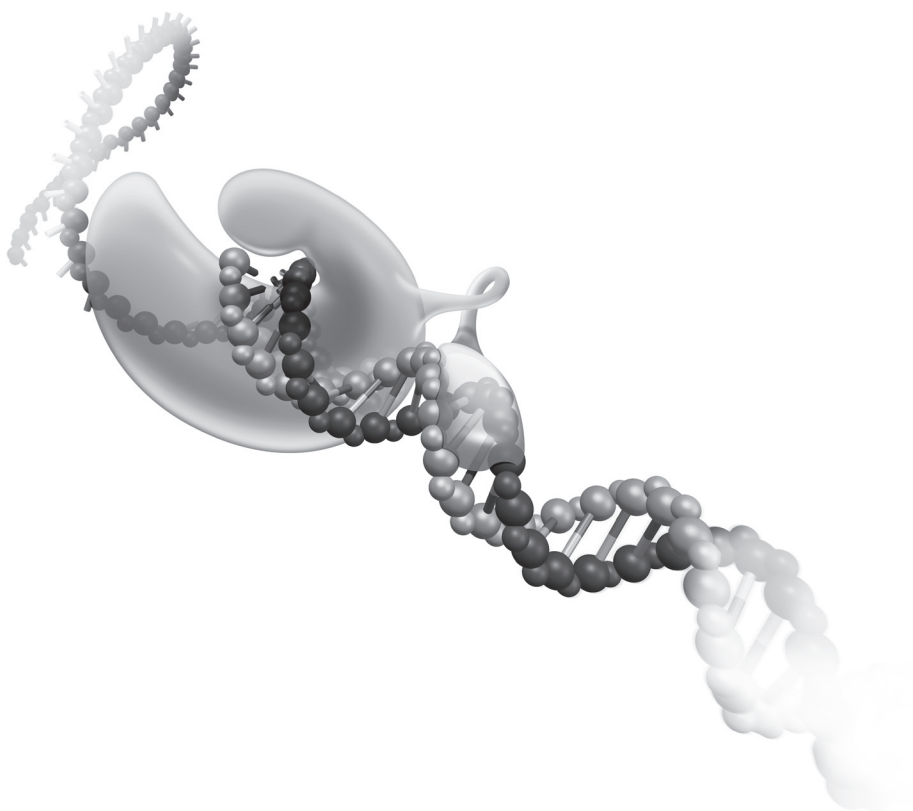


---

# PrimePCR™ Assays, Panels, and Controls

## Instruction Manual

For technical support, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.  
For research use only. Not for diagnostic procedures.



**BIO-RAD**



# Table of Contents

Introduction	1
Gene Expression Assay Protocol	2
Positive PCR Control Assay (PCR)	3
Reverse Transcription Control Assay (RT)	4
DNA Contamination Control Assay (gDNA)	5
RNA Quality Assay (RQ1 and RQ2)*	6
DNA Template	8

## Introduction

PrimePCR™ assays for real-time PCR are designed for SYBR® Green supermix-based gene expression analysis. Every primer assay has been wet-lab validated for guaranteed performance. Validation information is available for each assay at [www.bio-rad.com/PrimePCR](http://www.bio-rad.com/PrimePCR)

## Storage and Stability

PrimePCR assays are guaranteed for 12 months when stored at 4°C. For long term storage, store at –20°C.

## Gene Expression Assay Protocol

This manual describes how to use the PrimePCR assays, panels and controls for gene expression analysis. Please refer to the respective product manuals for additional products recommended for this protocol.

### Step 1: Isolate RNA

Purify total RNA from your samples(s) of interest. Bio-Rad recommends using the Aurum™ total RNA mini kit. This kit includes a DNase digest to ensure the removal of genomic DNA.

### Step 2: Generate cDNA

Perform reverse transcription to generate cDNA. For each sample use normalized concentrations of RNA. PrimePCR assays have been optimized for use with iScript™ cDNA synthesis products. All assays were wet-lab validated with iScript advanced cDNA synthesis kit for RT-qPCR.

### Step 3: Prepare Real-Time PCR Reaction Mix

PrimePCR assays are provided as a 20x stock in solution or lyophilized in a well on a plate. Scale up PCR reaction mix proportionally for multiple reactions. PrimePCR assays have been optimized for use with SsoAdvanced™ SYBR® Green supermix. iTaq™ Universal SYBR® Green supermix is recommended for use on real-time PCR instruments that require ROX-normalization. Control assay preparation is described below.

