PrimePCR™ Assays, Panels, and Controls for Real-Time PCR

Instruction Manual

BIO-RAD

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Contents

1 Introduction

In 2012, Bio-Rad's PrimePCR™ assays set a new standard for real-time PCR gene expression analysis. We collaborated with leaders in real-time PCR to design, optimize, and experimentally validate every primer pair. Products are available as individual SYBR® Green assays, individual probe assays, pathway and disease panels, and custom-configured plates. Reference gene and experimental control assays are also available. Assays are validated for use with Bio-Rad's amplification reagents. All PrimePCR assays and panels can be ordered online at **www.bio-rad.com/primepcr**.

PrimePCR Assays, Panels, and Controls

SYBR® Green Assays

Transcriptome-wide assays are available for gene expression analysis. Individual assays are provided as a 20x stock solution of 200, 1,000, or 2,500 reactions. Each PrimePCR assay provides a Unique Assay ID, which can be easily tracked and reported in publications.

Probe Assays

5' exonuclease probe assays are available for gene expression analysis. Each assay can be labeled with a FAM, HEX, Tex615, Cy5, or Cy5.5 fluorophore. Individual assays are provided as a 20x stock solution in 500, 1,000, or 2,500 reactions. Each PrimePCR assay provides a Unique Assay ID, which can be easily tracked and reported in publications.

Predesigned Pathway and Disease Panels

A wide range of pathway- or disease-specific gene panels are available in 96- or 384-well plates. Primer pairs are lyophilized in the wells on the plate. Plates are designed for use with SYBR® Green detection chemistry and include experimental controls and reference assays. Plates are available for all major real-time PCR instruments (see the list of compatible real-time instruments under Reagents and Equipment).

Custom Plates

96- and 384-well PCR plates can be user-designed with PrimePCR™ SYBR® Green assays. Custom primer assays can also be added. Primer pairs are lyophilized in the wells on the plate. Plates are available for all major real-time PCR instruments, and can be designed with or without experimental controls.

PreAmp Assays

PrimePCR PreAmp assays are available for unbiased preamplification of target genes from small quantities of cDNA. Each assay is provided as a 100x stock solution and corresponds to a SYBR® Green or probe gene expression assay.

DNA Templates

Assay-specific synthetic DNA templates are designed to give a positive PCR result when used with the corresponding SYBR® Green or probe assay. Templates are provided as a 20x stock solution of 200 reactions.

Experimental Control Assays

Experimental control assays are available for RNA quality assessment, genomic DNA contamination, reverse transcription, and PCR performance. Controls can be ordered as individual assays or preplated on predesigned or custom PCR plates.

Reference Gene Assays

Commonly used reference gene assays are available to normalize for variation in the amount of input messenger RNA (mRNA) among samples. Assays can be ordered as individual assays or added to a custom plate. Predesigned reference gene panels are available in 96- and 384-well plates.

Droplet Digital™ PCR (ddPCR™) Probe Assays

Copy number variation and mutation detection assays have been expertly designed and validated for use with the QX100™ and QX200™ Droplet Digital PCR platform. Probe assays are labeled with a FAM or a HEX fluorophore, and are available in 200, 1,000, and 2,500 reactions. Please see the PrimePCR ddPCR product inserts for detailed procedure and assay information.

Shipping and Storage

PrimePCR assays and plates are shipped at room temperature. Upon receipt, store product at 4°C (up to 12 months). For long-term storage, store at –20°C.

If storing assay at –20°C, prevent oligonucleotide degradation by aliquoting the total volume into smaller volumes to minimize the number of freeze-thaw cycles.

cDNA synthesis kits and supermixes are shipped on dry ice and should be stored at –20°C. Refer to individual product manuals for more details.

Reagents and Equipment

Consumables and Equipment Needed for Gene Expression Analysis

- cDNA synthesis kit
- Centrifuge
- Gene expression analysis software
- Microfuge tubes
- Nuclease-free pipet tips and tubes
- Nuclease-free water
- Optical seals
- PCR plates or strips (if not using a preplated panel)
- Pipets — single channel and multichannel
- Purified RNA samples
- Real-time PCR detection system
- SYBR® Green or probe supermix
- TE buffer (pH 7.5, nuclease-free) — use for dilution of reverse transcription (RT) control template
- TE buffer with 10 ng/ml tRNA (pH 8.0, nuclease-free) — use for standard curve dilutions

Guaranteed Performance

PrimePCR assays are guaranteed to perform with the following qPCR reagents:

- iScript™ advanced cDNA synthesis kit for RT-qPCR*
- iScript cDNA synthesis kit
- iScript reverse transcription supermix for RT-qPCR
- SsoAdvanced™ PreAmp supermix
- SsoAdvanced™ SYBR® Green supermix*
- SsoAdvanced universal probes supermix
- SsoAdvanced™ universal SYBR® Green supermix
- iTaq™ universal probes supermix
- iTaq™ universal SYBR® Green supermix

* Reagents used for validation report

Compatible Real-Time PCR Instruments

PrimePCR assays and panels are available for the following real-time PCR detection systems:

Bio-Rad

CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, CFX Connect™, Chromo4™, iQ™, iQ™5, MyiQ™, MyiQ™2, Opticon™, Opticon™ 2

ABI

7300, 7500 (standard and Fast), 7900 (standard and Fast, 96- and 384-well), ViiA 7 (standard and Fast, 96- and 384-well), QuantStudio (standard and Fast, 96- and 384-well), StepOnePlus

Stratagene Mx

Eppendorf realplex

Roche LightCycler 480 (96- and 384-well)

Important: Verify instrument and fluorophore compatibility.

Tips for Success

For a complete description of how to optimize a gene expression experiment, please visit the gene expression workflow section of our Applications and Technologies pages at **www.bio-rad.com/en-us/applications-technologies/gene-expression-analysis**.

Ensure High RNA Quality and Quantity

To obtain optimal real-time PCR results, start with high-quality RNA from all samples. RNases, salts, proteins, and other contaminants can compromise the integrity of RNA samples and may impact the performance of downstream enzymes used in reverse transcription and PCR reactions.

