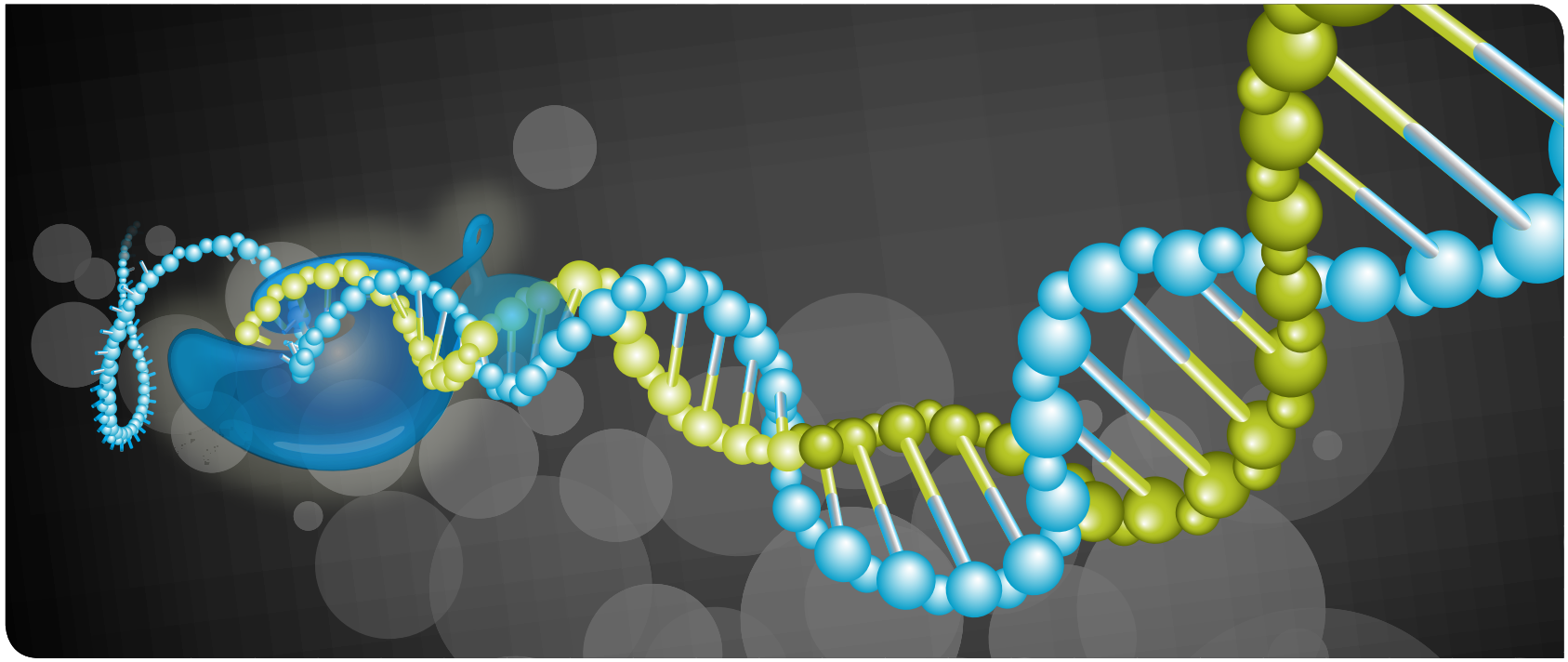


PrimePCR™ Assays and Panels

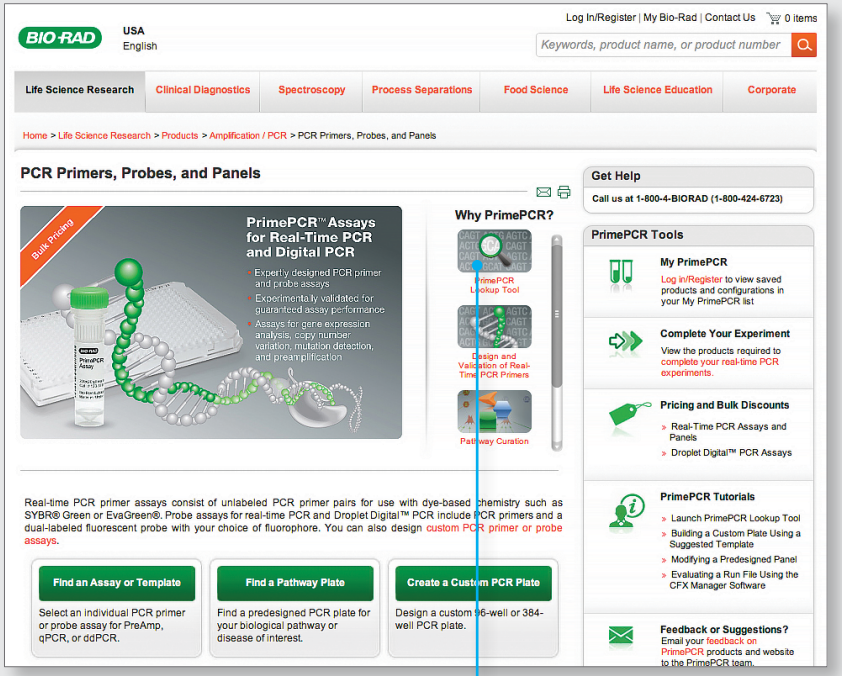
BIO-RAD

PrimePCR™ Assays and Panels

We focused on the details so you can focus on what really matters — your results.

- Expertly designed PCR primer and probe assays for quantitative PCR (qPCR) and Droplet Digital™ PCR (ddPCR™)
- Experimentally validated for guaranteed performance
- Assays for gene expression analysis, copy number variation, and mutation detection

Interactive PDF. Drag cursor over text to see symbol.



Click on this icon to use the new PrimePCR Lookup Tool to find assays and panels for your genes of interest.

bio-rad.com/PrimePCRLookup

Visit **bio-rad.com/PrimePCR** for assay and ordering information.

Table of Contents

PrimePCR™ Real-Time PCR Products

Predesigned SYBR® Green Assays
Transcriptome-wide human and mouse primer assays for SYBR® Green gene expression analysis are available in 200, 1,000, or 2,500 reaction sizes.

DNA Templates
Gene-specific synthetic DNA templates are designed to give a positive real-time PCR result when used with the corresponding gene assay.

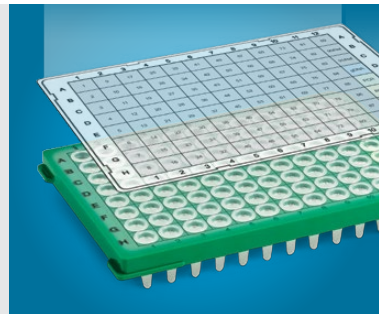
Predesigned Probe Assays
Transcriptome-wide probe assays for gene expression analysis are available in 500, 1,000, or 2,500 reaction sizes.

Experimental Controls
Control assays are available for reverse transcription, RNA quality, genomic DNA (gDNA) contamination, and PCR performance.

