delta C_T$ ) or normalized expression ( $\Delta\Delta C_T$ ) using multiple reference genes and individual reaction efficiencies. If you are performing a comprehensive study with multiple experiments, you can use CFX Manager software to combine and analyze all data at once.



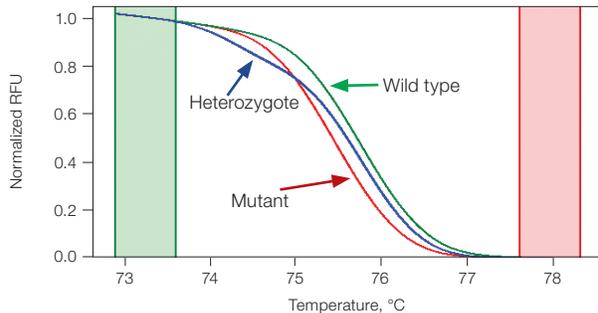
CFX Manager software data analysis module, showing well C6 highlighted.

## Precision Melt Analysis Software

Precision Melt Analysis software is a convenient, easy-to-use application that imports and analyzes data files generated from the CFX96 or CFX384 real-time PCR detection system to genotype samples based on the thermal denaturation properties of double-stranded DNA. The software can be used for a variety of genotyping applications, including scanning for new gene variants, screening DNA samples for SNPs, identifying insertions/deletions or other unknown mutations, and determining the percentage of methylated DNA in unknown samples.

Precision Melt Analysis software saves analysis time by assigning sample genotypes automatically based on cluster analysis, or manually using multiple data view options to tailor the software to the appropriate analysis. Difference curve plots of a sample fluorescence versus a selected control at each temperature transition provide a convenient visual aid to interpret the data.

Precision Melt Analysis software enables data comparison between multiple file runs by combining data into a single Melt Study. Develop a standard library of melt curve runs to analyze an unlimited number of melt experiments without having to export data.



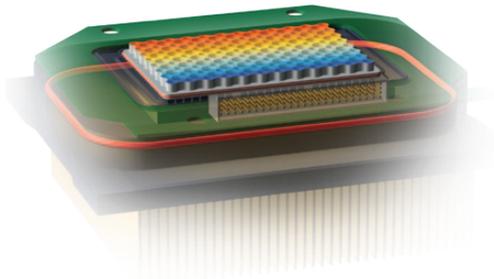
## SNP genotyping by Precision Melt Analysis software using data generated by the CFX96 real-time PCR detection system.

Discrimination of human factor V coagulation SNP genotypes (C to T substitution) using SsoFast EvaGreen supermix. Data from homozygous wild type (■), mutant (■), and heterozygote (■) samples are shown on a normalized melt curve plot. RFU, relative fluorescence units.

# Efficient Optimization

## Thermal Gradient

Determining the optimal temperature for primer annealing is crucial for efficient and specific amplification of product. With the CFX96 system's thermal gradient feature, you can determine the optimal temperature for primer annealing in a single experiment, minimizing the use of precious samples and reagents and saving valuable research time. At any step in a protocol, you can program a temperature gradient of up to 24°C across the reaction block. The thermal cycler provides exceptional temperature uniformity and reproducibility within each gradient zone, and the temperatures can easily be programmed and viewed onscreen in the software, so you can quickly identify the optimal incubation temperature.

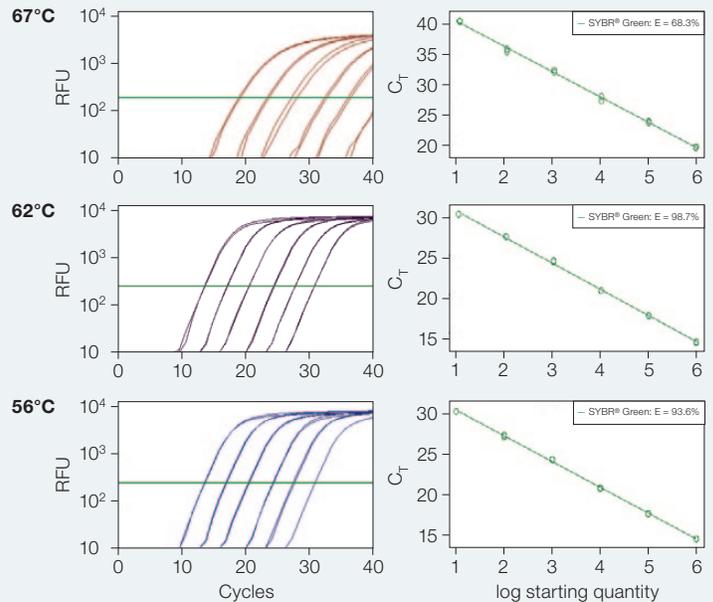


## qbase<sup>PLUS</sup> Software

qbase<sup>PLUS</sup> software is a powerful tool that imports and analyzes data generated from the CFX96 or CFX384 real-time PCR detection system. This platform-independent software package is available for major computer operating systems, such as Microsoft Windows, Macintosh, and Linux. qbase<sup>PLUS</sup> accelerates real-time PCR data analysis and provides independent validation of gene expression results using proven, qualified algorithms.

