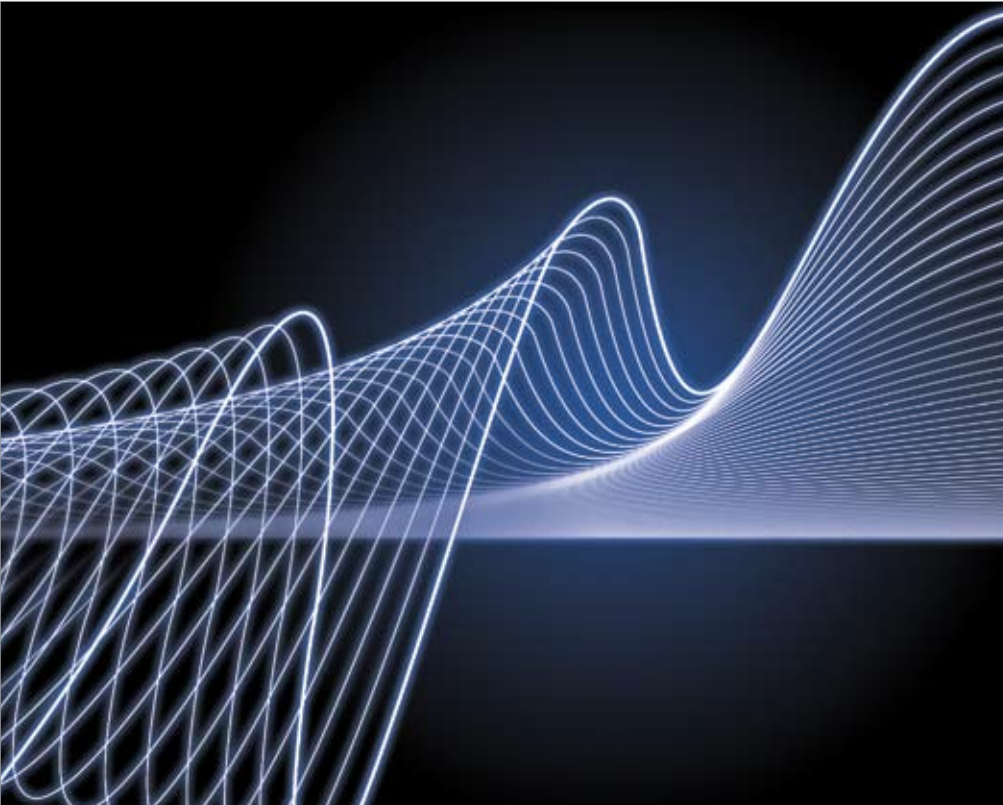


## Real-Time PCR: CFX96 System



## CFX96™ Real-Time PCR Detection System

Designed for the Way You Work



# 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** — reliable 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 e-mail notification with attached data file when the run is finished
- **Easily expand your throughput when you need to**— up to 4 instruments can be controlled by a single computer

# Genomic Research Solutions From Bio-Rad

Bio-Rad's PCR instruments, reagents, and plastics 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. iScript™ cDNA synthesis kits and iScript one-step RT-PCR kits minimize the potential for primer-dimer formation and other nonspecific PCR artifacts, delivering maximum sensitivity and consistent results every time. iQ™ multiplex powermix makes multiplex real-time PCR easier by minimizing the need to optimize buffer, enzyme, or primer concentrations.

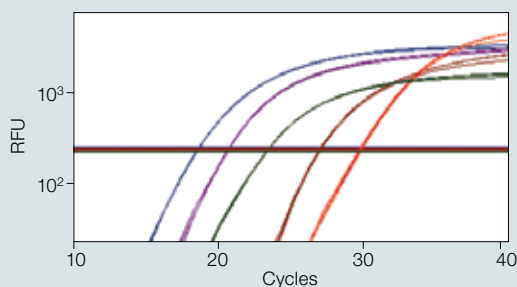
## Don't Worry About Your Consumables

Bio-Rad's full line of plastic consumables has been validated to deliver reliable, reproducible results, leaving you one less thing to worry about. Each box of Bio-Rad tubes, plates, and caps is process-sampled and tested to be negative for DNase, RNase, and DNA.