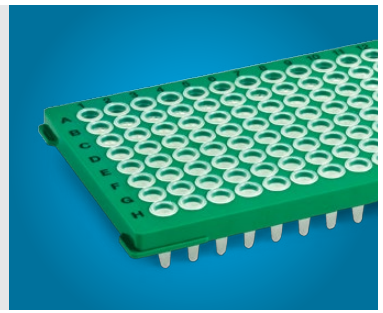
PreAmp Assays
Concentrated primers are available for target-specific preamplification of up to 100 SYBR® Green or probe assays.

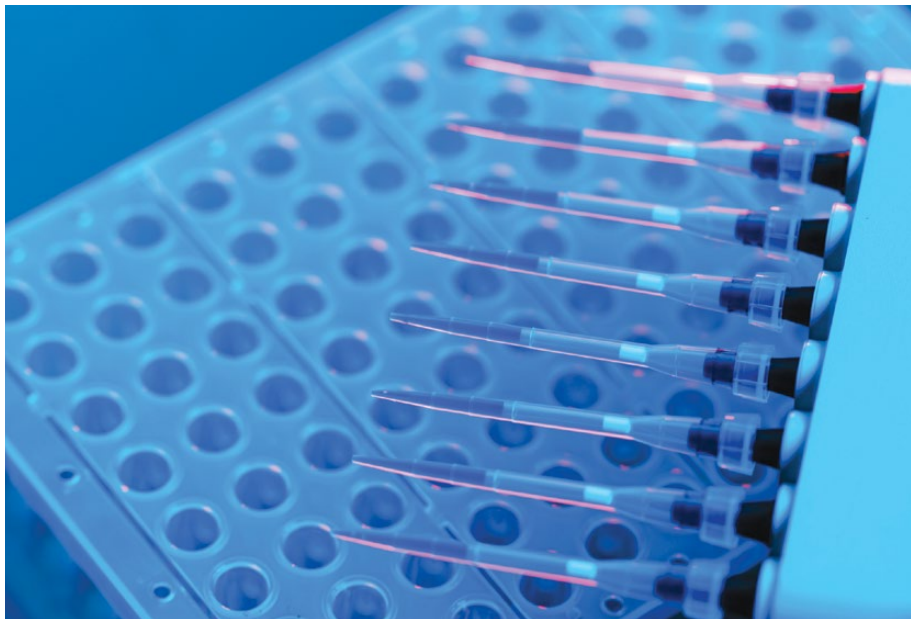
Custom Assays
User-defined primer and probe sequences can be ordered.

Predesigned Pathway Panels
A large selection of predesigned disease- and pathway-specific panels are available.

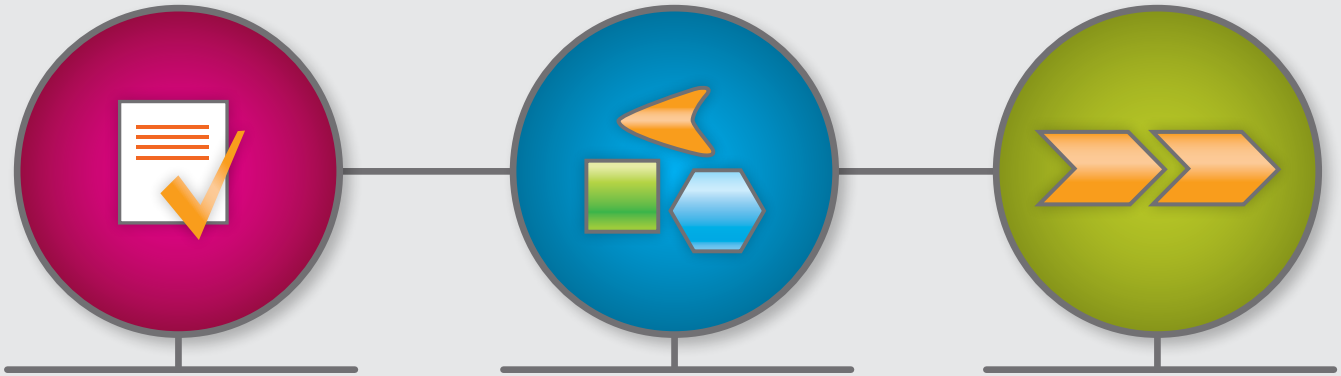


Custom PCR Plates
Custom-configured 96- and 384-well PCR plates can be ordered with SYBR® Green assays.





Why PrimePCR?



Wet-Lab Validation of Every Primer Pair

- Offers guaranteed performance
- Eliminates time-consuming optimization
- Aids in minimum information for publication of quantitative real-time PCR experiments (MIQE) compliance

Wide Range of Predesigned Disease- and Pathway-Specific Panels

- Expertly curated to include the most biologically relevant gene targets
- Customizable to include different gene targets of interest
- Integrated with CFX Manager™ Software

Complete Solution for Real-Time PCR

- Aurum™ Total RNA Kits
- iScript™ cDNA Synthesis Kits
- Supermixes for qPCR
- PCR plates and tubes
- Real-time PCR instruments
- CFX Manager Software

Assay Design

- Assay specificity confirmed by next-generation sequencing
- Avoided common single nucleotide polymorphisms in target regions
- Designed intron-spanning assays whenever possible
- Avoided secondary structures in primer annealing sites
- Maximized fraction of transcript isoforms being detected
- Compatible with standard assay conditions

Experimental Controls and Reference Gene Assays

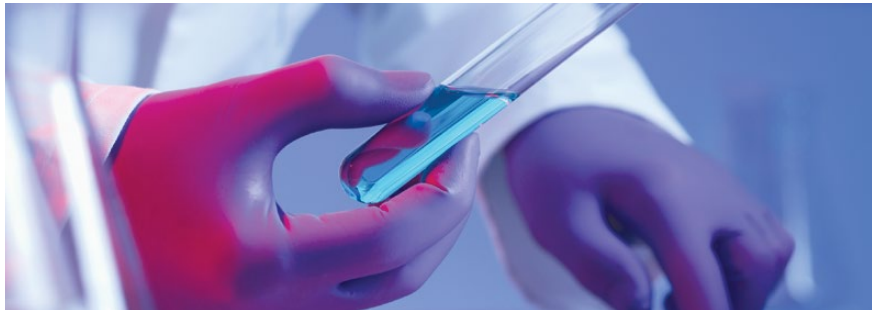
Controls

Experimental control assays and synthetic DNA templates are designed to assess the key experimental factors that may impact your real-time PCR results.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	1	9	17	25	33	41	49	57	65	73	81	89	A
B	2	10	18	26	34	42	50	58	66	74	82	90/Ref	B
C	3	11	19	27	35	43	51	59	67	75	83	91/Ref	C
D	4	12	20	28	36	44	52	60	68	76	84	gDNA	D
E	5	13	21	29	37	45	53	61	69	77	85	PCR	E
F	6	14	22	30	38	46	54	62	70	78	86	RQ 1	F
G	7	15	23	31	39	47	55	63	71	79	87	RQ 2	G
H	8	16	24	32	40	48	56	64	72	80	88	RT	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Reference Gene Assays

We have suggested a set of commonly used reference genes that can be used individually, or easily screened using our preplated 96-well and 384-well reference gene panels. Reference gene assays may also be added to custom-designed plates.



gDNA

DNA Contamination Control Assay

The PrimePCR DNA Contamination Control Assay is designed to determine if gDNA is present in a sample at a level that may affect PCR results.