Key benefits of qbase<sup>PLUS</sup> software include:

- Reliable validation — based on proven solutions for quality control, normalization, and inter-run calibration
- Easy-to-use program — intuitive navigation with automated calculations and error propagation
- Efficient analysis — fast calculations of results with direct import of instrument data; use the integrated data organizer to streamline managing results
- Flexible programming — intuitive to use for small experiments; powerful for big experiments



### Thermal gradient experiment for optimizing annealing temperature.

A 10-fold dilution series (10<sup>6</sup> to 10 copies) of plasmid containing *GAPDH* template was amplified in the presence of SYBR<sup>®</sup> Green dye using a protocol with an annealing thermal gradient ranging from 55 to 68°C. Results are presented for three temperatures, showing 62°C as the optimal in this case, with early C<sub>T</sub> values and the highest standard curve efficiency. C<sub>T</sub>, threshold cycle; RFU, relative fluorescence units.



Data analysis window in qbase<sup>PLUS</sup> software.

## Specifications

### Thermal Cycler

Chassis	C1000
Maximum ramp rate	5°C/sec
Average ramp rate	3.3°C/sec
Heating and cooling method	Peltier
Lid	Heats up to 105°C
Temperature	
Range	0–100°C
Accuracy	±0.2°C of programmed target at 90°C
Uniformity	±0.4°C well-to-well within 10 sec of arrival at 90°C
Gradient	
Operational range	30–100°C
Programmable span	1–24°C

### Optical Detection

Excitation	6 filtered LEDs
Detection	6 filtered photodiodes
Range of excitation/emission wavelengths	450–730 nm
Sensitivity	Detects 1 copy of target sequence in human genomic DNA
Dynamic range	10 orders of magnitude
Scan time	
All channels	12 sec
FAM/SYBR® Green only	3 sec

### CFX Manager Software

Operating systems	Windows XP, Windows Vista
Memory	Minimum 1 GB
Multiplex analysis	Up to 5 targets per well
Data analysis modes	PCR quantitation with standard curve Melt curve analysis Gene expression analysis by relative quantity ( $\Delta C_T$ ) or normalized expression ( $\Delta\Delta C_T$ ) with multiple reference genes and individual reaction efficiencies Multiple file gene expression analysis for comparison of an unlimited number of $C_T$ values Allelic discrimination End-point analysis
Data export	Save, copy, and print all graphs and spreadsheets from right-click menu Export results to Microsoft Excel Copy and paste directly into Microsoft Excel, Word, or PowerPoint files Customizable reports containing run settings, data graphs, and spreadsheets can be directly printed or saved as PDFs

### System

Licensed for real-time PCR	Yes
Sample capacity	96 wells
Sample size	1–50 $\mu$ l (10–25 $\mu$ l recommended)
Communications	USB 2.0
Electrical approvals	IEC, CE
Dimensions (W x D x H)	33 x 46 x 36 cm (13 x 18 x 14")
Weight	21 kg (47 lb)

## Ordering Information

Catalog #	Description
184-1000	<b>C1000 Thermal Cycler Chassis</b> , includes USB key, power cord; does not include reaction module
184-5096	<b>CFX96 Optical Reaction Module</b> , for use with C1000 thermal cycler chassis, includes CFX Manager software, license for qbase <sup>PLUS</sup> software, communication cable, reagents, consumables
185-5096	<b>CFX96 Real-Time PCR Detection System</b> , includes C1000 thermal cycler chassis, CFX96 optical reaction module, CFX Manager software, license for qbase <sup>PLUS</sup> software, communication cable, reagents, consumables
184-5001	<b>CFX Manager Software, Security Edition</b> , includes 1 user license, installation CD, HASP HL key
184-5025	<b>Precision Melt Analysis Software</b> , includes 2 user licenses, installation CD, 2 HASP HL keys, melt calibration kit
184-5008	<b>CFX Manager Software, Chinese Edition</b> , includes 3 user licenses, installation CD, 3 HASP HL keys
184-5028	<b>CFX Manager Software, Russian Edition</b> , includes 3 user licenses, installation CD, 3 HASP HL keys
184-5072	<b>CFX Automation System</b> , includes robotic plate handler, base tray, bar code scanner, CFX automation control software CD
172-5200	<b>SsoFast EvaGreen Supermix</b> , 200 x 20 $\mu$ l reactions, 2x mix contains dNTPs, Sso7d fusion polymerase, MgCl <sub>2</sub> , EvaGreen dye, stabilizers
170-8890	<b>iScript™ cDNA Synthesis Kit</b> , 25 x 20 $\mu$ l reactions, includes 5x iScript reaction mix, iScript reverse transcriptase, nuclease-free water
172-5848	<b>iQ Multiplex Powermix</b> , 50 x 50 $\mu$ l reactions, 2x mix contains dNTPs, 11 mM MgCl <sub>2</sub> , iTaq™ DNA polymerase, stabilizers

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Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of these products includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of Licensed Probe. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Purchase of this instrument conveys a limited non-transferable immunity from suit for the purchaser's own internal research and development and for use in human in vitro diagnostics and all other applied fields under one or more of U.S. Patent Numbers 5,656,493; 5,333,675; 5,475,610 (Claims 1, 44, 158, 160–163, and 167 only); and 6,703,236 (Claims 1–7 only), or corresponding claims in their non-U.S. counterparts, owned by Applera 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.

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's United States Patent Number 6,814,934 B1 for use in research, human in vitro diagnostics, and all other fields except veterinary diagnostics.

Bio-Rad's thermal cyclers and real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.



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**Bio-Rad  
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