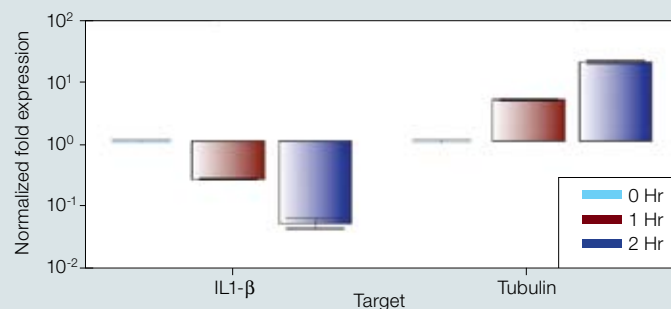


## Successful Gene Expression

Striving to make genomic technologies accessible to every researcher, Bio-Rad offers a complete line of products to ensure successful target quantitation, whether you are determining the number of microbes in a given amount of soil or measuring the fold change of an siRNA target in response to knockdown using RNAi. Identify the role of target genes in specific cellular processes or signaling pathways using a combination of genomic and proteomic analysis tools from Bio-Rad.



**Accurate five-target multiplex gene expression.** Human spleen RNA was transcribed into cDNA using the iScript cDNA synthesis kit. 50 ng cDNA was amplified in four replicate 50  $\mu$ l reactions using five reporter dyes to monitor fluorescence data from five targets. ■, FAM/actin (18.02  $\pm$  0.02); ■, Cy5/tubulin (20.07  $\pm$  0.03); ■, HEX/GAPDH (22.81  $\pm$  0.02); ■, Quasar 705/IL1- $\beta$  (26.39  $\pm$  0.04); ■, Texas Red/IL2 (29.17  $\pm$  0.07). Average C<sub>T</sub> values are shown in parentheses. RFU, relative fluorescence units.

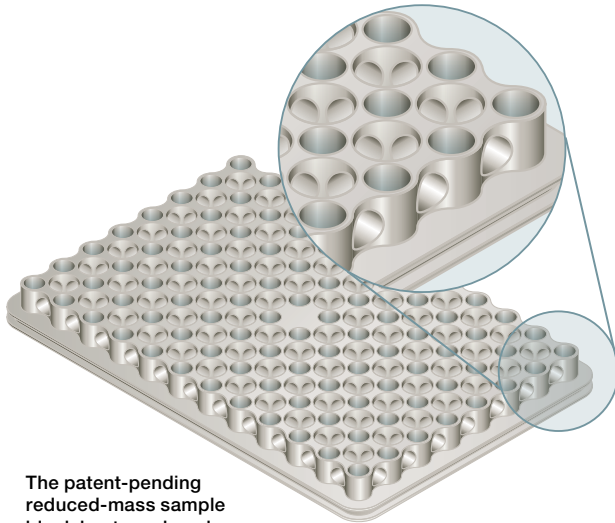


**Decrease in IL1- $\beta$  expression and increase in tubulin expression over experiment time-course.** The built-in gene expression analysis module in CFX Manager™ software lets you immediately turn your results into answers, without data export.

# Fast Thermal Cycling

## Superior Uniformity

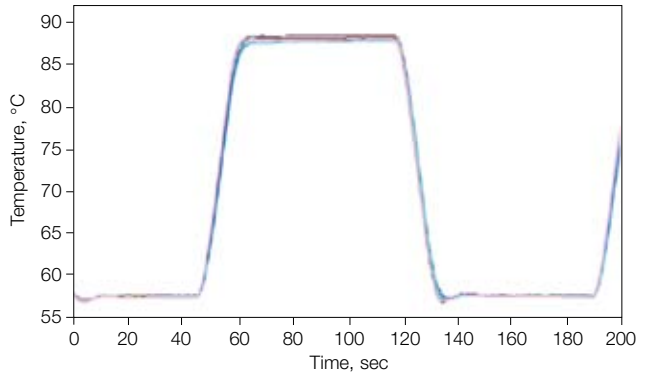
The rate and efficiency of PCR depend critically on the precision of the temperature steps. To obtain reliable, consistent results, all sample wells must maintain proper temperature throughout each incubation step. The CFX96 system uses six independently controlled thermal electric modules (TEs), the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



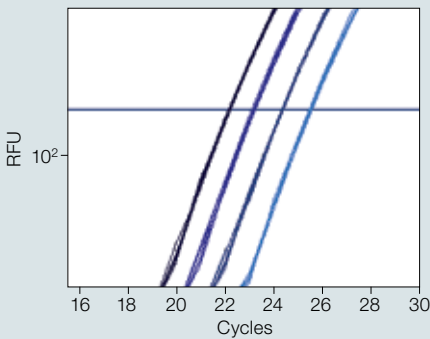
The patent-pending reduced-mass sample block heats and cools more quickly than standard blocks, so average ramp rates are increased and overall run times are reduced.