PCR reaction mix	Volume per reaction		
	Individual assays	96-well plate	384-well
PrimePCR assay (20x)	1 µl	Dried in well	Dried in well
SsoAdvanced™ SYBR® Green supermix (2x)	10 µl	10 µl	5 µl
cDNA sample	Variable	Variable	Variable
RNase/DNase-free water	Variable	Variable	Variable
<b>Total reaction volume</b>	<b>20 µl</b>	<b>20 µl</b>	<b>10 µl</b>

Transfer the appropriate volume of the PCR reaction mix into each well: 20 µl for a 96-well plate, or 10 µl for a 384-well plate. Seal the plate with the appropriate seal. Briefly centrifuge the plate to remove bubbles.

### Step 4: Real-Time PCR Run

Perform amplification and collect fluorescence data on your real-time PCR instrument using the following cycling protocol. This protocol can be used for standard and fast cycling instruments.

#### PrimePCR Cycling Protocol

Step	Temperature	Time	# Cycles
Activation	95°C	2 min	1
Denaturation	95°C	5 sec	40
Anneal/Extension	60°C	30 sec*	40
Melt Curve	65–95°C (0.5°C increments)	5 sec/step	1

\* The annealing and extension step can be shortened to 15 seconds.

### Step 5: Data Analysis

Process your data and evaluate results using CFX Manager™ 3.0 or other analysis software.

## Positive PCR Control Assay (PCR)

**Purpose** The positive PCR control assay (PCR) targets a synthetic DNA 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 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 of PrimePCR positive PCR control assay (20x stock solution includes primers and DNA template)

#### 96-Well or 384-Well Plate

- Primers are lyophilized in the designated well(s) on the plate (DNA template is provided in a separate tube)
- 200 reactions of PrimePCR positive control DNA template (20x stock solution)

### Use

#### Individual Assay

For each cDNA sample, prepare a positive PCR control reaction mix as follows:

PrimePCR positive PCR control assay (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>20 <math>\mu</math>l</b>

#### 96-Well or 384-Well Plate

1. For each cDNA sample, prepare a positive PCR control reaction mix as follows:

##### 96-Well

PrimePCR positive PCR control DNA template (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>20 <math>\mu</math>l</b>

##### 384-Well

PrimePCR positive PCR control DNA template (20x)	0.5 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	5 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>10 <math>\mu</math>l</b>

2. Add reaction mix to corresponding PCR control well(s) on the plate

### Interpretation

#### For a Single Sample

$C_q \geq 30$ : poor qPCR performance; will likely compromise gene expression results.

#### For Two or More Samples

Designate one of the positive PCR control assays as your control sample.

To determine the  $\Delta C_q$  between the control sample and each remaining sample, use the following equation:

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

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

## Reverse Transcription Control Assay (RT)

### Purpose

The reverse transcription control assay introduces a synthetic RNA transcript into the cDNA synthesis reaction to evaluate RT performance. The sequence of the synthetic RNA template is not present in the transcriptome.

Designed to qualitatively assess:

- Performance of the 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 PrimePCR reverse transcription control primer assay (20x stock solution)
- 200 reactions of PrimePCR reverse transcription control RNA template\* (40 x 10<sup>6</sup> lyophilized copies/tube)

#### 96-Well or 384-Well Plate

- Primers are lyophilized in the designated well(s) on the plate
- 200 reactions of PrimePCR reverse transcription control RNA template\* (40 x 10<sup>6</sup> lyophilized copies/tube)

\*Resuspend lyophilized RNA template in 100 µl TE buffer pH 7.5 before use. Synthetic RNA is intended for use upon resuspension.

### Use

#### Reverse Transcription

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

#### Individual Assay

For each cDNA sample, prepare a reverse transcription control reaction mix as follows:

PrimePCR RT control primer assay (20x)	1 µl
SsoAdvanced™ SYBR® Green supermix (2x)	10 µl
cDNA sample with RNA template	1 µl
RNase/DNase-free water	8 µl
<b>Total reaction volume</b>	<b>20 µl</b>

#### 96-Well or 384-Well Plate

1. For each cDNA sample, prepare a reverse transcription control reaction mix as follows:

##### 96-Well

SsoAdvanced™ SYBR® Green supermix (2x)	10 µl
cDNA sample with RNA template	1 µl
RNase/DNase-free water	9 µl
<b>Total reaction volume</b>	<b>20 µl</b>

##### 384-Well

SsoAdvanced™ SYBR® Green supermix (2x)	5 µl
cDNA sample with RNA template	0.5 µl
RNase/DNase-free water	4.5 µl
<b>Total reaction volume</b>	<b>10 µl</b>

2. Add reaction mix to corresponding RT control well(s) on the plate.

continues

## Reverse Transcription Control Assay (RT) cont.

### Interpretation For a Single Sample

$Cq \geq 30$ : poor reverse transcription performance; will likely compromise gene expression results.