- Decontaminate the workspace of RNase, salt, and protein sources
- Limit the number of freeze-thaw cycles of RNA sample, ideally to one cycle
- Use screw-cap tubes for long-term RNA storage at –80°C
- Use nuclease-free plastics and solutions
- Determine concentration and purity of RNA prior to gene expression experiment
- Mass normalize RNA samples by dilution with nuclease-free water prior to the reverse transcription reaction

Prevent and Monitor Genomic DNA Contamination

Genomic DNA (gDNA) present in real-time PCR experiments can cause inaccurate gene expression quantification. Although PrimePCR assays were designed for intron-spanning regions when possible, elimination of gDNA contamination is a best practice for high-quality qPCR results.

- Perform a DNA digestion step with DNase during RNA purification
- Include a gDNA experimental control to determine if gDNA is present (see DNA Contamination Control Assay in Step 3 of Chapter 2, Gene Expression Protocols)

Select Appropriate Reference Genes

Choosing an appropriate reference gene(s) to normalize for variation in the amount of input messenger RNA (mRNA) among samples is important for accurate gene expression analysis. Consult Chapter 6, Reference Gene Selection and Reference Gene Panels, to learn how to determine a stably expressed gene target for your samples of interest.

Calibrate the Real-Time PCR Instrument for Dyes and Fluorophores

Prior to starting a qPCR experiment, verify that the real-time PCR instrument is compatible and calibrated correctly for the dyes and fluorophores chosen. CFX Manager™ software has a Dye Calibration Wizard with an easy guide to calibration (**Tools > Dye Calibration Wizard**). Bio-Rad's CFX instruments are currently calibrated with pure dyes to correspond to PrimePCR fluorophore options before installation. Refer to the instrument manual to determine calibration settings, and calibrate accordingly if necessary.

Fluorophore/Dye	Excitation, nm	Emission, nm
SYBR® Green	494	521
5' FAM	495	520
5' HEX	538	555
5' TEX615	596	613
5' Cy5	648	668
5' Cy5.5	685	706

Use Optical PCR Plate Seals

Use optical seals for accuracy when collecting real-time PCR data. Adhesive seals and heat seals are available options. Bio-Rad recommends using the PX1™ PCR plate sealer with optical heat seals for consistently sealed PCR plates. Seals must fully enclose the PCR plate to prevent evaporation from the wells during thermal cycling. Evaporation is identified by analyzing technical repeats of data — specifically, if a well near the edge of the plate yields variable data, evaporation has likely occurred.

Determine the Threshold Setting

Determining placement of the threshold line is important for accurate quantification of gene expression levels. For precise comparisons of gene expression levels between samples, determine C_q values for a gene only when the threshold line has been defined uniformly for all samples being analyzed. While the threshold can be set manually or automatically using the instrument's software, it is important that the same numerical threshold value be used across all samples, whether they are on the same or multiple plates. The threshold is typically placed at least 10 standard deviations above the average baseline fluorescence level where the PCR reaction is undergoing exponential amplification. As an example, 300 rfu was used as the uniform threshold line setting for all PrimePCR assay validation experiments on a CFX384 instrument.

Multiplex Assay Considerations

PrimePCR probe assays have been optimized to work under the same thermal cycler conditions, allowing for multiple assays to be used in the same well with different fluorophores. To ensure data generated in a multiplex reaction are equivalent to data generated in a singleplex reaction, it is imperative to evaluate the assay performance in both multiplex and singleplex reactions. It is also important to understand the expression level of your target sequences, as this will impact the multiplex optimization method. Consult the SsoAdvanced Universal Probes Supermix Instruction Manual for more details on multiplexing.

Below is a brief list of considerations when conducting multiplex reactions:

- Determine expression levels of gene targets
- Use brighter fluorophores for lower expressing targets
- Compare standard curve of singleplex reaction to multiplex reaction
- Efficiency of multiplex reaction must be similar to minimize amplification bias
- Target abundance should not vary greatly between multiplex assays

2 Gene Expression Protocols

This chapter provides protocols for RNA isolation, cDNA synthesis, real-time PCR reaction setup (in each PrimePCR™ format), and the real-time PCR instrument run. Please follow the appropriate protocol for the format of your experiment (individual assays, 96- or 384-well plates). Remember to set up controls alongside reverse transcription and PCR reactions.

Overview: Real-Time PCR Workflow

Step 1: Isolate RNA

- Aurum™ total RNA mini kit
- Aurum total RNA fatty and fibrous kit

Step 2: Synthesize cDNA

- iScript™ family of reverse transcription reagents

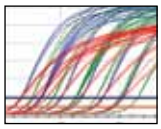
  Optional: Prepare Preamplification Reaction

  – PrimePCR PreAmp assays

  – SsoAdvanced PreAmp supermix

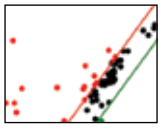
Step 3: Prepare Real-Time PCR Reaction

- PrimePCR assays and panels
- SsoAdvanced™ universal supermixes



Step 4: Cycle in Real-Time PCR Instrument

- CFX real-time PCR detection systems



Step 5: Analyze Gene Expression Data

- CFX Manager™ software
- PrimePCR analysis software

Step 1: Isolate RNA

1. Isolate and purify the RNA sample(s) using Aurum isolation kits or a similar product. The Aurum total RNA mini kit (catalog #732-6820) and the Aurum fatty and fibrous tissue kit (catalog #732-6830) enable RNA isolation from a wide range of cells and tissues and include a DNase digestion step to ensure DNA-free total RNA. Refer to the instruction manual for the detailed protocol.