## Rapid Arrival at Target Temperature

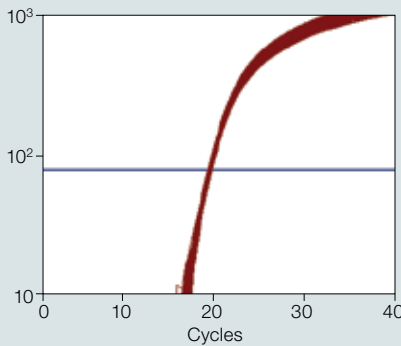
A key component of overall protocol run time is the time to reach target temperature, which is determined by the average ramp rate and the time for the sample block to reach thermal uniformity. Maximum ramp rate is less important, because it can fluctuate significantly during the ramp. The CFX96 system's 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.



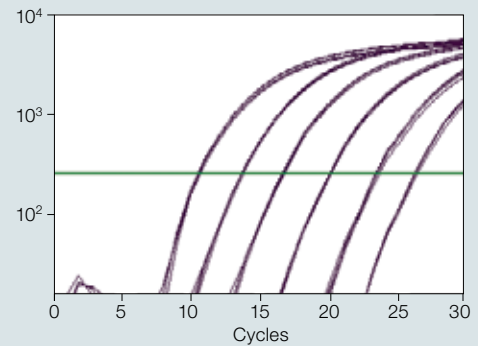
**Rapid arrival at target temperature and superior uniformity.** 1000-series thermal cyclers exhibit high average ramp rates, rapid settling time, and tight thermal uniformity throughout the ramp. This graph shows the temperature measured by probes in 15 wells across a sample block. The traces are nearly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



**Precise target discrimination.** One-cycle spacing between threshold cycle ( $C_T$ ) values is precisely maintained in a series of 2-fold dilutions of human genomic DNA from 120 to 15 ng. IL1- $\beta$  target was amplified using a FAM-labeled detection probe with iQ supermix. Graph shows 8 replicates for each dilution with the following average  $C_T$  values:  $22.15 \pm 0.02$ ,  $23.14 \pm 0.04$ ,  $24.28 \pm 0.03$ ,  $25.41 \pm 0.04$ . RFU, relative fluorescence units.



**Excellent uniformity.** IL1- $\beta$  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  $10^5$  pl reactions. Average  $C_T = 19.81 \pm 0.10$ .



**High speed performance.** Precise spacing between  $C_T$  values is maintained for a 10-fold serial dilution of tubulin plasmid target amplified using iQ™ SYBR® Green supermix. Data was acquired using the CFX96 system's fast scan mode in a two-step protocol that took less than 25 min for the amplification. Graph shows 4 replicates for each dilution.

# Innovative Optical Design

The CFX96 system's solid-state optical technology (six filtered LEDs and six filtered photodiodes) 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

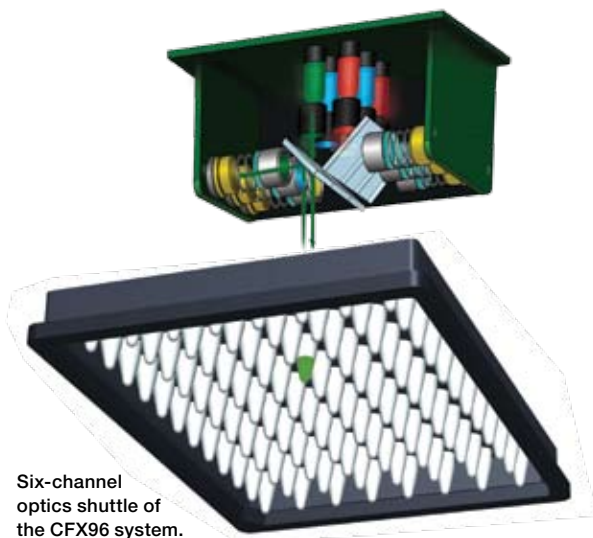
The CFX96 system can discriminate up to five targets in a single reaction well. The optical filter sets are designed to maximize fluorescence detection for specific dyes in specific channels. At every position and with every scan, the CFX96 optics shuttle is reproducibly centered above each well, so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