PCR

Positive PCR Control Assay

The PrimePCR Positive Control Assay is designed to assess how a given experimental sample may adversely affect PCR performance.

RQ 1

RQ 2

RNA Quality Assay

The PrimePCR RNA Quality Assay is designed to determine whether RNA integrity may adversely affect PCR results.

RT

Reverse Transcription Control Assay

The PrimePCR Reverse Transcription Control Assay is designed to qualitatively assess the performance of the RT reaction.

Assay Performance Standards

Sensitivity	Accurate detection of 20 copies
Specificity	Validated amplicon sequence with next-generation sequencing; minimal primer-dimer formation and gDNA cross-reactivity
Amplification efficiency	90–110%
Linear dynamic range	Minimum of 6 orders of magnitude; detection of a synthetic template standard curve from 20 to 20,000,000 copies
R^2	>0.98

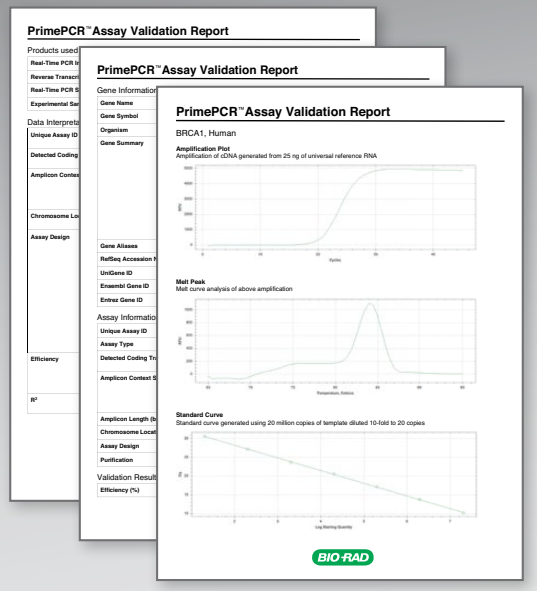
Assay Validation

Partnered with Experts

- We have collaborated with Biogazelle, leaders in real-time PCR, to design and experimentally validate gene expression assays for all human and mouse protein-coding genes
- Assays provide confidence in results while eliminating time-consuming design and optimization steps
- MIQE compliance made easy — validation information for each assay is available at **bio-rad.com/PrimePCR**

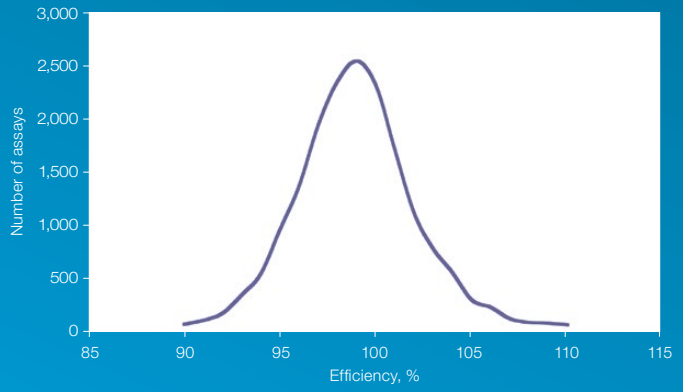
biogazelle

All primers were validated using iScript™ Advanced cDNA Synthesis Kit for RT-qPCR and SsoAdvanced™ SYBR® Green Supermix on an automated CFX384 Touch™ Real-Time PCR Detection System.



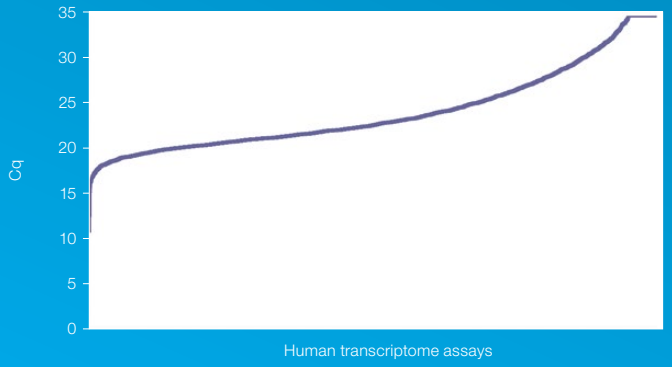
Assay Performance and Compatibility

Superior Assay Performance



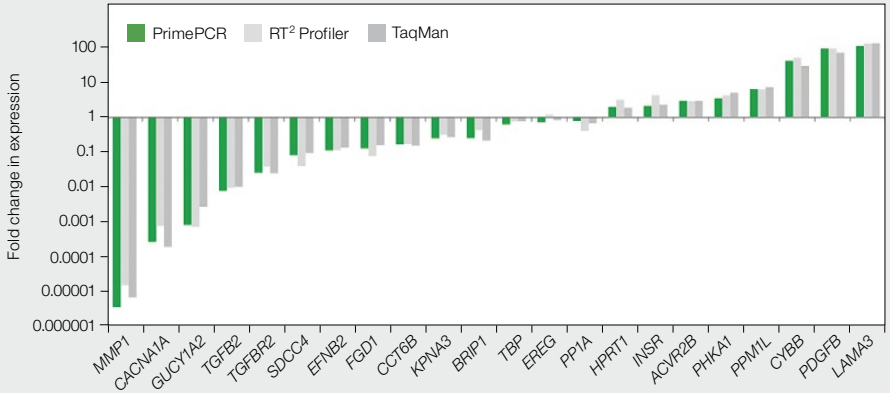
Distribution of PrimePCR Assay efficiencies. A large majority of assays fall between 95 and 105%.

Broad Dynamic Range



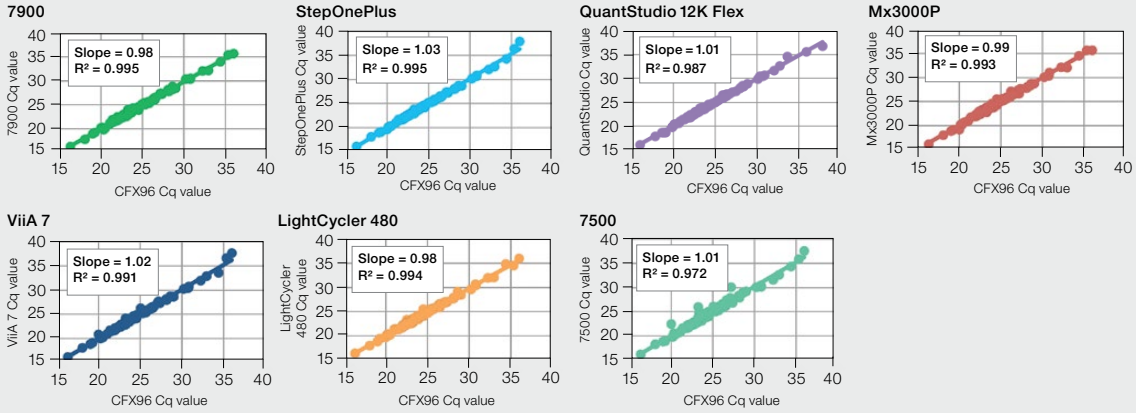
Dynamic range of PrimePCR Assays. Quantification of human reference total RNA revealed a wide range of expression levels across all genes. The lowest quantification cycle (Cq) value, observed for *RPS23* mRNA, equals 13. This results in a Cq range of 22 cycles (assuming a single transcript copy has a Cq of 35), or an observed expression range of at least 4,000,000-fold.