### For Two or More Samples

Designate one of the reverse transcription control assays as your control sample. To determine the  $\Delta Cq$  between the control sample and each remaining sample, use the following equation:

$$|(RT\ Cq\ for\ designated\ control\ sample) - (RT\ Cq\ for\ sample)| = \Delta Cq$$

$\Delta Cq > 1$ : samples differ in their reverse transcription performance; will likely compromise gene expression results.

## DNA Contamination Control Assay (gDNA)

### Purpose

The DNA contamination control assay (gDNA) targets a non-transcribed region of the genome.

Designed to:

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

### Format

#### Individual Assay

- 200 reactions of PrimePCR DNA contamination control assay (20x stock solution)

#### 96-Well or 384-Well Plate

- Primers are lyophilized in the well(s) on the plate

### Use

#### Individual Assay

For each cDNA sample, prepare a DNA contamination control reaction mix as follows:

PrimePCR gDNA control primer assay (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>20 <math>\mu</math>l</b>

#### 96-Well or 384-Well Plate

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

##### 96-Well

SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>20 <math>\mu</math>l</b>

##### 384-Well

SsoAdvanced™ SYBR® Green supermix (2x)	5 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total reaction volume</b>	<b>10 <math>\mu</math>l</b>

2. Add reaction mix to corresponding gDNA control well(s) on the plate.

continues

## DNA Contamination Control Assay (gDNA) cont.

### Interpretation For a Single Sample

$Cq \geq 35$ : below single copy detection. No gDNA present.

$Cq < 35$ : 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  $Cq$  value for a given gene of interest (GOI) to the  $Cq$  value for the DNA contamination control assay using the following equation:

$$|(GOI\ Cq) - (gDNA\ Cq)| = \Delta Cq$$

#### gDNA contribution to GOI signal

$\Delta Cq$	Percent Contribution
1	50%
2	25%
3	12.5%
4	6.25%
5	3.125%
6	1.5625%
7	0.78125%

### For Two or More Samples

Designate one of the DNA contamination control assays as your control sample. To determine the  $\Delta Cq$  between the control sample and each remaining sample, use the following equation:

$$|(gDNA\ Cq\ for\ designated\ control) - (gDNA\ Cq\ for\ sample)| = \Delta Cq$$

$\Delta Cq < 1$ : samples have similar levels of gDNA contamination; gDNA contamination will likely have little to no effect on results.

$\Delta Cq \geq 1$ : 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 (RQ1 and RQ2) targets two mRNA transcripts that can indicate when RNA degradation may be negatively impacting gene expression results.

Designed to:

- Determine if RNA integrity may adversely affect PCR results of a single sample
- Compare the relative RNA integrity between different samples in order to determine if PCR results may be affected

\* The RQ1 and RQ2 assays must be used as a pair for each cDNA sample.

### Format

#### Individual Assay

- 200 reactions of PrimePCR RQ1 RNA quality assay (20x stock solution)
- 200 reactions of PrimePCR RQ2 RNA quality assay (20x stock solution)

#### 96-Well or 384-Well Plate

- The RQ1 and RQ2 primers are lyophilized in the designated wells on a plate.

continues

## RNA Quality Assay (RQ1 and RQ2) cont.

### Use

#### Individual Assay

For each cDNA sample, prepare the RQ1 and RQ2 RNA quality assay reaction mixes as follows:

PrimePCR RQ1 assay (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ1 reaction volume</b>	<b>20 <math>\mu</math>l</b>

PrimePCR RQ2 assay (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ2 reaction volume</b>	<b>20 <math>\mu</math>l</b>

#### 96-Well or 384-Well Plate

1. For each cDNA sample, prepare the RQ1 and RQ2 RNA quality assay reaction mixes as follows. The RQ1 and RQ2 reaction mixes can be combined to streamline PCR reaction preparation.