## Fast Scan Option

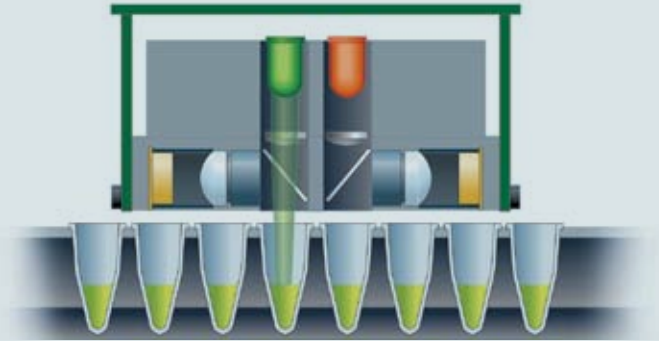
You can complete SYBR Green I and single-color FAM protocols even faster using the fast scan mode, which reads single-channel fluorescence from all 96 wells in just 3 seconds.

## Fluorescence Resonance Energy Transfer (FRET)

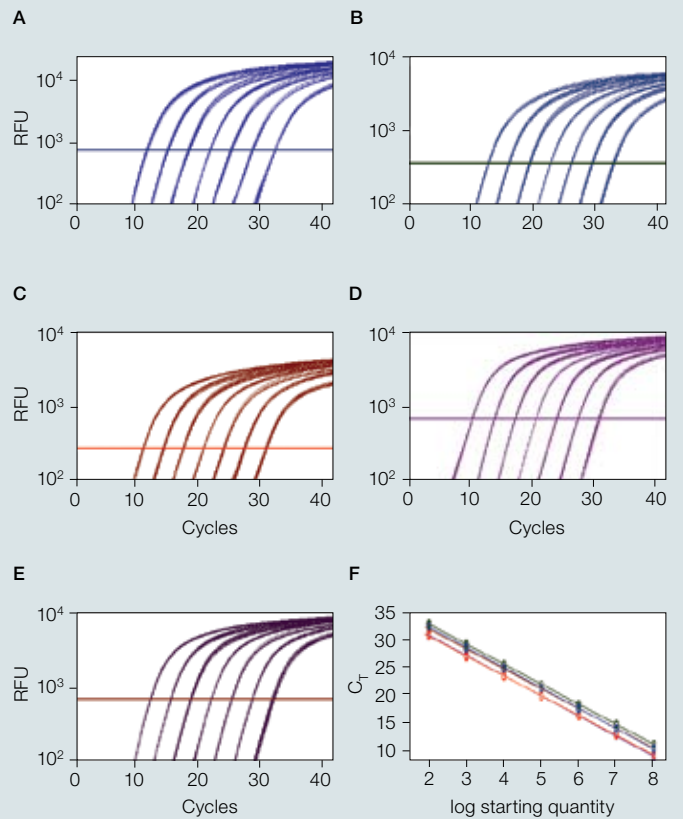
The CFX96 system's optics shuttle includes one channel with an LED-filter photodiode combination dedicated for FRET singleplex experiments, further expanding your experimental options.



Six-channel optics shuttle of the CFX96 system.



As the CFX96 optics shuttle travels across the plate, light is focused directly into the center of each sample well.



**Linearity of five-target multiplex detection.** A–E, fluorescence data from a series of 10-fold dilutions of plasmid DNA (10<sup>8</sup>–10<sup>2</sup> copies) amplified using reporter dyes to monitor five targets. ■, FAM/actin; ■, HEX/GAPDH; ■, Texas Red/cyclophilin; ■, Cy5/tubulin; ■, Quasar 705/IL1-β; F, standard curves generated from data in A–E, reaction efficiencies range from 97% to 103%. C<sub>T</sub>, 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, including unique log-in names, gene and sample name libraries, and data analysis preferences.

## Getting Started

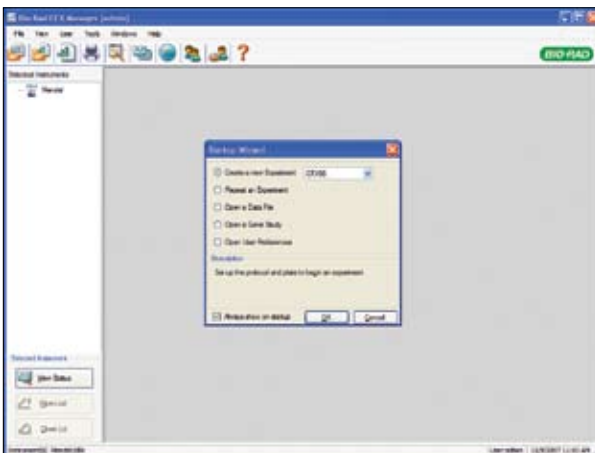
CFX Manager software's convenient wizards and intuitive navigation make it easy to get started and to get great real-time PCR results. You can use the express-loading feature to instantly access your experiment files and files that are preloaded in the software. To further speed up your process, get the experiment started with the Quick Plate feature, and then enter or edit well information on your own schedule — before, during, or after the run has finished.

