CFX Connect™ Real-Time PCR
Detection System

BIO-RAD

CFX CONNECT REAL-TIME PCR DETECTION SYSTEM

ADVANCING qPCR TOGETHER

The CFX Connect Real-Time PCR Detection System offers two-target analysis, excellent thermal cycler specifications, and the same reliable performance as the CFX96 Touch™ System. The system incorporates innovative optical technologies with powerful software to provide maximal reliability and efficiency for all your real-time PCR needs.

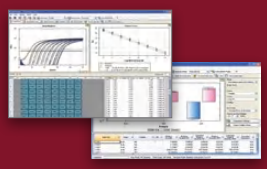
Sample Preparation → Reverse Transcription and Real-Time PCR Reagents → Real-Time Data Collection → Data Analysis and Optimization

- SingleShot™ Cell Lysis RT-qPCR Kits
- Aurum™ Total RNA Mini Kit
- Aurum Total RNA 96 Kit
- Aurum Total RNA Fatty and Fibrous Tissue Kit

- iScript™ Reverse Transcription Kits
- SsoAdvanced™ Universal Supermixes
- iTaq™ Universal Supermixes
- iTaq Universal One-Step Kits
- PrimePCR™ Assays and Panels

- CFX384 Touch™ Real-Time PCR Detection System
- CFX96 Touch Real-Time PCR Detection System
- CFX96 Touch Deep Well Real-Time PCR Detection System
- CFX Connect Real-Time PCR Detection System

- CFX Maestro™ Software
- Precision Melt Analysis™ Software
- qbase+ Software



Visit **bio-rad.com/amplification1** for more information.

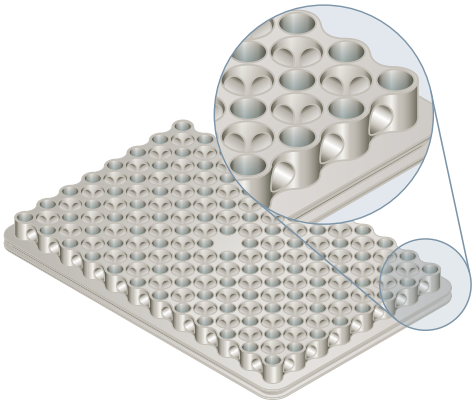
Have Confidence in Your Entire Genomics Workflow

Bio-Rad offers a complete suite of research tools for your experiments that utilize real-time PCR detection. Generating accurate, reproducible results is reliant on each preceding step in the workflow as documented in the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines (Bustin et al. 2009). Appropriate selection of methods and analyses results in robust, repeatable data and conclusions. Bio-Rad's suite of genomics research tools can help you achieve this goal.

UNIFORM THERMAL CYCLING

Superior Uniformity

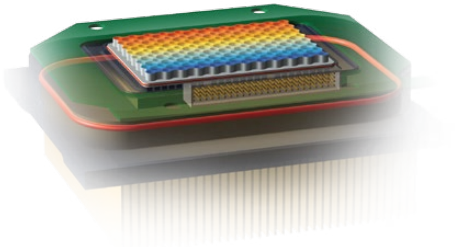
The 96-well block of the CFX Connect System offers excellent thermal performance and uniformity across the entire block. Precision of the temperature steps is critical for the rate and efficiency of PCR. To obtain reliable, consistent results, all sample wells must maintain proper temperature throughout each incubation step. The CFX Connect System achieves precision by using six independently controlled thermal electric modules, the heating and cooling elements of the thermal cycler, to maintain tight temperature uniformity at all points during a run — even while ramping. A high average ramp rate allows the system to rapidly reach its target temperature, thus shortening run time with an unsurpassed 10 sec settling time.



