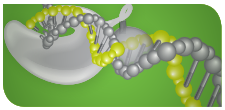

PrimePCR™ ASSAYS AND PANELS

PrimePCR Assay
Quick Guide

Real-Time PCR Workflow

For a complete guide to PrimePCR Assays, Panels, and Controls, visit **bio-rad.com/PrimePCR** to download the instruction manual.

Step 1: Isolate RNA

- Aurum™ Total RNA Mini kit
- Aurum Total RNA Fatty and Fibrous Tissue Kit

Step 2: Synthesize cDNA with reverse transcription (RT) control

- Prepare cDNA with an iScript™ reverse transcription kit
- Add 1 µl RT control template to each 20 µl cDNA synthesis reaction

Note: Before first use, suspend the RT control template in 200 µl nuclease-free TE buffer (pH 7.5) or the same reagent used to dilute the RNA samples. Keep the template on ice.

Step 3: Prepare real-time PCR reaction

1. Thaw and mix reagents.
2. Prepare all quantitative PCR (qPCR) reaction mixes according to Table 1 or 2.
3. Transfer the appropriate volume of the PCR reaction mix into each well.
4. Seal the plate with an appropriate seal and briefly centrifuge.
5. Load the PCR plate into a real-time PCR instrument. Follow the PrimePCR cycling protocol in Table 3.

Table 1. Individual assay (SYBR® Green or probe) reaction setup.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR Assay or PrimePCR Control Assay	1 µl	0.5 µl	1x
2x SsoAdvanced™ Universal 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**	**—**

Table 2. Predesigned or custom plate (SYBR® Green) reaction setup.

Component	Volume per Reaction		Final Concentration
	96-Well	384-Well	
20x PrimePCR Assay or PrimePCR Control Assay*	Dried in well	Dried in well	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**	**—**

* For the PrimePCR PCR Control Assay, add 1 µl for 96-well plates or 0.5 µl for 384-well plates to the well(s) designated for PCR control. The total volume will be 21 µl in a 96-well plate or 10.5 µl in a 384-well plate for the PrimePCR PCR Control Assay reaction. Additional volume will not affect the qPCR reaction.

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Step 4: Perform real-time PCR

Table 3. PrimePCR cycling protocol.

Step	Temperature	Time	Number of Cycles
Activation	95°C	2 min	1
Denaturation	95°C	5 sec	40
Annealing/extension	60°C	30 sec	
Melt curve*	65–95°C (0.5°C increments)	5 sec/step	1

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

Step 5: Analyze gene expression data

- CFX Manager™ Software
- PrimePCR Analysis Software
- Analyze PrimePCR Controls according to Table 4

Table 4. Analysis of PrimePCR Controls.

PrimePCR Control	Purpose	Pass, Cq	Fail, Cq
PCR control assay	Tests performance of qPCR reaction with sample	<30	≥30
Reverse transcription control assay	Tests performance of reverse transcription reaction	<30	≥30
DNA contamination control assay	Tests for genomic DNA in sample	≥35	<35
RNA quality assay	Tests RNA integrity in sample	ΔCq ≤3*	ΔCq >3*

* ΔCq = |(RQ2 Cq) – (RQ1 Cq)|
Cq, quantification cycle; RQ, RNA quality assay.