

ClearPeak 2x SYBR Master Mix

Troubleshooting Guide

100 rxn, 500 rxn

Cat No: SMM1, SMM5

Use this guide for common SYBR Green qPCR amplification, specificity, template, primer, and handling problems with ClearPeak 2x SYBR Master Mix. The premix uses an antibody-based hot-start EcoTaq DNA Polymerase and is designed for rapid, sensitive real-time qPCR.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles; after thawing the datasheet permits up to 6 months at 2-8°C under dark conditions.
2. Protect the SYBR-containing mix from strong light.
3. For 20 uL use 10 uL 2x SYBR Master Mix, forward/reverse primers, template, and water to volume.
4. The typical final primer concentration is 0.2 uM; 0.1-1.0 uM is given as an optimization range.
5. The usual template guidance is 10-100 ng genomic DNA or 1-10 ng cDNA.
6. Use two-step cycling first; use the three-step option for lower-T_m primers or when target conditions require it.

Problem	Most likely cause	Recommended action
No amplification is detected	Template amount, primer amount, or reaction composition may not be appropriate.	Confirm the 20 uL setup and use template within the datasheet guidance: 10-100 ng genomic DNA or 1-10 ng cDNA.
Amplification efficiency is low	Primer concentration may be too low or the cycling program may not suit the target.	Use 0.2 uM as the typical primer concentration and optimize within 0.1-1.0 uM; use three-step cycling when needed.
Non-specific amplification is observed	Primer concentration may be too high or reaction conditions may not suit the primer/template combination.	Reduce primer concentration within the stated 0.1-1.0 uM range and optimize cycling according to the datasheet notes.
Ct is late	Template amount may be too low for the target copy number.	Perform a template gradient within the recommended mass range to determine the optimal input.
Replicate Ct values vary	Pipetting, template amount, or mixing may not be consistent.	Mix gently without foaming and standardize primer concentration, template amount, and final reaction volume across wells.
SYBR fluorescence is weak after storage	The mix may have been exposed to strong light or repeated freeze-thaw.	Protect from light, minimize freeze-thaw, and follow the -20°C or permitted dark 2-8°C storage conditions.
Two-step cycling performs poorly	Primer T _m may be too low for the two-step program.	Use the datasheet three-step program and optimize the annealing step for the primer set.

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Problem	Most likely cause	Recommended action
Amplicon >200 bp amplifies poorly with rapid timing	Extension time may be too short for the fragment size.	The datasheet notes a 10-sec minimum for amplicons <200 bp and recommends 30 sec when the amplicon exceeds 200 bp.
Reaction volume is incorrect	The 2x mix may not have been used at the intended 1x final concentration.	Use 10 uL of 2x SYBR Master Mix in a 20 uL final reaction.
Foam or bubbles are present	The tube may have been mixed too vigorously.	Mix gently by inversion and avoid foaming before dispensing reactions.
Template-to-template results differ strongly	Different targets/species may have different copy numbers.	Use gradient dilution of template to determine the optimal input for each target rather than assuming one mass is universal.
Run-to-run performance changes	Storage, light exposure, primer concentration, template input, or cycling may not be standardized.	Standardize dark storage, primer concentration, template amount, and the selected cycling program across runs.

Still having trouble? Recheck light-protected storage, 10 uL master mix in a 20 uL reaction, primer concentration within 0.1-1.0 uM, template mass, amplicon-dependent timing, and two-step versus three-step cycling.