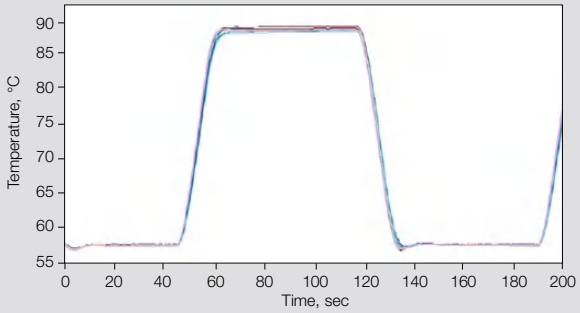
The patented* reduced-mass sample block heats and cools more quickly than standard blocks, so average ramp rates are increased and overall run times are reduced.

Efficient Optimization

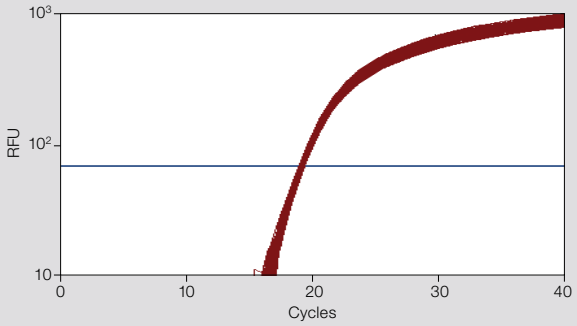
Determining the optimal temperature for primer annealing is crucial for efficient and specific target amplification. The thermal gradient feature of the CFX Connect System quickly assists with optimizing your assay in a single experiment, minimizing the use of precious samples and reagents and saving valuable research time. At any step in a protocol, you can program a temperature gradient of up to 24°C across the reaction block with exceptional temperature uniformity and reproducibility within each gradient zone.



* U.S. patent 7,632,464.

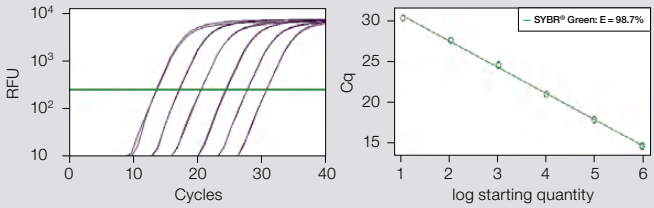


Rapid arrival at target temperature and superior uniformity for reproducible results. The CFX Connect System exhibits high average ramp rates, rapid settling time, and tight thermal uniformity throughout the ramp. This graph shows the temperature measured by probes in 15 wells across a sample block. The traces are nearly indistinguishable due to the tight uniformity. Note the consistent high average ramp rate throughout heating and cooling.

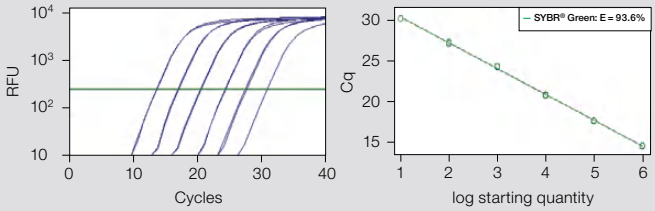


Excellent uniformity. *IL-1β* plasmid template diluted to 10^5 copies/reaction amplified in the presence of a FAM-labeled detection probe with iQ Supermix. Graph shows 96 replicates of 10 µl reactions. Average quantification cycle (Cq) = 19.81 ± 0.10. RFU, relative fluorescence units.

62°C



56°C



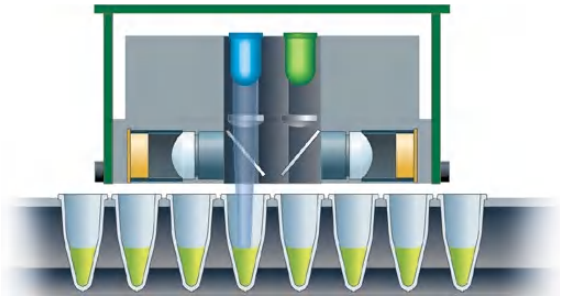
Thermal gradient experiment for optimizing annealing temperature. A tenfold dilution series (10^6 to 10 copies) of plasmid containing *GAPDH* template was amplified in the presence of SYBR® Green using a protocol with an annealing thermal gradient ranging from 55 to 68°C. Results are presented for two temperatures, showing 62°C as the optimal in this case. Cq, quantification cycle; RFU, relative fluorescence units.