2. Analyze the RNA sample for integrity and purity.

   a. Use Bio-Rad's Experion™ automated electrophoresis system, the Agilent Bioanalyzer, or similar system to evaluate the integrity of the RNA sample. For gene expression analysis, ensure that the RNA quality indicator (RQI) or RNA integrity number (RIN) values are similar between samples to yield accurate qPCR results. An RQI value of 10 indicates intact RNA, whereas a value of 1 indicates degraded RNA.

   b. Use an agarose gel to assess RNA integrity if the above systems are not available. High-quality eukaryotic RNA yields two clean peaks, 18S and 28S. Degraded RNA appears as a smear on the gel.

   c. To assess purity, evaluate the following spectrophotometer readings:

      A_{260}/A_{280} > 2.0 for pure RNA (assesses DNA contamination)

      A_{260}/A_{230} ~2.0 for pure RNA (assesses protein contamination)

      Lower ratios indicate the presence of contaminants such as salts, carbohydrates, peptides, proteins, phenols, and guanidine thiocyanate.

3. Assess total RNA concentration using a SmartSpec™ Plus spectrophotometer or similar instrument.

4. Normalize RNA concentration across samples by diluting in nuclease-free water.

5. Store RNA sample(s) at –80°C in single-use aliquots and at equivalent concentrations.

Step 2: Synthesize cDNA

Use the iScript family of reverse transcription reagents for cDNA synthesis. The iScript advanced cDNA synthesis kit was used during wet-lab validation of PrimePCR assays. In a single 20 µl reaction, this two-tube kit provides increased data throughput and the widest possible linear dynamic range through its loading capacity of up to 7.5 µg total RNA.

1. Thaw kit contents and normalized RNA samples on ice. Mix components thoroughly, centrifuge briefly to collect solutions at the bottoms of tubes, and then store on ice.

2. Use the same amount of RNA in each cDNA synthesis reaction. Typically 1–5 µg total RNA is sufficient, but may be adjusted depending on abundance of target transcript.

Reagents	Description
5x iScript advanced reaction mix (red cap)	5x reaction mix with dNTPs, oligo(dT), random primers, buffer, $MgCl_2$, enhancers, and stabilizers
iScript advanced reverse transcriptase (orange cap)	iScript MMLV-RT (RNase H^+) and RNase inhibitor
Nuclease-free water	

3. For each RNA sample, prepare a cDNA synthesis reaction on ice.

4. If using the PrimePCR RT control assay, dilute the lyophilized RT control assay template in 200 µl nuclease-free TE (pH 7.5) or the same reagent used to dilute RNA samples. Keep the template on ice. It will degrade similar to RNA samples if exposed to multiple freeze-thaw cycles, ambient temperatures, or RNases. When preparing the cDNA synthesis reaction, add 1 µl of the diluted RT control template.

Component	Volume per Reaction
5x iScript advanced reaction mix	4 µl
iScript advanced reverse transcriptase	1 µl
RNA (100 fg to 7.5 µg)*	Variable
RT control assay template	1 µl
Nuclease-free water	Variable
Total volume	**20 µl**

* It may be necessary to optimize the amount of RNA input due to differences in target gene expression levels and sample availability. See step 6 for an example calculation.

5. Incubate the complete reaction mix in a thermal cycler using the protocol below:

   Reverse transcription: 30 min at 42°C

   RT inactivation: 5 min at 85°C

6. Dilute the cDNA reaction to the desired volume for qPCR reactions. The ideal range of input cDNA per qPCR reaction is 1–25 ng. Altering the amount of input RNA or the level of cDNA dilution can optimize gene expression results for a given collection of genes. Below is a step-by-step example calculation from starting RNA input through amount of cDNA per qPCR reaction used for a PrimePCR 96-well panel with 96 unique gene targets:

   1 µg RNA input per 20 µl RT reaction = 50 ng/µl

   Dilute cDNA to 100 µl = 10 ng/µl

   Use 1 µl diluted cDNA per 20 µl qPCR reaction = 10 ng cDNA per reaction

Prepare and Run Preamplification Reaction (Optional)

For the unbiased amplification of small quantities of cDNA, follow the preamplification protocol prior to preparing the real-time PCR reaction.

Prepare Preamplification Assay Pool with PrimePCR PreAmp Assays

1. Add 5 µl of each PreAmp assay (up to 100 assays) to a microcentrifuge tube.

2. Bring total volume of assay pool up to 500 µl with nuclease-free water.

3. Mix thoroughly, briefly centrifuge, and store on ice.

4. Use 5 µl of the assay pool in a 50 µl reaction.

Note: Assay pools are stable at 4°C for up to 30 days and at –20°C for up to 1 year. PrimePCR control assays are currently not compatible with preamplification.

Prepare Preamplification Reaction

1. Thaw SsoAdvanced PreAmp supermix and cDNA samples. Mix thoroughly by inversion or by a 15 sec vortex. Centrifuge briefly to collect solutions at the bottom of each tube and then store on ice and protected from light.

2. Prepare preamplification reaction mix on ice. Good pipetting practice must be employed to ensure assay precision and accuracy.

Component	Volume per Reaction	Final Concentration
2x SsoAdvanced PreAmp supermix	25 µl	1x
PrimePCR PreAmp pool	5 µl	1x
cDNA sample	Variable	250 ng–100 pg
Nuclease-free water	Variable	—
Total volume	**50 µl**	—

3. Mix the reaction mix thoroughly to ensure homogeneity and dispense into a PCR tube or into the wells of a PCR plate.

4. Load the PCR tubes or plate into a thermal cycler and start the PCR run with the following thermal cycling protocol.

Step	Temperature	Time	# of Cycles
Activation	95°C	3 min	1
Denaturation	95°C	15 sec	10–12
Annealing/extension	58°C	4 min	10–12
Hold	4°C	∞	1

5. After run completion, the preamplification reaction can be stored at –20°C for up to 12 months or at 4°C for up to 72 hr.

6. The completed preamplification reaction should be diluted to a minimum of 1:5 with TE buffer. However, a larger dilution volume may be required, depending on the number of assays planned for downstream qPCR and the number of technical replicates. For example, a 1:15 dilution will provide enough volume for 100 qPCR assays using technical triplicates and 2 µl per reaction.

   100 qPCR assays in triplicate = 100 assays x 3 replicates x 2 µl = 600 µl needed

   1:15 preamplification reaction dilution = 750 µl

   150 µl excess

7. Use 2 µl of the dilution per 20 µl qPCR reaction or 1 µl per 10 µl qPCR reaction for a 96- or a 384-well plate, respectively.