Comparison of Gene Expression Data



PrimePCR Assays yield data comparable to other leading gene expression assays. Relative quantification analysis for 22 genes was performed on two samples using the entire respective reverse transcription quantitative PCR (RT-qPCR) workflows for PrimePCR, RT² Profiler (QIAGEN), and TaqMan (Life Technologies Corporation) assays. Data collection for PrimePCR and RT² Profiler was performed on a CFX96™ Real-Time PCR Detection System. Data collection for the TaqMan assays was performed on an Applied Biosystems 7900HT fast real-time PCR system using a 96-well fast block. All of the manufacturer's recommendations were followed and 25 ng of cDNA was used in each qPCR reaction.

Instrument Compatibility



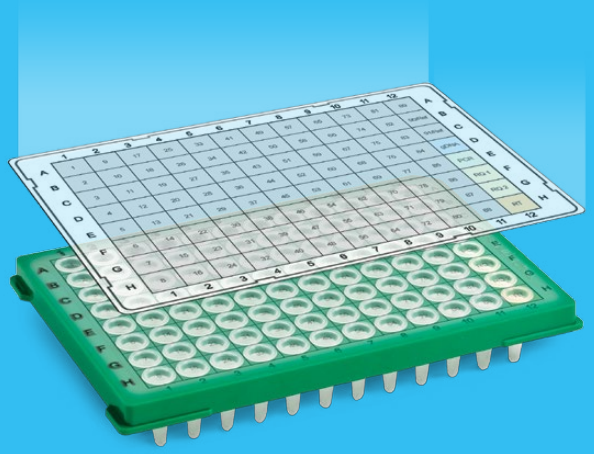
PrimePCR Panels produce comparable results on all instrument platforms. Reference total RNA was analyzed using the Cancer Tier 1 H96 Panel on a CFX96™ System and seven other platforms. The RT-qPCR was performed using the iScript™ Advanced cDNA Synthesis Kit for RT-qPCR and SsoAdvanced™ Universal SYBR® Green Supermix. The Cq values were normalized to three reference genes (*TBP*, *GAPDH*, and *HPRT1*) before comparison. Cq, quantification cycle.

PrimePCR Predesigned Panels

The Most Focused Approach for Real-Time PCR

Bio-Rad collaborated with Thomson Reuters to expertly design an extensive range of predesigned panels. Each real-time PCR plate contains the most biologically relevant gene targets in a canonical pathway, disease, or biological process.

- Obtain complete pathway data from a single experiment
- Visualize biological interactions using interactive pathway maps
- Gain new insights using integrated data analysis tools



Target Ranking

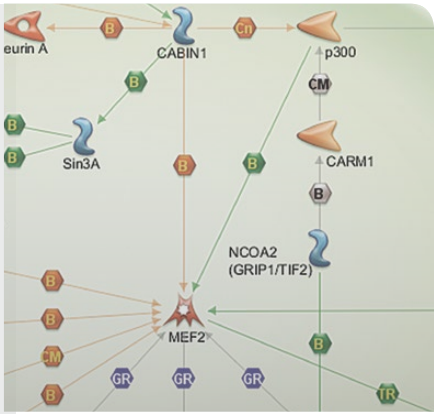
Gene assays present on predesigned panels have been prioritized based on three main criteria:

- How often a gene changes expression level in transcriptome studies
- How much attention was paid to this gene in the overall scientific research
- How interesting the scientific community found this gene in the last 2 years

Pathway Panels

Pathway-Focused Analysis

PrimePCR Pathway Panels were designed for more than 300 canonical pathways. Pathway panels enable complete pathway analysis for differentially expressed, top-ranked gene targets.



Collection Panels

Broad Target Exploration

PrimePCR Collection Panels represent the top-ranked gene targets for differential gene expression analysis, allowing for a more general survey of gene targets across a biological process or group.

Disease Panels

Disease-Focused Analysis

PrimePCR Disease State Panels were designed by referencing the National Library of Medicine MeSH database. Disease state panels allow for the thorough investigation of previously published, differentially expressed genes within a specified pathology.

More than 1,000 unique panels are available in these categories.

PrimePCR Panels for a Broad Range of Pathways and Disease States

Diseases

- Bacterial infections and fungal mycoses
- Cancer and neoplasms
- Cardiac hypertrophy
- Cardiovascular diseases
- Congenital, hereditary, and neonatal diseases and abnormalities
- Cystic fibrosis
- Digestive system diseases
- Endocrine system diseases
- Eye diseases
- Female urogenital diseases and pregnancy complications
- Hemic and lymphatic diseases
- Immune system diseases
- Male urogenital diseases
- Mental disorders
- Musculoskeletal diseases
- Nervous system diseases
- Nutritional and metabolic diseases
- Otorhinolaryngologic diseases
- Parasitic diseases
- Pathological conditions, signs, and symptoms
- Respiratory tract diseases
- Skin and connective tissue diseases
- Stomatognathic diseases
- Virus diseases
- Wounds and injuries

Processes

- Apoptosis and survival
- Blood coagulation
- Cell adhesion
- Cell cycle
- Chemotaxis
- Cytoskeleton remodeling
- Development
- DNA damage
- Hypoxia response
- Immune response
- Muscle contraction
- Neurophysiological process
- Oxidative stress
- Proteolysis
- Reproduction
- Transcription
- Translation
- Transport

Metabolism

- Amino acid metabolism
- Carbohydrate metabolism
- Lipid metabolism
- Nucleotide metabolism
- Regulation of lipid metabolism
- Regulation of metabolism
- Steroid metabolism
- Vitamin and cofactor metabolism
- Xenobiotic metabolism

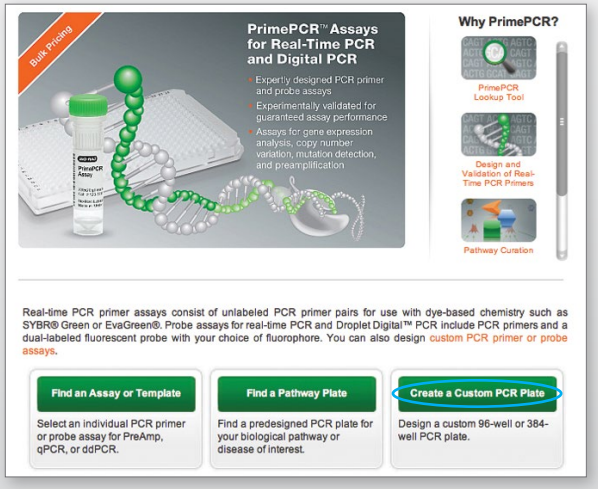
Protein Function

- Cytokines and chemokines
- G proteins
- Growth factors
- Hormones
- Kinases
- Phosphatases
- Second messengers
- Transcription factors

