

PrimePCR™ PreAmp Assay Quick Guide

Real-Time PCR Preamplification

For a complete guide to PrimePCR assays, panels, and controls, visit bio-rad.com/PrimePCR to download the instruction manual.



Step 1: Isolate RNA

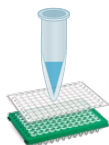
Step 2: Synthesize cDNA



Step 3: Pool PrimePCR PreAmp assays

1. Transfer 5 µl of each assay (up to 100 assays) into a microcentrifuge tube.
2. Add nuclease-free water to bring the total volume of the assay pool up to 500 µl.
3. Mix and briefly centrifuge.
4. Use 5 µl of the pool in the preamplification reaction.

Note: The remainder of the assay pool is stable at 4°C for up to 30 days and at -20°C for up to 1 year.



Step 4: Prepare and cycle preamplification reaction

1. Thaw and mix reagents.
2. Prepare the preamplification reaction mix on ice according to Table 1.
3. Mix the reaction mix thoroughly and transfer it to a PCR tube or plate.
4. Load the reaction(s) into a thermal cycler and program the instrument according to Table 2.

Table 1. Preamplification reaction setup.

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

Table 2. Preamplification thermal cycler protocol.

| Step | Temperature, °C | Time | Number of Cycles |
|---------------------|-----------------|--------|------------------|
| Activation | 95 | 3 min | 1 |
| Denaturation | 95 | 15 sec | 12 |
| Annealing/extension | 58 | 4 min | 12 |
| Hold | 4 | ∞ | 1 |



Step 5: Prepare real-time PCR reaction



1. Dilute the preamplification reaction 1:5 to 1:20, depending on the number of assays planned.
2. Use 2 μ l of the dilution per 20 μ l reaction or 1 μ l per 10 μ l reaction.
3. Prepare the setup for all corresponding PrimePCR reactions according to the PrimePCR instruction manual.
4. Transfer the appropriate volume of the PCR reaction mix into each well.
5. Seal the plate and briefly centrifuge.

Step 6: Cycle in real-time PCR instrument



1. Use the PrimePCR thermal cycler protocol (Table 3).
2. Analyze the gene expression data using CFX Manager™ software or PrimePCR analysis software.

Table 3. PrimePCR thermal cycler protocol.

| Step | Temperature, °C | Time | Number of Cycles |
|---------------------|--------------------------|------------|------------------|
| Activation | 95 | 2 min* | 1 |
| Denaturation | 95 | 5 sec | 40 |
| Annealing/extension | 60 | 30 sec | 40 |
| Melt curve** | 65–95 (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.



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Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

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