## Getting Answers

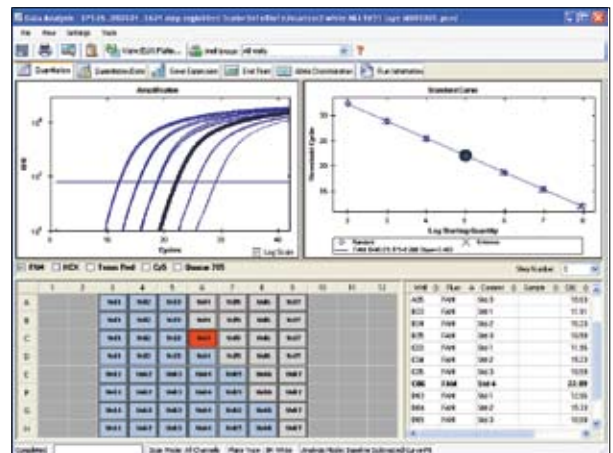
CFX Manager software can send e-mail notification with an attached data file when the run is finished, so you can analyze your results when and where you want.

The software has built-in data analysis modules with automatic baseline subtraction and threshold setting. You can easily customize how your data are analyzed and displayed, and you can use 1-step export from any graph or table to export results or images to Excel spreadsheets or PowerPoint presentations. A well-grouping feature is available to analyze multiple experiments from a single plate.

CFX Manager software lets you take your results a step further without having to export data and set up your own analysis macro. 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 main application window launched with the Startup Wizard and one attached instrument.

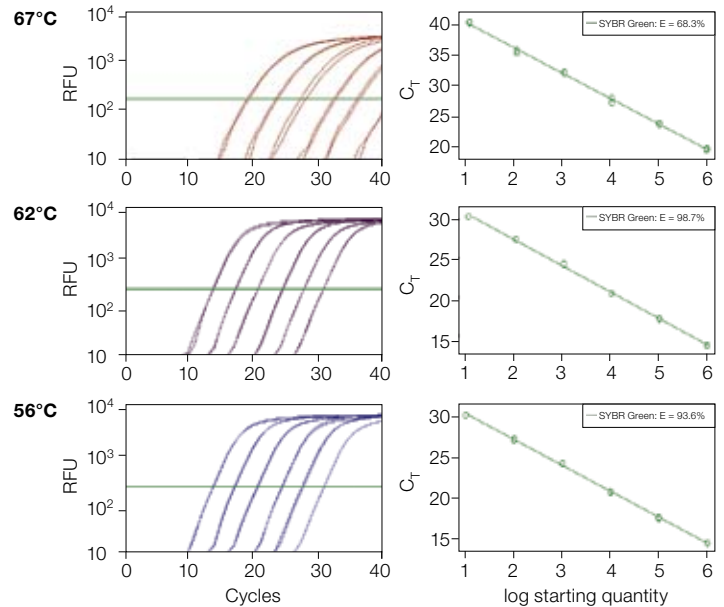
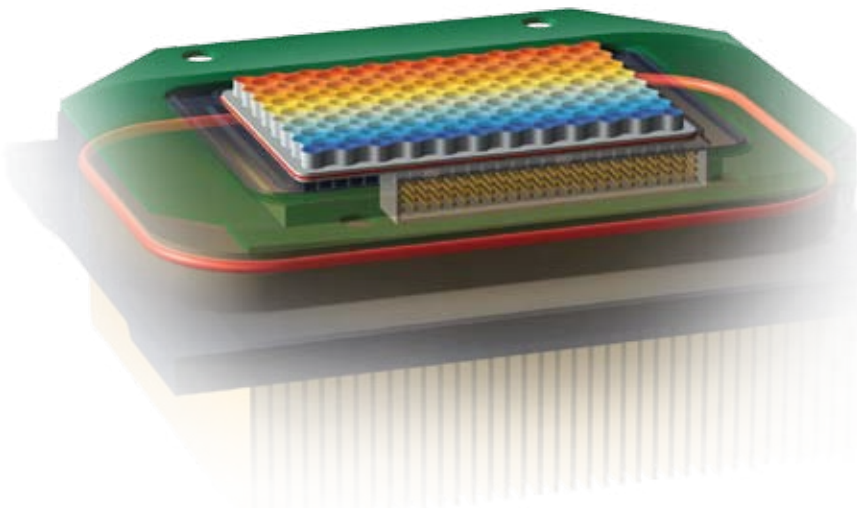