Note: When using SsoAdvanced universal SYBR® Green supermix for qPCR after preamplification, run the activation and denaturation steps at 98°C if delayed Cq values or decreased efficiency occur.

Step 3: Prepare Real-Time PCR Reaction

Protocols for preparing real-time PCR reaction mix are described according to format (96-well plates, 384-well plates, or individual assays). Protocols for PrimePCR control assays are incorporated into the reaction setup. Please refer to Chapter 3 for interpretation of experimental control assays.

Pathway/Disease Panel or Custom Plate — 96-Well Format

Primers are lyophilized in each well; therefore, add all remaining components to the PCR reaction mix.

1. Thaw SsoAdvanced™ universal SYBR® Green supermix, cDNA samples, and positive PCR control DNA template (if a PCR control assay is on the plate). Mix thoroughly by inversion or a 15 sec vortex, centrifuge briefly to collect solutions at the bottoms of tubes, and then store on ice and protected from light.
2. Allow the PrimePCR plate to come to room temperature prior to removing the seal.
3. Dilute each cDNA sample to a total volume of 100 µl if preparing a 96-well plate with unique targets.
4. Prepare (on ice or at room temperature) enough reaction mix for all qPCR reactions by adding all required components. Scale up proportionally for multiple reactions. CFX Manager™ software has an easy-to-use master mix calculator that provides scale-up calculations for your experiment.

Component	Volume per Reaction	Final Concentration
20x PrimePCR assay	Dried in well	1x
2x SsoAdvanced™ universal SYBR® Green supermix	10 µl	1x
cDNA sample	1–4 µl*	100 ng–100 fg*
Nuclease-free water	Variable	—
Total volume	**20 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume (4 µl) of cDNA sample to avoid excessive carryover of PCR inhibitors.

5. Transfer 20 µl of the PCR reaction mix into each well.
6. **Optional:** Add 1 µl PCR control assay template into the appropriate well (total volume will be 21 µl — additional volume will not affect the qPCR reaction).
7. Seal the plate with an optical seal. Optional: briefly centrifuge to remove bubbles (4,000 rpm for 2 min at room temperature).
8. Load the PCR plate into the real-time PCR instrument.

Pathway/Disease Panel or Custom Plate — 384-Well Format

Primers are lyophilized in each well; therefore, add all remaining components to the PCR reaction mix.

1. Thaw SsoAdvanced™ universal SYBR® Green supermix, cDNA samples, and positive PCR control DNA template (if a PCR control assay is on the plate). Mix thoroughly by inversion or a 15 sec vortex, centrifuge briefly to collect solutions at the bottoms of tubes, and then store on ice and protected from light.
2. Allow the PrimePCR plate to come to room temperature prior to removing the seal.
3. Dilute each cDNA sample to a total volume of 400 µl if preparing a 384-well plate with unique targets.
4. Prepare (on ice or at room temperature) enough reaction mix for all qPCR reactions by adding all required components. Scale up proportionally for multiple reactions. CFX Manager software has an easy-to-use master mix calculator that provides easy scale-up calculations for your experiment.

Component	Volume per Reaction	Final Concentration
20x PrimePCR assay	Dried in well	1x
2x SsoAdvanced™ universal SYBR® Green supermix	5 µl	1x
cDNA sample	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	—
Total volume	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

5. Transfer 10 µl of the PCR reaction mix into each well.
6. **Optional**: Add 0.5 µl PCR control assay template into the appropriate well (total volume will be 10.5 µl — additional volume will not affect the qPCR reaction).
7. Seal the plate with an optical seal. Optional: briefly centrifuge to remove bubbles (4,000 rpm for 4 min at room temperature).

   Note: Be careful to not introduce bubbles during pipetting steps, since it is difficult to remove bubbles from 384-well plates.
8. Load the PCR plate into the real-time PCR instrument.

Individual Assays

Protocols for primer assays, probe assays, and control assays follow. PrimePCR individual assays come ready to use as a 20x stock solution.

SYBR® Green Assay

1. Thaw SsoAdvanced™ universal SYBR® Green supermix, PrimePCR™ SYBR® Green assay, cDNA samples, and positive PCR control (if used) to room temperature. Mix thoroughly by inversion or a 15 sec vortex, centrifuge briefly to collect solutions at the bottoms of tubes, and then store on ice and protected from light.

2. Prepare (on ice or at room temperature) enough reaction mix for all qPCR reactions by adding all required components. PrimePCR™ SYBR® Green assays are provided as a 20x stock solution. Scale up proportionally for technical replicates. CFX Manager software has an easy-to-use master mix calculator that provides easy scale-up calculations for your experiment.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR assay	1 µl	0.5 µl	1x
2x SsoAdvanced universal SYBR® Green supermix	10 µl	5 µl	1x
cDNA sample	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

3. Transfer the appropriate volume of the PCR reaction mix into each tube or well: 20 µl for a 96-well plate, or 10 µl for a 384-well plate.

4. **Optional**: Set up control reactions to determine sample quality and PCR and RT reaction performance.

5. Seal the plate with an optical seal. Optional: briefly centrifuge to remove bubbles (4,000 rpm for 2 min at room temperature).

6. Load the PCR plate (or tubes) into the real-time PCR instrument.

Probe Assay

1. Thaw SsoAdvanced universal probes supermix, PrimePCR probe assay, cDNA samples, and positive PCR control (if used) to room temperature. Mix thoroughly by inversion or a 15 sec vortex, centrifuge briefly to collect solutions at the bottoms of tubes, and then store on ice and protected from light.

2. Prepare (on ice or at room temperature) enough reaction mix for all qPCR reactions by adding all required components. PrimePCR probe assays are provided as a 20x stock solution. Scale up PCR reaction mix proportionally for technical replicates. CFX Manager software has an easy-to-use master mix calculator that provides easy scale-up calculations for your experiment.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR assay	1 µl	0.5 µl	1x
2x SsoAdvanced universal probes supermix	10 µl	5 µl	1x
cDNA sample	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

3. Transfer the appropriate volume of the PCR reaction mix into each tube or well: 20 µl for a 96-well plate, or 10 µl for a 384-well plate.
4. **Optional**: Set up control reactions to determine sample quality and PCR and RT reaction performance.
5. Seal the plate with an optical seal. Optional: briefly centrifuge to remove bubbles (4,000 rpm for 2 min at room temperature).
6. Load the PCR plate (or tubes) into the real-time PCR instrument.

