

ClearPeak 2x Probe Master Mix

Troubleshooting Guide

100 rxn, 500 rxn

Cat No: PMM1, PMM5

Use this guide for common probe-qPCR setup, template, probe, and cycling problems with ClearPeak 2x Probe Master Mix. The 2x premix contains EcoTaq DNA Polymerase, PCR buffer, dNTPs, Mg²⁺, enhancers, and stabilizers; templates, primers, and probes are added by the user.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles.
2. For a 20 uL reaction use 10 uL 2x Probe Master Mix, the probe assay, template, and water to volume.
3. The datasheet gives 10-100 ng genomic DNA or 1-10 ng cDNA as usual template amounts.
4. Mix gently by inversion and avoid foaming.
5. Use the two-step program first; a three-step program may be attempted for primers with lower T_m values.
6. Probe concentration depends on instrument, probe type, and fluorophore; follow the instrument/probe requirements.

Problem	Most likely cause	Recommended action
No amplification is detected	Template amount, probe/primer setup, or cycling conditions may not be appropriate.	Confirm the 20 uL setup and evaluate template within the datasheet ranges: 10-100 ng genomic DNA or 1-10 ng cDNA.
Ct is late or amplification is weak	Template input may be suboptimal for the target copy number.	Perform a template gradient within the stated range to determine the optimal input for the target and species.
Non-specific amplification is observed	Primer/cycling conditions may not suit the target.	Use the datasheet recommendation to optimize setup and cycling for the template, primer structure, and target fragment size; consider the three-step program for lower-T _m primers.
Probe fluorescence is weak	Probe concentration may not be appropriate for the instrument, probe chemistry, or fluorescent label.	Adjust probe concentration according to the real-time PCR instrument manual and the specific probe requirements.
Results vary between replicate wells	Pipetting, mixing, or template amount may not be consistent.	Mix gently without foaming and standardize template, assay components, and total reaction volume across replicates.
Performance declines after repeated use	Repeated freeze-thaw may have affected the master mix.	Store at -20°C and minimize freeze-thaw cycles.
Two-step PCR gives poor results	Primer T _m may not be compatible with the two-step program.	Use the datasheet three-step option when lower-T _m primers do not perform well with two-step cycling.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Reaction volume is incorrect	The mix may not have been used at 1x final concentration in a 20 uL reaction.	Use 10 uL of the 2x master mix in a 20 uL final reaction and adjust the remaining volume with the other components and water.
Template-to-template performance differs	Different targets/species can have different copy numbers.	Use gradient dilution of the template to determine optimal input rather than assuming the same mass is optimal for every target.
Foam or bubbles are present in the mix	The tube may have been mixed too vigorously.	Mix gently by inversion as specified and avoid foaming before dispensing reactions.
Probe signal is inconsistent between instruments	Probe concentration requirements can vary by instrument and fluorescent label.	Recheck instrument-specific probe guidance and maintain the same instrument/probe configuration when comparing runs.
Fast cycling gives poor target performance	The target may require reaction-condition optimization despite the mix supporting rapid qPCR.	Optimize PCR setup and reaction timing according to target fragment size and primer structure, following the datasheet notes.

Still having trouble? Recheck -20°C storage, 10 uL master mix in a 20 uL reaction, template amount, instrument-specific probe concentration, gentle mixing, and the two-step versus three-step cycling choice.