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.

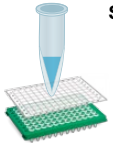
Step 1: Isolate RNA

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

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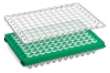
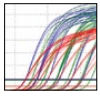
Step 5: Prepare real-time PCR reaction



1. Dilute the preamplification reaction 1:5 to 1:20, depending on the number of assays planned.
2. Use 2 µl of the dilution per 20 µl reaction or 1 µl per 10 µl 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 Laboratories, Inc.

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Web site *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 3065 7550* ***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*** *81 3 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*** *026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *1800 88 22 88* ***United Kingdom*** *020 8328 2000*

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