##### 96-Well

SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ1 reaction volume</b>	<b>20 <math>\mu</math>l</b>

SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ2 reaction volume</b>	<b>20 <math>\mu</math>l</b>

##### 384-Well

SsoAdvanced™ SYBR® Green supermix (2x)	5 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ1 reaction volume</b>	<b>10 <math>\mu</math>l</b>

SsoAdvanced™ SYBR® Green supermix (2x)	5 $\mu$ l
cDNA sample	Variable
RNase/DNase-free water	Variable
<b>Total RQ2 reaction volume</b>	<b>10 <math>\mu</math>l</b>

2. Add the RQ1 reaction mix to corresponding RQ1 control well(s) on the plate, and the RQ2 reaction mix to the RQ2 control well(s) on the plate.

continues

## RNA Quality Assay (RQ1 and RQ2) cont.

### Interpretation For a Single Sample

To determine the  $\Delta Cq$  between the RQ1 and RQ2 assay, use the following equation:

$$|(RQ2\ Cq) - (RQ1\ Cq)| = \Delta Cq$$

$Cq > 3.0$ : RNA integrity may compromise gene expression results.

### For Two or More Samples

Designate one of the RNA quality assays as your control sample. To determine the  $\Delta\Delta Cq$  between the control sample and each remaining sample, use the following equation:

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

$\Delta\Delta Cq = 0$  to 1.5: samples are similar in quality; will likely have little or no effect on gene expression results.

$\Delta\Delta Cq = 1.5$  to 4.0: RNA integrity differs; will likely have a slight to moderate effect on gene expression results.

$\Delta\Delta Cq > 4.0$ : RNA integrity differs significantly; will likely have a significant effect on gene expression results.

## DNA Template

### Purpose

PrimePCR DNA templates are single stranded synthetic DNA templates that are complementary to their corresponding gene-specific primer assay.

Designed to:

- Produce a positive real-time PCR result when used with the corresponding gene-specific PrimePCR assay
- Generate a standard curve

### Format

4 x 10<sup>9</sup> copies/200 $\mu$ l (20 x 10<sup>6</sup> copies/ $\mu$ l)

### Use

#### PCR Control

For a PrimePCR assay, prepare the reaction mix as follows:

PrimePCR assay for gene of interest (20x)	1 $\mu$ l
SsoAdvanced™ SYBR® Green supermix (2x)	10 $\mu$ l
PrimePCR DNA template for gene of interest (20x)	1 $\mu$ l
RNase/DNase-free water	8 $\mu$ l
<b>Total reaction volume</b>	<b>20 <math>\mu</math>l</b>

#### Standard Curve

Perform a standard curve using a seven-point, tenfold serial dilution series from 20 million copies down to 20 copies. It is strongly recommended to include 90 ng of carrier tRNA in each dilution point.

### Interpretation When Used as a qPCR Control

$Cq < 30$ : assay performance is not affected.

$Cq > 30$ : poor PCR performance; will likely compromise results.



## Ordering Information

To order PrimePCR products, visit [www.bio-rad.com/PrimePCR](http://www.bio-rad.com/PrimePCR)

## Related Products

To learn more about Bio-Rad's complete solution for real-time PCR, visit our website: [www.bio-rad.com/amplification](http://www.bio-rad.com/amplification)

## Customer Service and Technical Support

For customer service or technical support, please contact us at 1-800-4-BIORAD (1-800-424-6723).

### Notice To Purchaser:

SYBR is a trademark of Life Technologies Corporation. Bio-Rad Laboratories, Inc. is licensed by Life Technologies Corporation to sell reagents containing SYBR Green I for use in real-time PCR for research purposes only.



**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**



*Life Science  
Group*

**Web site** [www.bio-rad.com](http://www.bio-rad.com) **USA** 800 424 6723  
**Australia** 61 2 9914 2800 **Austria** 01 877 89 01 **Belgium** 09 385 55 11  
**Brazil** 55 11 5044 5699 **Canada** 905 364 3435 **China** 86 21 6169 8500  
**Czech Republic** 420 241 430 532 **Denmark** 44 52 10 00  
**Finland** 09 804 22 00 **France** 01 47 95 69 65 **Germany** 089 31 884 0  
**Greece** 30 210 9532 220 **Hong Kong** 852 2789 3300  
**Hungary** 36 1 459 6100 **India** 91 124 4029300 **Israel** 03 963 6050  
**Italy** 39 02 216091 **Japan** 03 6361 7000 **Korea** 82 2 3473 4460  
**Mexico** 52 555 488 7670 **The Netherlands** 0318 540666  
**New Zealand** 64 9 415 2280 **Norway** 23 38 41 30  
**Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700  
**Russia** 7 495 721 14 04 **Singapore** 65 6415 3188  
**South Africa** 27 861 246 723 **Spain** 34 91 590 5200  
**Sweden** 08 555 12700 **Switzerland** 061 717 95 55  
**Taiwan** 886 2 2578 7189 **Thailand** 800 88 22 88  
**United Kingdom** 020 8328 2000