Positive PCR Control Assay (PCR)

1. For each cDNA sample, prepare a positive PCR control reaction mix (template and assay in the same tube).

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR positive PCR control assay	1 µl	0.5 µl	1x
2x SsoAdvanced supermix	10 µl	5 µl	1x
cDNA sample	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

2. Follow the remainder of the PrimePCR cycling protocol.
3. Assess performance of the qPCR reaction (see Chapter 3, Interpretation of Experimental Control Assays).

Reverse Transcription Control Assay (RT)

1. Resuspend lyophilized RT control assay template in 200 µl nuclease-free TE (pH 7.5) or the same reagent used to dilute RNA samples. Keep template on ice. It will degrade similar to RNA samples if exposed to multiple freeze-thaw cycles, ambient temperatures, or RNases.

2. For each RNA sample, include 1 µl of the control RNA template in each 20 µl cDNA synthesis reaction, and proceed with the reverse transcription reaction.

3. Once cDNA is synthesized and diluted to working stock, prepare a reverse transcription control reaction mix.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR RT control assay	1 µl	0.5 µl	1x
2x SsoAdvanced supermix	10 µl	5 µl	1x
cDNA sample with RT control template	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

4. Follow the remainder of the PrimePCR cycling protocol.

5. Assess performance of the RT reaction (see Chapter 3, Interpretation of Experimental Control Assays).

DNA Contamination Control Assay (gDNA)

1. For each cDNA sample, prepare a DNA contamination control reaction mix.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR gDNA control assay	1 µl	0.5 µl	1x
2x SsoAdvanced supermix	10 µl	5 µl	1x
cDNA sample with RT control template	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

2. Follow the remainder of the PrimePCR cycling protocol.

3. Assess performance of the RT reaction (see Chapter 3, Interpretation of Experimental Control Assays).

RNA Quality Assay (RQ1 and RQ2)

1. For each cDNA sample, prepare RQ1 and RQ2 RNA quality assay reaction mixes.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR RQ1 assay	1 µl	0.5 µl	1x
2x SsoAdvanced supermix	10 µl	5 µl	1x
cDNA sample	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR RQ2 assay	1 µl	0.5 µl	1x
2x SsoAdvanced supermix	10 µl	5 µl	1x
cDNA sample	1–4 µl*	0.5–2 µl*	100 ng–100 fg*
Nuclease-free water	Variable	Variable	—
Total volume	**20 µl**	**10 µl**	—

* Calculated during Step 2, cDNA synthesis. Avoid adding >20% reaction volume of cDNA sample to avoid excessive carryover of PCR inhibitors.

2. Follow the remainder of the PrimePCR cycling protocol.
3. Assess performance of the qPCR reaction (see Chapter 3, Interpretation of Experimental Control Assays).

Step 4: Cycle in Real-Time PCR Instrument

Load the PCR tubes or plate on the real-time PCR instrument and program the thermal cycling protocol according to the following table. Start the real-time PCR run.

Step	Temperature	Time	No. of Cycles
Activation	95°C	2 min*	1
Denaturation	95°C	5 sec	40
Annealing/Extension	60°C	30 sec	40
Melt Curve**	65–95°C (0.5°C increments)	5 sec/step	1

* Activation can be reduced to 30 sec. Do not use a 10-min activation time with Bio-Rad supermixes.

** Melt curve step is for SYBR® Green analysis only.

Step 5: Analyze Gene Expression Data

Process your data and evaluate results using CFX Manager or other analysis software. See Chapter 5, CFX Manager Quick Guide, or consult your instrument and software manuals for detailed instructions. If PrimePCR control assays were used, refer to Chapter 3 to interpret sample quality and reaction performance.

3 Interpretation of Experimental Control Assays

PrimePCR™ experimental control assays are designed to help assess the quality of your sample(s) and how this may affect the performance of the reverse transcription and qPCR reactions. They work alongside any assay from any source. This chapter explains how to interpret control assays. For control assay reaction setup, refer to the format (plate or individual assay) in Chapter 2, Gene Expression Protocols.

Positive PCR Control Assay (PCR)

Purpose: The positive PCR control assay (PCR) targets a synthetic DNA template to determine if samples contain inhibitors or other factors that may negatively affect your gene expression results. The sequence of the synthetic DNA template is not present in the human or mouse genome.

Designed to qualitatively assess:

- Performance of a qPCR reaction associated with a single sample
- Relative performance of the qPCR reactions associated with different samples

Format:

- Individual assay — 200 reactions (20x stock solution includes primers and DNA template)
- 96-well or 384-well plate — primers are lyophilized in designated well(s) on the plate, and the DNA template is provided in a separate tube

Interpretation:

- Single sample — $C_q \geq 30$ indicates poor PCR performance; will likely compromise gene expression results
- Two or more samples — designate one of your samples as the control. To determine the ΔC_q between the control sample and each remaining sample, use the following equation:

  $|(\text{PCR } C_q \text{ for control sample}) - (\text{PCR } C_q \text{ for sample})| = \Delta C_q$

  $\Delta C_q > 1$ indicates samples differ in their impact on qPCR performance and may compromise gene expression results.

Reverse Transcription Control Assay (RT)

Purpose: The reverse transcription (RT) control assay introduces a synthetic RNA template into the cDNA synthesis reaction to evaluate the RT performance. The sequence of the synthetic RNA template is not present in the human or mouse transcriptome.