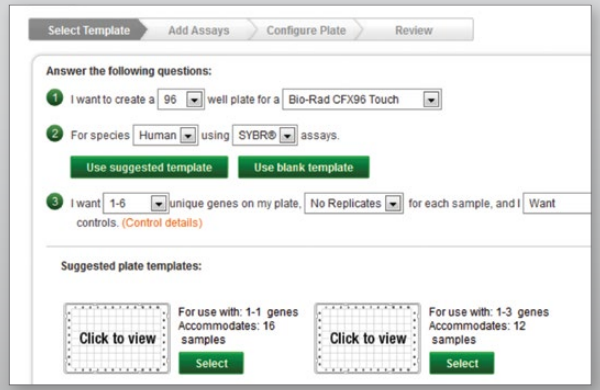
Custom Plates

Design a custom plate with PrimePCR Assays using 96-well or 384-well plates, which are available for every major real-time PCR instrument. Customize your plate design layout or use a suggested plate template as a guide, then select PrimePCR Assays or add your own custom assays.

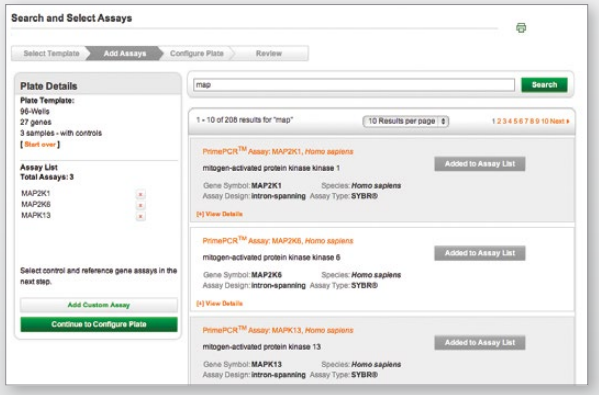
1 Visit **bio-rad.com/PrimePCR** and click **Create a Custom PCR Plate** button.



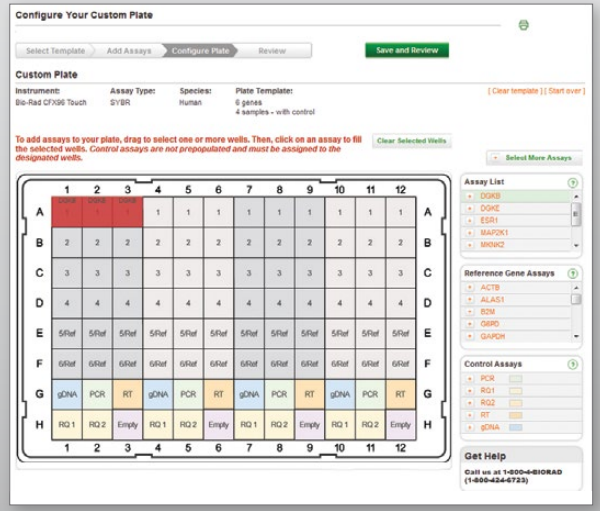
2 Select a plate template.



3 Search and select PrimePCR Assays.



4 Lay out assays, controls, and references.



5 Review configuration. Plate configurations are automatically saved to your My PrimePCR hot list for easy modifications or reorder.

Bulk discounts are available. Go to bio-rad.com/PrimePCR for more information.

Gene Expression Workflow for Real-Time PCR

Assay Performance and Compatibility

Superior Assay Performance
Bio-Rad offers a complete workflow solution for real-time PCR that includes RNA isolation kits, reverse transcription kits, real-time PCR supermixes, real-time PCR detection systems, and data analysis software.

1 Isolate RNA from Sample

2 Generate cDNA

Preamplification (optional)

Aurum™ Total RNA Mini Kit
The Aurum Total RNA Mini Kit produces high-quality DNA-free total RNA from a wide range of starting materials, including cultured cells, bacteria, and yeast, as well as animal and plant tissues.

For more information, visit **bio-rad.com/rna-isolation** and request or download **bulletin 2920**.

iScript™ Advanced cDNA Synthesis Kit for RT-qPCR
The iScript Advanced cDNA Synthesis Kit for RT-qPCR is a simple, fast, and sensitive first-strand cDNA synthesis kit for gene expression analysis using real-time qPCR.

For more information, visit **bio-rad.com/iscript** and request or download **bulletin 6125**.

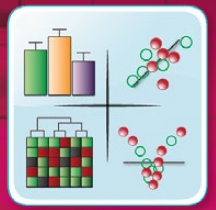
PrimePCR™ PreAmp Assays
Experimentally validated gene-specific primers are available for the unbiased preamplification of small quantities of cDNA for subsequent use in downstream gene expression analysis.

SsoAdvanced™ PreAmp Supermix
SsoAdvanced PreAmp Supermix is a ready-to-use reaction master mix optimized for unbiased target-specific preamplification of nucleic acid. It has been formulated to preamplify a pool of up to 100 PrimePCR™ PreAmp SYBR® Green– or probe-based qPCR assays to aid the accurate detection of small quantities of cDNA.

3 Prepare PCR Reaction

4 Perform Amplification

5 Analyze Results



PrimePCR™ Assays

Experimentally validated real-time PCR primer assays are available as individual SYBR® Green and probe assays, preplated pathway- and disease-specific panels, or custom-configured plates.

SsoAdvanced™ Universal Supermixes

SsoAdvanced Universal Supermixes are available for SYBR® Green– and probe-based detection chemistries. They are formulated to provide superior inhibitor tolerance, increased processivity, and greater speed without affecting qPCR sensitivity, efficiency, or reproducibility.

PCR Plastic Consumables

A large selection of thin-wall polypropylene PCR tubes, PCR plates, sealers, and accessories are precisely manufactured for optimal fit and cycling performance.

For more information, visit **bio-rad.com/PrimePCR**, **bio-rad.com/supermixes**, and **bio-rad.com/pcrplastics** and request or download **bulletins 6090**, **6136**, **6262**, and **6263**.

Real-Time PCR Detection Systems

Real-time PCR detection systems combine thermal cyclers with optical reaction modules for singleplex and multiplex detection of fluorophores. All systems feature thermal gradient functionality.

For more information, visit **bio-rad.com/realtime** and request or download **bulletins 6093**, **6096**, and **6105**.

CFX Manager™ Software

CFX Manager Software allows for the collection and analysis of real-time data. Data analysis tools include ΔCq, ΔΔCq, bar chart, clustergram, scatter plot, volcano plot, and heat map.

PrimePCR Analysis Software

A stand-alone data analysis tool is available for non–Bio-Rad instruments. Visit **bio-rad.com/PrimePCR** to download the software.