PRECISE DETECTION

The solid-state optical technology of the CFX Connect System enables precise excitation and detection of fluorophores. Scanning just above the sample plate, the optics shuttle, containing light-emitting diodes (LEDs) and photodiodes, 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.

Multiple Data Acquisition Modes

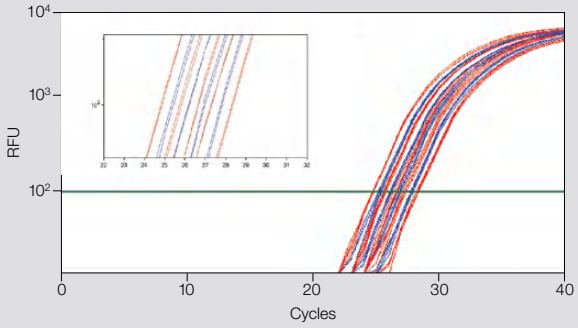
The CFX Connect System can acquire data using several modes. Fast scan mode acquires data for SYBR® Green I, EvaGreen®, and single-color FAM protocols while all channel mode acquires data for duplex experiments. The CFX Connect System includes one channel with an LED-filter photodiode combination designated for single-color fluorescence resonance energy transfer (FRET) experiments, further expanding your experimental options.



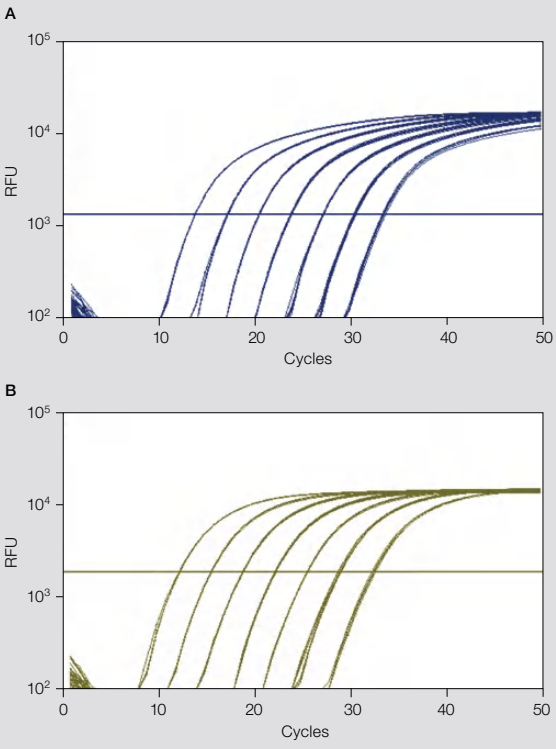
As the optics shuttle of the CFX Connect System travels across the plate, light is focused directly into the center of each sample well. Side view of the optics shuttle shows the blue LED firing over a well.

Accurate Two-Target Multiplexing

The optical design of the CFX Connect System provides flexibility in fluorophore selection. The optical filter sets are designed to maximize fluorescence detection for specific dyes in specific channels. At every position and with every scan, the 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.



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–511 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^2 = 0.996. Inset is a magnified view showing robust discrimination and reproducible amplification. RFU, relative fluorescence units.



Excellent linearity of duplex detection. A–B, fluorescence data from a series of tenfold dilutions of plasmid DNA (10^8–10^2 copies) amplified using reporter dyes to monitor two targets: ■, FAM/cyclophilin; ■, VIC/*IL-1β*. RFU, relative fluorescence units.

POWERFUL SOFTWARE

CFX Maestro Software

CFX Maestro Software for CFX Real-Time PCR Instruments is easy-to-use, yet flexible and powerful software for data collection, data analysis, and graphing of real-time PCR data.