Designed to qualitatively assess:

- Performance of a reverse transcription reaction associated with a single sample
- Relative performance of the reverse transcription reactions associated with different samples

Format:

- Individual assay — 200 reactions of 20x stock solution PrimePCR reverse transcription control primer assay and 200 reactions lyophilized RNA template
- 96-well or 384-well plate — primers are lyophilized in designated well(s) on the plate, and the lyophilized RNA template is provided in a separate tube

Interpretation:

- Single sample — $C_q \geq 30$ indicates poor reverse transcription reaction performance; will likely compromise gene expression results
- Two or more samples — designate one of your samples as the control. To determine the ΔC_q between the control sample and each remaining sample, use the following equation:

  $|(\text{RT } C_q \text{ for control sample}) - (\text{RT } C_q \text{ for sample})| = \Delta C_q$

  $\Delta C_q > 1$ indicates that samples differ in their impact on reverse transcriptase performance and may compromise gene expression results.

DNA Contamination Control Assay (gDNA)

Purpose: The DNA contamination control assay (gDNA) is a species-specific control assay that targets a nontranscribed region of the genome.

Designed to qualitatively assess:

- Whether genomic DNA (gDNA) is present in a sample at a level that may affect qPCR results
- Relative levels of gDNA contamination present in different samples to determine if qPCR results may be affected

Format:

- Individual assay — 200 reactions (20x stock solution)
- 96-well or 384-well plate — primers are lyophilized in designated well(s) on the plate

Interpretation:

Single sample:

- $C_q \geq 35$: indicates below single copy detection; no gDNA present
- $C_q < 35$: indicates the sample is contaminated with gDNA and gene expression results may be affected. The relative contribution of gDNA contamination to a sample's signal can be determined by comparing the C_q value for a given gene of interest (GOI) to the C_q value for the DNA contamination control assay using the following equation:

$$|(\text{GOI } C_q) - (\text{gDNA } C_q)| = \Delta C_q$$

ΔC_q	Percent Contribution
1	50%
2	25%
3	12.5%
4	6.25%
5	3.13%
6	1.56%
7	0.78%

Two or more samples: Designate a sample as the control. To determine the ΔC_q between the control sample and each remaining sample, use the following equation:

$$|(\text{gDNA } C_q \text{ for control sample}) - (\text{gDNA } C_q \text{ for sample})| = \Delta C_q$$

- $\Delta C_q < 1$: samples have similar levels of gDNA contamination; gDNA contamination will likely have little to no effect on results
- $\Delta C_q \geq 1$: samples have different levels of gDNA contamination; gDNA contamination may affect gene expression results

RNA Quality Assay (RQ1 and RQ2)

Purpose: The RNA quality assay is a pair of assays (RQ1 and RQ2) that target the same transcript at different locations with different amplicon sizes. The RQ1 and RQ2 assays must be used as a pair for each cDNA sample. Differences in Cq values can indicate whether RNA degradation may be negatively impacting gene expression results.

Designed to qualitatively assess:

- Whether RNA integrity may adversely affect PCR results of a single sample
- Relative RNA integrity between samples to determine if qPCR results may be affected

Format:

- Individual assay — 200 reactions (20x stock solution) of RQ1 RNA quality assay and 200 reactions (20x stock solution) of RQ2 RNA quality assay
- 96-well or 384-well plate — RQ1 and RQ2 primers are lyophilized in designated well(s) on the plate

Interpretation:

- Single sample — to determine the ΔCq between the RQ1 and RQ2 assays, use the following equation:

  $|(\text{RQ2 } C_q) - (\text{RQ1 } C_q)| = \Delta C_q$

  $\Delta C_q \leq 3.0$: RNA degradation is minimal and will likely have little to no effect on gene expression results

  $\Delta C_q > 3.0$: RNA integrity may compromise gene expression results

- Two or more samples — designate one of your samples as your control sample. To determine the $\Delta\Delta C_q$ between the control sample and each remaining sample, use the following equation:

  $[\ |(\text{RQ2 } C_q \text{ for designated control sample}) - (\text{RQ1 for designated control sample } C_q)|\ ] - [\ |(\text{RQ2 } C_q \text{ for sample}) - (\text{RQ1 for sample } C_q)|\ ] = \Delta\Delta C_q$

  $\Delta\Delta C_q = 0$ to 1.0: samples are similar in quality; will likely have little to no effect on gene expression results

  $\Delta\Delta C_q = 1.0$ to 2.0: RNA integrity differs; will likely have slight to moderate effects on gene expression results

  $\Delta\Delta C_q > 2.0$: RNA integrity differs; will likely have significant effects on gene expression results

4 DNA Templates

PrimePCR™ DNA templates are single-stranded synthetic DNA templates that are complementary to corresponding gene-specific PrimePCR assays. Each template is linked to the corresponding assay with a Unique Assay ID. Templates for use with SYBR® Green assays are 60 base pairs in length, and those for use with probe assays are 90 base pairs in length. They are designed to give a positive real-time PCR result when used with the correct PrimePCR assay and can also be used to generate a standard curve.

Format: individual template — 200 reactions (20x stock solution) of assay-specific template (20 x 10^6 copies/µl)

Procedure:

1. For use as a qPCR control, prepare the reaction mix.

Component	Volume per Reaction		Final Concentration
	96-Well	**384-Well**	
20x PrimePCR assay for gene of interest	1 µl	0.5 µl	1x
2x SsoAdvanced™ supermix	10 µl	5 µl	1x
20x PrimePCR cDNA template for gene of interest	1 µl	0.5 µl	20 x 10^6 copies/µl
Nuclease-free water	8 µl	4 µl	—
Total volume	**20 µl**	**10 µl**	—

2. Follow the PrimePCR cycling protocol and evaluate results to determine assay performance.

Interpretation: When used as a qPCR control

- C_q < 30: assay performance is not affected
- C_q > 30: poor PCR performance; will likely compromise results

Standard Curve:

Perform a standard curve using a seven-point, tenfold serial dilution series from 20 million copies to 20 copies. Prepare serial dilutions of a stock solution of 10 ng/ml tRNA in TE (pH 8.0).

5 CFX Manager™ Software Quick Guide

For detailed instructions, please refer to your CFX instrument manual or CFX Manager software manual.