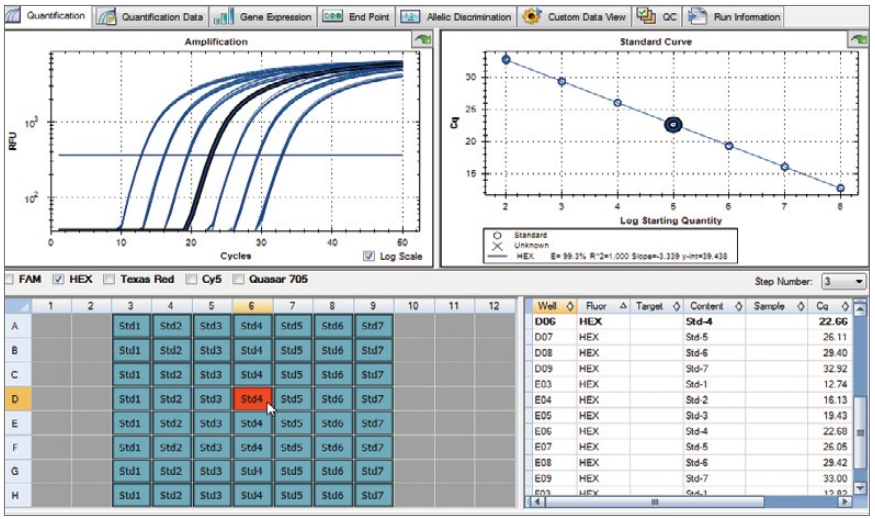
For more information, visit **bio-rad.com/amplification-software** and request or download **bulletin 5990**.

PrimePCR™ Data Analysis

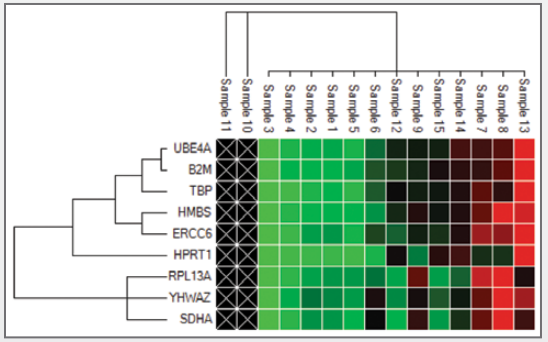
CFX Manager™ Software

Ordering a PrimePCR Plate or Assay is just the beginning of a seamless and integrated workflow, from reaction setup to data acquisition and analysis using CFX Manager Software. The expert design and wet-lab validation of all PrimePCR Assays ensure optimal assay performance so that time once spent optimizing runs can now be devoted to analyzing and interpreting experimental results.

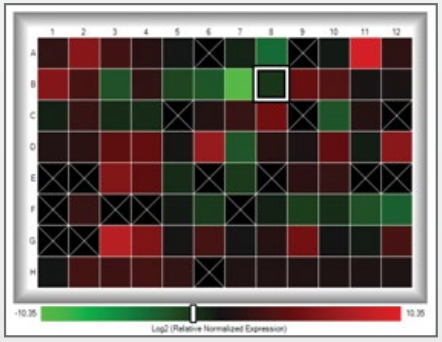
- Select PrimePCR and start a run with a single click
- Begin analyzing data in a single step by importing target, reference, and control information from a PrimePCR run file directly into the plate layout
- Combine data from multiple plates into a Gene Study to rapidly screen large numbers of targets or samples
- Use powerful data visualization tools, such as hierarchical clustering and color-matched expression levels, to identify individual targets or clusters to consider for further investigation



A

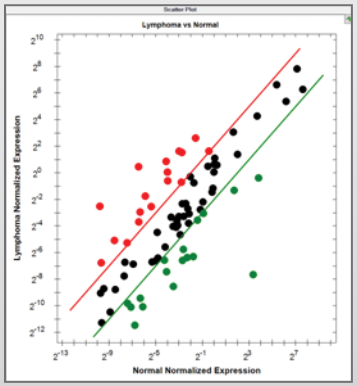


B

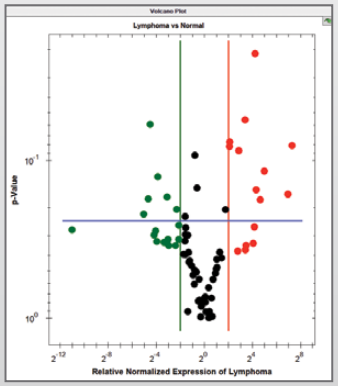


Identify complex regulation patterns at a glance. A, clustergram with color indicative of degree of upregulation (■) or downregulation (■). Targets or samples with similar regulation are clustered together. **B**, heat map shows regulation based on location within a plate.

A



B



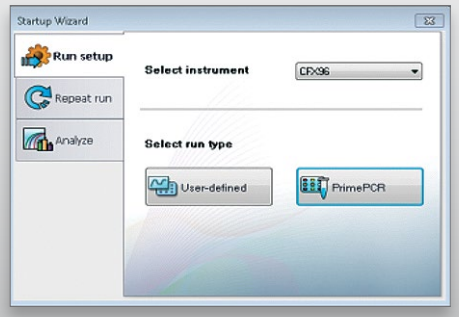
Rapidly discover regulated targets for downstream analysis. Scatter plot (**A**) and volcano plot (**B**) with upregulated (■) and downregulated (■) targets; *P* value indicates statistical significance.

Easily identify specific samples. In every data analysis window, multipane highlighting allows you to correlate information in one panel with that in another.

PrimePCR™ Runs from Start to Finish

Start runs quickly by choosing PrimePCR in the Startup Wizard to select the validated PrimePCR run protocol, then click Start Run. CFX Manager™ Software is fully integrated with PrimePCR products for a fast, streamlined path from data generation to data analysis.

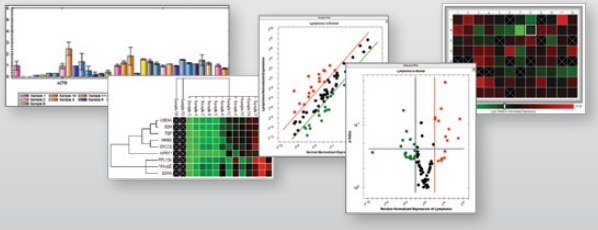
1 Start run



2 Apply run file

The run file autopopulates the plate layout with gene names, making multiplate data analysis quick and simple.