With CFX Maestro Software you can:

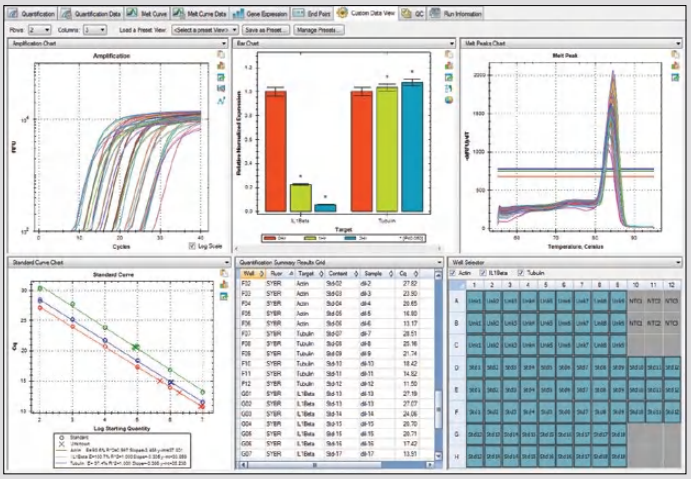
- **Perform automatic statistical analysis in seconds** — with just a few mouse clicks you can perform t-tests or analyze your data with one-way ANOVA
- **Extract more meaningful information from your run** — analyze data using bar chart, box and whisker plot, dot plot, clustergram, scatter plot, or volcano plot
- **Create and export publication-ready graphics** — annotate graphs with P values, text, and arrows to call out specific data. Change colors, fonts, and legends. Export graphs at any size or resolution for presentations, posters, or for publication
- **Easily integrate PrimePCR™ Assays** — use PrimePCR Primers and Plates to save time on primer design with predesigned and validated primers. Post run, use the PrimePCR controls analysis tool to ensure run quality from integrated controls
- **Work anywhere, on a PC or Mac** — with both PC and Mac versions of CFX Maestro, you can analyze your data on your own computer, anytime, without the need for an internet connection (Mac version is for data analysis only and does not provide instrument control.)
- **Perform further data analysis using: qbase+ Software** — CFX Maestro Software comes with a premium license for qbase+ Software to further enhance your data analysis capabilities

Precision Melt Analysis Software

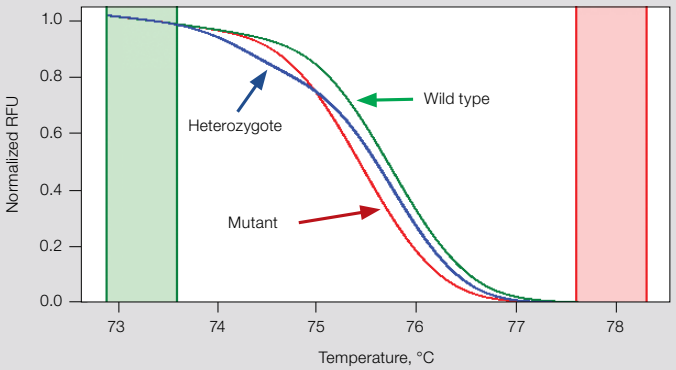
Precision Melt Analysis Software imports and analyzes data files generated by the CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, or CFX384 Touch Real-Time PCR Detection System to genotype samples based on their DNA thermal denaturation properties. The software can be used for a variety of applications, including scanning for new gene variants, screening DNA samples for single nucleotide polymorphisms (SNPs), identifying insertions/deletions or other unknown mutations, and determining the percentage of methylated DNA in unknown samples.

qbase+ Software

qbase+ Software is a powerful tool that imports and analyzes data generated by the CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, or CFX384 Touch System. This platform-independent software package is available for major computer operating systems such as Microsoft Windows, Macintosh, and Linux.



Custom data view. With custom data view, your most relevant data can be viewed and analyzed in one screen.



Quickly and accurately genotype samples using Precision Melt Analysis Software. 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.