PrimePCR™ Run Setup

1. To begin a PrimePCR protocol, select the PrimePCR button from the Startup Wizard and go to **File > New > PrimePCR run**, or drag and drop a PrimePCR run file* onto the main window of CFX Manager.
2. Once a PrimePCR run has been selected, the Run Setup window opens in the Start Run tab. The PrimePCR default protocol and plate layout are loaded.
3. For SYBR® Green or FAM experiments, select the SYBR/FAM scan mode. For probe assays using other fluorophores (HEX and Tex615) or for multiplex experiments, open the Plate tab and use the Edit Selected option to choose the specific fluorophores and select the All Channels scan mode.
4. For SYBR® Green assays, select **Include Melt Step** in the Protocol tab; deselect it for probe assays.
5. Once the plate and protocol are loaded, click **Close Lid** and **Start Run**.

* PrimePCR run files for predesigned and custom plates can be downloaded from **www.bio-rad.com/primepcr**. Custom plates are saved under My PrimePCR once configured and purchased. Predesigned panels have a Download Run File button on the Review Plate page.

Data Analysis for Gene Expression Experiments

1. Drag and drop the PrimePCR run file for a predesigned or custom plate before or after a protocol run in order to populate the assay names for data analysis. Alternatively, open a data file by selecting **File > Open > Data file**. If analyzing data from multiple plates, skip to step 5 and set up a gene study.

2. The plate should contain sample and target information, as well as controls. Select reference targets in Experiment Settings.

3. Once the above requirements are met for normalized gene expression analysis, the Quantification and Quantification Analysis tabs of the Data Analysis window display a table of C_q values, averages, standard deviations, and normalized values.

4. Bar charts, clustergrams, scatter plots, volcano plots, and heat maps are available under the Gene Expression tab.

5. Create a gene study to compare gene expression data from one or more real-time PCR experiments using an inter-run calibrator to normalize between experiments.

   a. Go to **File > New > Gene Study**.

   b. A Gene Study window opens. To add data files for the gene study, select **File > Add Data File** and choose the .pcrd data files you would like to analyze.

   c. CFX Manager software automatically attempts an inter-run calibration (IRC) to normalize for variation between different plates. An inter-run calibrator must have the same sample and target between plates. If no such well exists on the PrimePCR plate, consider designating a positive PCR control well as the IRC, and be sure that the sample and target are labeled the same (for example, edit sample name to IRC and target to Positive PCR).

6. After gene expression, generate analysis reports by selecting **Tools > Reports…** or export the data to Microsoft Excel by selecting **Export > Export All Datasheets to Excel**.

Note: PrimePCR Analysis Software is available for real-time platforms not made by Bio-Rad. This enables easy PrimePCR plate file population with data (C_q values) generated on any real-time PCR instrument. Visit **www.bio-rad.com/PrimePCR** to download the software and instruction manual.

6 Reference Gene Selection and Reference Gene Panels

To ensure proper normalization of loaded RNA in a gene expression experiment, use a stable, validated reference gene(s). The reference gene(s) should maintain consistent expression across all samples in the project regardless of treatment, source, or extraction method. The number used and specific choice of reference genes are key factors in determining the magnitude of change in expression that can be detected. To detect large changes (greater than fourfold), a single reference gene may be sufficient. For smaller changes (less than fourfold), use multiple well–validated reference genes to prevent confounding effects produced by fluctuations in basal or uninduced expression in any single reference gene. In addition, validate reference genes for stability using this method:

1. Choose candidate reference genes.

Select a candidate list of reference genes using published references as guidance. Use at least five reference genes for evaluation. For your convenience, Bio-Rad offers preplated reference gene panels using our validated and optimized PrimePCR™ assays. Each reference gene panel contains 14 reference genes commonly used in gene expression studies. The 96-well plate can accommodate up to six unique samples (or three samples in duplicate or two samples in triplicate), while the 384-well plate can accommodate 24 unique samples (or twelve samples in duplicate, eight samples in triplicate).

2. Select representative samples across the groups.

Select samples to represent all the conditions to be used in the study (for example, treatments, tissues, time courses), ensuring that all variables within the sample groups are evaluated.

3. Isolate the RNA.

Isolate the RNA from the samples and treat with DNase using the same protocol for all samples. Quantify and normalize the RNA to the same concentration.

4. Perform reverse transcription to cDNA.

Perform a reverse transcription reaction for each sample using the same kit, column, and concentration. Dilute the cDNA, as needed, treating each sample the same to ensure there are no differences from sample to sample in terms of volume and concentration from the initial RNA input.

5. Analyze expression using real-time PCR.

Perform a real-time PCR experiment using the samples and the reference gene panel, or your selected reference gene assays.

6. Evaluate stability.

Statistical validation of reference gene stability is detailed in a study by Vandesompele et al. (2002) using an iterative test of pairwise variation. CFX Manager™ software includes this method as an automated dialog. To calculate the M value for each reference gene tested, open the data file in the Gene Expression tab and select **Target Stability**.
The M values will be presented in tabular format in a dialog box. The lowest M value corresponds to the most stable expression in the tested samples, and recommended cutoff values for M are <0.5 (for homogenous sample sets) and <1 (for heterogenous sample sets). Reference gene stability can also be calculated using Biogazelle's geNormPLUS or qbase+ software. Alternatively, use coefficient of variation (CV) or analysis of variance (ANOVA) methods to evaluate stability.

Reference

Vandesompele J et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3, RESEARCH0034.

7 MIQE Compliance

The minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines allow for greater transparency in qPCR data across laboratories. These guidelines allow scientists to investigate the quality of results presented and the ability to repeat experiments precisely. Bio-Rad's PrimePCR™ assays make MIQE compliance easy. Each PrimePCR assay provides a Unique Assay ID, which can be easily tracked and reported in publications. These IDs are provided on the website when ordering and on the specifications sheets accompanying the product when shipping. Additionally, each PrimePCR assay has a thorough validation report that includes which transcripts for a given gene are targeted, and a context sequence where the amplicon can be found. For more information on how PrimePCR assays meet MIQE guidelines, refer to bulletin 6262 (found under the Documents tab at **www.bio-rad.com/primepcr**) or review the original publication (Bustin et al. 2009).