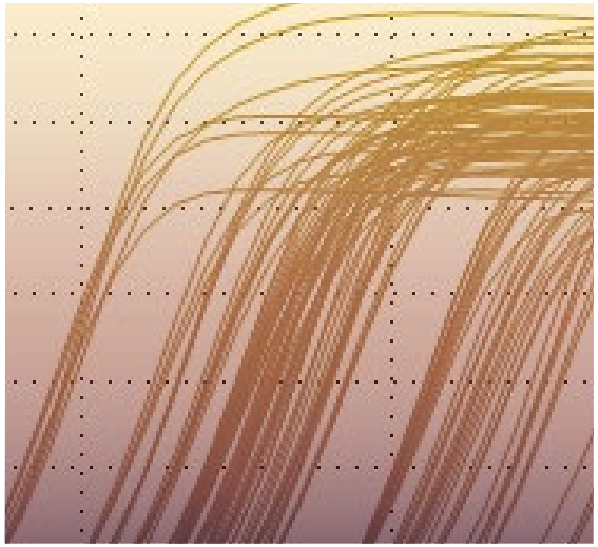
3 Automated data analysis



PrimePCR
PrimePCR Analysis

PrimePCR Analysis Software

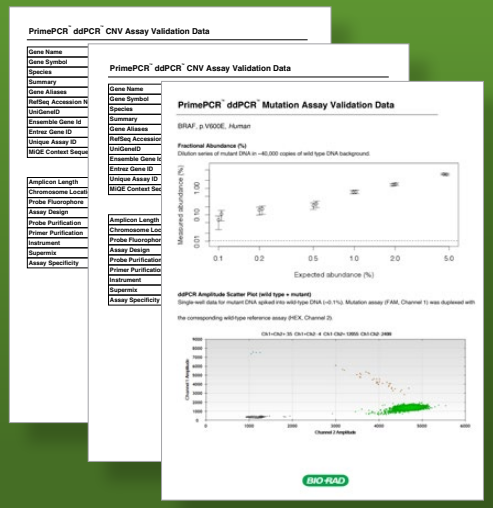
For non-CFX platforms, Bio-Rad offers a PrimePCR data analysis solution. Visit **bio-rad.com/PrimePCR** to download the software. Easily upload Cq values and quickly generate meaningful information from your gene expression experiment.



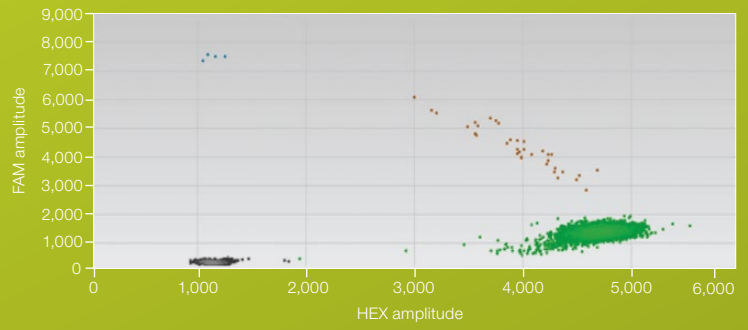
PrimePCR™ ddPCR™ Assays

PrimePCR ddPCR Assays are fully wet-lab validated assays, expertly designed to resolve small fold changes without the use of a standard curve. Researchers can combine the power of ddPCR with the guaranteed performance of these assays for applications involving mutation detection and copy number variation (CNV) analysis.

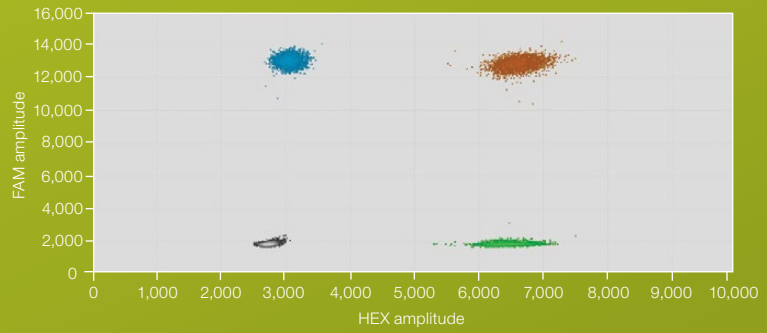
For more information, request or download **bulletin 6512**.



CNV assay and mutation assay validation data.



ddPCR amplitude scatter plot (wild type + mutant). Single-well data for mutant DNA spiked into wild-type DNA (approximately 0.1%) are shown. The mutation assay (FAM, channel 1) was duplexed with the corresponding wild-type reference assay (HEX, channel 2).



2-D amplitude scatter plot. Single-well data are shown for a target copy number assay (FAM, channel 1) duplexed to an *EIF2C1* reference assay (HEX, channel 2).

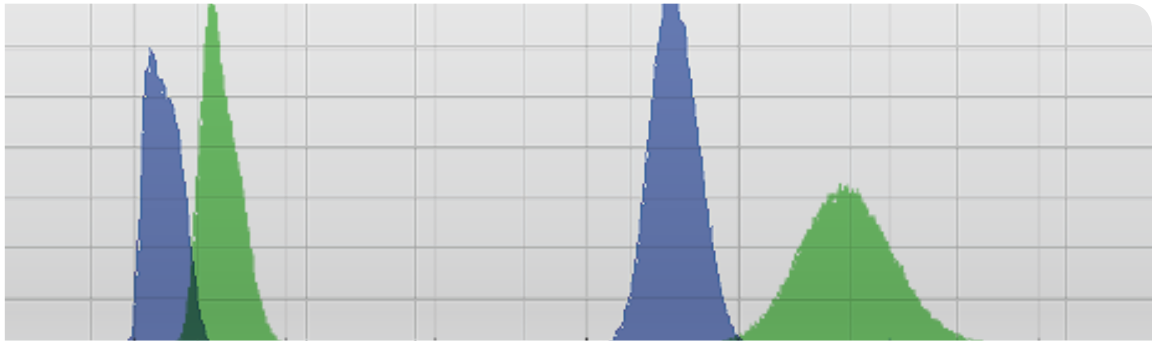
Available with a FAM or HEX fluorophore, these assays were designed with uniform cycling conditions and a primer/probe strategy for both CNV and mutation detection assays, as well as a universal restriction enzyme digestion strategy for copy number assays. The ease of use and reliable results these PrimePCR ddPCR Assays provide will accelerate discovery and optimize research strategies.

ddPCR™ Reagents

ddPCR Supermix for Probes (no dUTP)
The ddPCR Supermix for Probes (no dUTP) is a ready-to-use 2x supermix that has been tested for reliable amplification over a wide dynamic range of sample input, including gDNA, cDNA, and plasmid. The QX200™ Droplet Generator partitions a 20 µl PCR reaction containing the sample, primer pairs, and ddPCR Supermix for Probes (no dUTP) into 20,000 water-in-oil droplets. During the partitioning, target DNA and background DNA are randomly distributed among the droplets. After PCR amplification in a standard thermal cycler, nucleic acids are recovered from the droplets for other downstream applications.

Combined with Bio-Rad's QX200 Droplet Digital PCR System, the ddPCR Supermix for Probes (no dUTP) will:

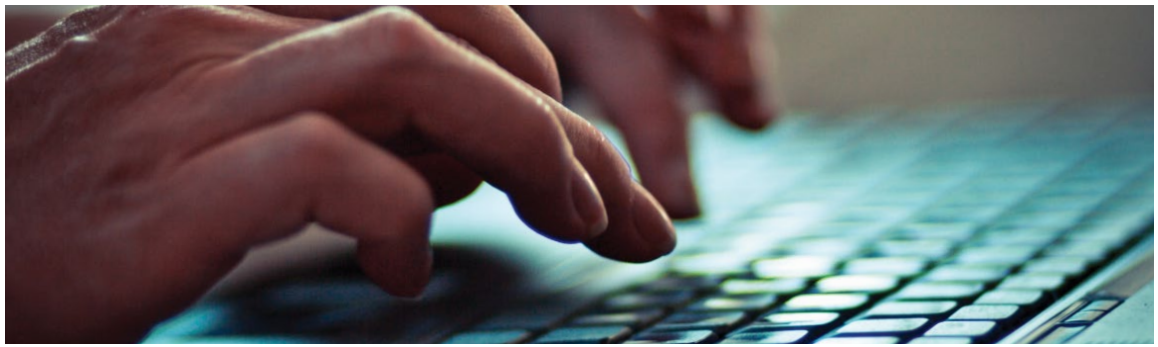
- Enrich for rare target DNA sequences during PCR amplification
- Limit nonspecific PCR amplification
- Allow for DNA recovery after amplification



ddPCR Software

QuantaSoft™ Software
QuantaSoft Software organizes and provides one-click access to the three main steps of droplet analysis in the left navigation bar, moving you through the entire workflow:

- **Set up** — enter information about the samples, assays, and experiments
- **Run** — start the run and control the instrument, if needed
- **Analyze** — compute nucleic acid concentration

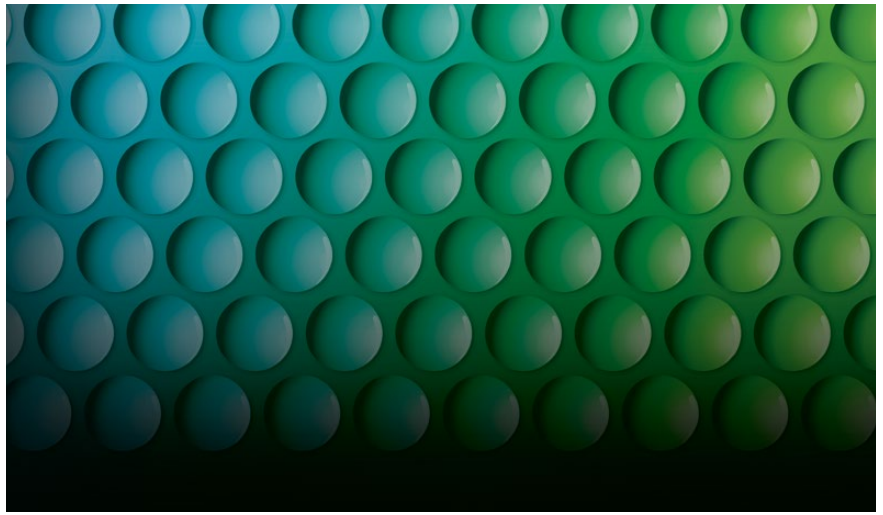


ddPCR™ Workflow

QX200™ Droplet Digital PCR System

The QX200 System consists of two instruments — a droplet generator and a droplet reader — and associated consumables. The droplet generator partitions each sample into 20,000 uniform nanoliter-sized droplets and, after PCR, droplets from each sample are analyzed individually on the droplet reader. PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form.

For more information, request or download **bulletin 6311**.



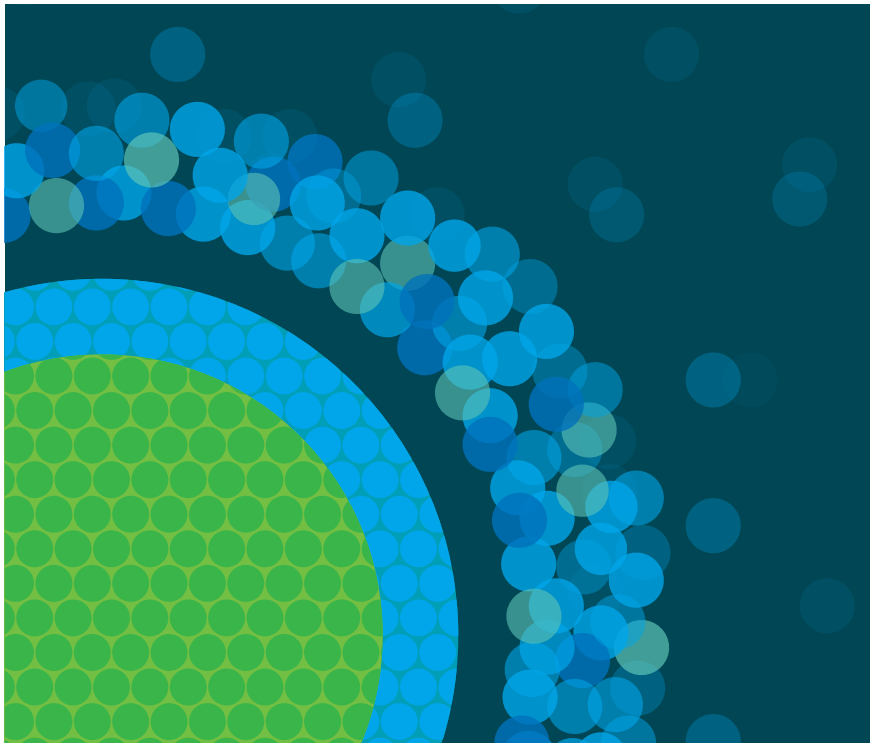
Key Benefits

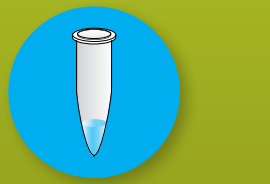
- Achieve absolute quantification without the use of a standard curve
- Design scalable assays for high sensitivity or high throughput
- Expand applications using flexible ddPCR chemistry — EvaGreen or probes

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

ddPCR™ Experiment Procedure

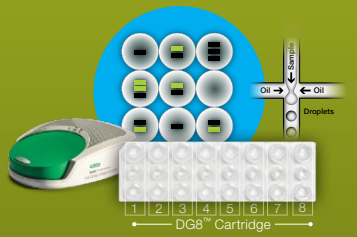
Droplet Digital PCR has a simple, user-friendly experiment setup that is designed for eight samples at a time. The process easily scales up to run a 96-sample experiment with minimal hands-on time. When higher throughput is required, multiple 96-sample experiments can be run in a day.





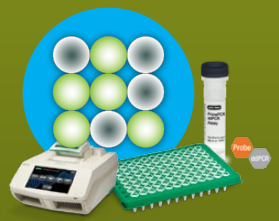
1 Prepare ddPCR reaction mix

- Combine DNA/RNA sample, primers, and/or probes with one of Bio-Rad's ddPCR Supermixes
- Fully validated PrimePCR™ ddPCR Assays can be used



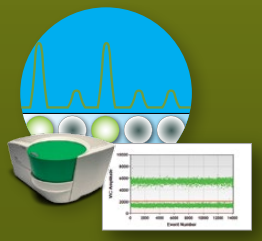
2 Generate droplets

- Load the ddPCR reaction mix into the wells of a droplet generator cartridge
- 8 x 20,000 droplets are generated from each run in the QX100™ or QX200™ Droplet Generator
- Target DNA (▬) and background DNA (▬) are randomly distributed in droplets



3 Perform PCR

- Transfer the droplets to a 96-well PCR plate and seal the plate
- Run the PCR protocol



4 Read and analyze results

- After PCR, load the 96-well PCR plate into the QX100 or QX200 Droplet Reader
- Positive and negative droplets in each sample are read
- Analyze concentrations with QuantaSoft™ Software

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