Key features of qbase+ Software:

- **Reliable validation** — based on proven solutions for quality control, normalization, and inter-run calibration
- **Efficient data analysis** — import and consolidate information from multiple runs and multiple instruments to quickly analyze your complete data set, and use a guided statistical wizard to determine significance
- **Streamlined publication submission** — export an RDML file containing annotations, such as sample and assay information, to conform to the MIQE guidelines

A COMPLETE SYSTEM

Bio-Rad offers optimized reagents and plastic consumables for all your quantitative PCR (qPCR) experiments. Obtain high-quality, contaminant-free RNA rapidly and efficiently with Aurum Total RNA Kits. Choose from a broad mix of reverse transcription qPCR (RT-qPCR) kits, supermixes, and plastic consumables to produce maximum sensitivity and consistent results every time.

RNA Isolation and Cell Lysis Kits

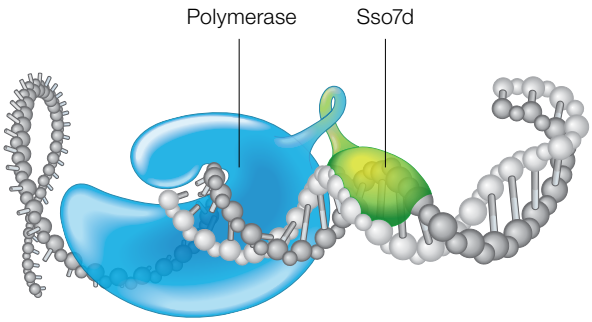
- Aurum Total RNA Kits are designed and formulated to assist in the isolation of highly pure and intact RNA from various starting materials
- SingleShot Cell Lysis RT-qPCR Kits provide a complete and fast solution for generation of lysates from cell cultures
  - Lysates are optimized for downstream one- or two-step qPCR reactions and do not require an RNA purification step
  - Kits are available in SYBR® Green or probe chemistries

Reverse Transcription Reagents

- Fast and efficient cDNA synthesis from 7.5 µg to 100 fg of total RNA
- High sensitivity enables single-copy detection utilizing an RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase and advanced formulation
- Unbiased 3' to 5' cDNA synthesis using an optimal blend of oligo(dT) and random primers
- Potent RNase A inhibitors protect RNA during setup and reverse transcription

Real-Time qPCR Reagents

- Patented* Sso7d fusion enzyme delivers superior data from GC- and AT-rich targets, challenging samples with known inhibitors, and target regions with high secondary structure
- Antibody-mediated hot-start polymerases enable instant activation and higher specificity
- Universal passive reference dyes enable use on all real-time PCR systems
- Broad range of thermal cycling conditions
- Flexible one-step and two-step RT-qPCR reagents



Bio-Rad's SsoAdvanced Universal Supermixes utilize patented* Sso7d fusion protein technology to provide enhanced processivity, speed, and tolerance to PCR inhibitors. SsoAdvanced™ Universal SYBR® Green Supermix delivers enhanced fluorescence compared to SYBR® Green alone. SsoAdvanced Universal Probes Supermix enables robust detection of two different gene targets under standard or fast cycling conditions.

PCR Plastic Consumables

- Precisely manufactured for optimal fit and cycling performance
- Produced in Class 10,000 or 100,000 cleanroom environment
- Certified to be free of DNase, RNase, and human genomic DNA
- Extremely uniform wells reduce well-to-well variability in real-time PCR
- Warp-free Hard-Shell® Plates are designed for optimum performance with automation

* U.S. patents 6,627,424; 7,541,170; and 7,560,260.

Bio-Rad's broad selection of vessels and sealers for PCR and real-time PCR.