Reference

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

8 Frequently Asked Questions (FAQs)

How did Bio-Rad wet-lab validate PrimePCR™ assays?

Every primer pair was experimentally tested by generating an amplification plot using universal RNA, conducting a melt curve analysis, and calculating the efficiency and dynamic range from a seven-point standard curve using 20–20,000,000 copies of synthetic template. Specificity was determined by next generation sequencing of the amplicon.

Where can I find validation reports?

At **www.bio-rad.com/primepcr**, each assay has a PDF with validation data, including assay information, transcripts detected, validation graphs and values, and a key for interpreting data.

Can I multiplex PrimePCR probe assays?

The fluorophores chosen for PrimePCR probes assays enable multiplex experiments; however, not every combination of probe assays has been validated. Compare singleplex and multiplex reactions to determine the performance of multiplex experiments. See Multiplex Assay Considerations in Chapter 1.

Do I need to preamplify my cDNA?

Preamplification is an optional step in the PrimePCR protocol. Performing preamplification will allow the user to perform more real-time PCR reactions from limited amounts of sample (10 pg–100 ng cDNA).

Will Bio-Rad provide primer sequence information?

No. The MIQE context sequence of the amplicon is provided in the validation information. Exact sequences are not provided.

What species does Bio-Rad offer?

Human and mouse for qPCR, human for ddPCR™.

Can PrimePCR products be ordered using PunchOut?

Yes.

What does guaranteed performance mean?

Assay performs well when used with Bio-Rad amplification reagents and samples of sufficient quality.

9 Troubleshooting

Troubleshooting guide

Symptom	Cause	Treatment
Positive gDNA experimental control	gDNA in sample	Perform DNase digestion during RNA purification step
Positive NTC (no template control)	DNA contamination in workflow	Identify and eliminate source of contamination (plastics, reagents, workspace, etc.)
Unexpectedly high C_q values and a positive PCR experimental control value	Possible PCR inhibitors in reaction	Identify and eliminate inhibitor (see list of common inhibitors below) by purifying samples using a post-isolation cleanup kit
Wells along edge of PCR plate are varying between technical repeats	Evaporation of PCR reactions	Seal plate better before placing in thermal cycler; use a heat sealer for best results
Variability in reference gene C_q values	Expression of reference gene is not stable in RNA sample	Analyze expression levels of multiple reference genes from all sample/condition combinations and choose a stably expressed target(s)
RNA quality control assay is outside acceptable range	RNA is degraded	Prepare and purify a new RNA sample, keeping it at –80°C until use Limit freeze-thaw cycles Eliminate RNases from workspace/reagents
Spikes in early cycles of amplification traces	Bubbles present in wells and popping during cycling	Take care when adding samples to plates, especially 384-well plates. Position the pipet tips almost to the bottom and dispense sample into wells, being careful not to go to the second stop, which will expel a small air bubble into the sample
Standard curve using synthetic template does not appear to be linear, or efficiency is not close to what is described in the validation data	Loss of synthetic template during serial dilutions	Use 10 ng/µl of tRNA in TE (pH 8.0) when diluting template

Common PCR inhibitors.

Source of Contamination	
Sample	**Isolation Method**
Melanin	Ethanol >1% v/v
Polysaccharides	Proteinase K
Hemoglobin	DMSO >5%
Chlorophyll	EDTA >50 mM
Polyphenolics	SDS >0.01% w/v
Heparin	Sodium acetate >5 mM
Humic acid	Mercaptoethanol
Hematin	Guanidinium
	Phenol >0.2% v/v
	DTT >1 mM

10 Ordering Information

To order PrimePCR™ products, visit **www.bio-rad.com/PrimePCR**. All products must be ordered and quoted online to ensure correct assays of interest and configuration of panels. To check for availability of genes and determine the best panel based on your targets of interest, visit **www.bio-rad.com/primepcr_lookup** and use our easy lookup tool.

PrimePCR Assays, Panels, and Plates

Catalog#	Description
100-25636 —100-25641	SYBR® Green Assays
100-31225 — 100-31239	qPCR Probe Assays
100-31240 — 100-31251	ddPCR™ Probe Assays
100-25220 — 100-25225	Custom Primer Assay
100-31261 — 100-31275	Custom qPCR Probe Assay
100-31276 — 100-31281	Custom ddPCR Probe Assay
100-25031 — 100-40427	Predesigned Pathway/Disease Panels
100-25216 — 100-25219	Custom 96-Well Plates
100-25210 — 100-25212	Custom 384-Well Plates
100-25695	Reverse Transcription Control Assay
100-25694 and 100-29103	RNA Quality Assay
100-25591 and 100-29101	Positive PCR Control Assay
100-25352 and 100-25352	Genomic DNA Control Assay
100-31286	Reverse Transcription Control Probe Assay
100-31287 and 100-32858	RNA Quality Control Probe Assay

Catalog#	Description
100-31288	Positive PCR Control Probe Assay — FAM
100-31289	Positive PCR Control Probe Assay — HEX
100-31290 and 100-32859	Genomic DNA Control Probe Assay
100-25716	Synthetic DNA SYBR® Control Templates
100-31285	Synthetic DNA Probe Control Templates
100-41594	Custom PreAmp Assay
100-41595	SYBR® Green PreAmp Assay
100-41596	Probe PreAmp Assay

Bulk Discounts

Please note that pricing and bulk discounting are subject to change.

Assay and Control Bulk Discounts

25% off 5 or more assays

Pathway and Collection Panel Bulk Discounts

25% off 5–9 plates

45% off 10–19 plates

60% off 20 or more plates

Custom Plate Bulk Discounts

Custom 96-well and 384-well plates with 1–96 unique genes:

20% off 10–19 plates

35% off 20 or more plates

Custom 384-well plates with 97 or more unique genes:

20% off 20–29 plates

35% off 30 or more plates

Related Products

To learn more about Bio-Rad's complete solution for real-time PCR, visit **www.bio-rad.com/amplification**.

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