CFX Manager software data analysis module, showing well C6 highlighted.

# 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.

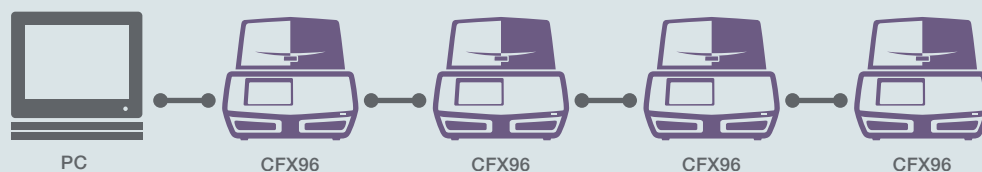


**Thermal gradient experiment for optimizing annealing temperature.** A 10-fold dilution series ( $10^9$  to 10 copies) of plasmid containing GAPDH template was amplified in the presence of SYBR Green dye using a protocol with an annealing thermal gradient ranging from 55 to 68°C. Results are presented for three temperatures, showing 62°C as the optimal in this case, with early threshold cycle ( $C_T$ ) values and the highest standard curve efficiency. RFU, relative fluorescence units.

## A System That Grows With Your Research

The flexibility of the 1000-series platform allows your setup to grow and evolve 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 real-time instruments and C1000 or S1000™ thermal cyclers. You may start a run on one instrument while another is running a different experiment, or you can start the same experiment on all four instruments at once.



## 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 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 one 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

### Software

Operating systems	Windows XP, Windows Vista
Multiplex analysis	Up to 5 targets per well
Data analysis modes	PCR quantitation with standard-curve and melt-curve analysis
	Gene expression analysis by $\Delta C_T$ or $\Delta\Delta C_T$ with multiple reference genes and individual reaction efficiencies
	Multiple file gene expression analysis
	Allelic discrimination
	Endpoint 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 (recommend 10–25 $\mu$ l)
Communications	USB 2.0
Electrical approvals	IEC, CE
Dimensions (W x D x H)	13 x 18 x 14" (33 x 46 x 36 cm)
Weight	21 kg (47 lb)

## Ordering Information

Catalog #	Description
184-1000	<b>C1000 Thermal Cycler Chassis</b> , includes USB flash drive, power cord, instructions; does not include reaction module
184-5096	<b>CFX96 Optical Reaction Module</b> , includes CFX96 optics shuttle, CFX Manager software, communication cable, reagent and consumable samples, instructions
185-5096	<b>CFX96 Real-Time PCR Detection System</b> , includes C1000 thermal cycler chassis, CFX96 optical reaction module, CFX Manager software, communication cable, power cord, reagent and consumable samples, instructions
184-5000	<b>CFX Manager Software</b> includes installation CD, instructions
184-5001	<b>CFX Manager Software, Security Edition</b> , includes 1 user license, installation CD, HASP HL key, instructions
184-5010	<b>CFX Manager Software, Security Edition</b> , includes 10 user licenses, 10 installation CDs, 10 HASP HL keys, 10 instruction manuals
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
170-8892	<b>iScript™ One-Step RT-PCR Kit With SYBR® Green</b> , 50 x 50 $\mu$ l reactions, includes iScript reverse transcriptase for one-step RT-PCR, 2x SYBR Green RT-PCR reaction mix, nuclease-free water
170-8894	<b>iScript One-Step RT-PCR Kit for Probes</b> , 50 x 50 $\mu$ l reactions, includes iScript reverse transcriptase for one-step RT-PCR, 2x probes RT-PCR reaction mix, 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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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 applied fields other than Human In Vitro Diagnostics under one or more of U.S. Patents Nos. 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 No. 6,814,934 B1 for use in research and for all other fields except the fields of human diagnostics and veterinary diagnostics.

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