PrimePCR Assays and Panels

- Expertly designed and wet-lab validated for proven performance; each assay for the human, mouse, and rat genomes was experimentally tested for optimal efficiency, specificity, sensitivity, and linear dynamic range
- Available as individual assays, predesigned pathway and disease panels, and custom plates
- Wide selection of reference gene and control assays to assess the key experimental factors that may impact your real-time PCR results
- Integrated with CFX Maestro Software for a fast, streamlined approach, from data generation to data analysis

Specifications

Thermal Cycler	
Chassis	CFX Connect
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	3 filtered LEDs
Detection	3 filtered photodiodes
Range of excitation/emission wavelengths	450–580 nm
Sensitivity	Detects 1 copy of target sequence in human genomic DNA
Dynamic range	10 orders of magnitude
Scan time	
All channels	12 sec
Single channel fast scan	3 sec
CFX Maestro Software	
Operating systems	Windows 7, Windows 8, Windows 10, Mac OS X El Capitan, Mac OS Sierra
Memory	Minimum 1 GB
Data analysis modes	PCR quantification with standard curve
	Melt curve analysis
	Gene expression analysis by relative quantity (ΔCq) or normalized expression (ΔΔCq) with multiple reference genes and individual reaction efficiencies
	Data analysis options include bar chart, box and whisker plot, dot plot, clustergram, scatter plot, volcano plot
	Statistical analysis with *t*-tests and one-way ANOVA
	Multiple file gene expression analysis for comparison of an unlimited number of Cq values for multi-plate studies
	Allelic discrimination
	End-point analysis
Image export	Image size: any
	Resolution: 72–600 dpi
	Image format: jpg, png, bmp
Data export	Export specified data in multiple formats
	Copy and paste into Microsoft Word, Excel, or PowerPoint file
	Customizable reports containing run settings, data graphs, and spreadsheets can be printed directly or saved as PDFs

Ordering Information

Catalog #	Description
1855200	**CFX Connect Real-Time PCR Detection System with Starter Package**, includes CFX Connect Thermal Cycler Chassis, CFX Connect Optical Reaction Module, CFX Maestro Software, license for qbase+ Software, communication cable, reagents, consumables
1855201	**CFX Connect Real-Time PCR Detection System**, includes CFX Connect Thermal Cycler Chassis, CFX Connect Optical Reaction Module, communication cable
12004110	**CFX Maestro Software**
12004128	**CFX Maestro Software for Mac**
12005258	**CFX Maestro Software, Security Edition**, includes 1 user license, installation CD, HASP HL key
1845025	**Precision Melt Analysis Software**, includes 2 user licenses, installation CD, 2 HASP HL keys, melt calibration kit
1845075	**CFX Automation System II**, includes plate handler and barcode scanner, mounting plate, automation software
1814000	**PX1™ PCR Plate Sealer**, includes heat sealing instrument
1814030	**Optically Clear Heat Seal**, for use with PX1 PCR Plate Sealer, 100
MSB1001	**Microseal® 'B' Adhesive Seals**, optically clear, 100
HSP9655	**Hard-Shell Low-Profile 96-Well Skirted PCR Plates**, white well, white shell, 50
HSP9955	**Hard-Shell Low-Profile 96-Well Skirted PCR Plates**, white well, white shell, barcoded, 50
1708840	**iScript™ Reverse Transcription Supermix for RT-qPCR**, 25 x 20 µl reactions, includes 100 µl 5x iScript RT Supermix, iScript RT Supermix No-RT Control
1725037	**iScript Advanced cDNA Synthesis Kit for RT-qPCR**, 25 x 20 µl reactions, includes 100 µl 5x iScript Advanced Reaction Mix, 25 µl iScript Advanced Reverse Transcriptase
1725270	**SsoAdvanced™ Universal SYBR® Green Supermix**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions, 2x qPCR mix, contains Sso7d fusion polymerase, ROX Normalization Dyes
1725280	**SsoAdvanced Universal Probes Supermix**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions, 2x qPCR mix, contains Sso7d fusion polymerase, ROX Normalization Dyes
1725160	**SsoAdvanced PreAmp Supermix**, 1.25 ml (1 x 1.25 ml vial), 50 x 50 µl reactions, 2x PreAmp Mix, contains dNTPs, Sso7d fusion polymerase, salts, enhancers, stabilizers, other proprietary components
1725095	**SingleShot™ SYBR® Green One-Step Kit**, 100 x 50 µl reactions

Bustin SA et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611–622.