

ClearPeak SYBR Green qPCR Assays Troubleshooting Guide

250 rxn

Cat No: qPP250

Use this guide for common amplification problems with ClearPeak SYBR Green qPCR Assays. These are pre-designed, pre-validated gene-specific primer pairs intended for real-time PCR with an intercalating dye system such as ClearPeak 2x SYBR Master Mix.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles; the datasheet states stable storage for 12 months at -20°C.
2. Confirm the correct gene-specific Assay ID.
3. For 20 uL use 10 uL 2x SYBR Master Mix, 1 uL SYBR Green qPCR Assay, 2-5 uL cDNA, and water to 20 uL.
4. Use the provided two-step or three-step qPCR reaction conditions.

Problem	Most likely cause	Recommended action
No amplification is detected	The wrong gene-specific assay may have been selected or the reaction may not match the specified setup.	Confirm the Assay ID and prepare the 20 uL reaction using 10 uL 2x SYBR Master Mix, 1 uL assay, and 2-5 uL cDNA.
Ct is late	cDNA input may be at the low end of the stated range or reaction setup may differ between wells.	Evaluate cDNA within the 2-5 uL range while keeping the rest of the 20 uL setup constant.
Replicates are inconsistent	Pipetting or freeze-thaw history may vary.	Use consistent pipetting, maintain -20°C storage, and minimize freeze-thaw cycles.
Two-step cycling is unsatisfactory	The assay may perform better with the provided three-step program.	Use the three-step program listed in the datasheet rather than changing multiple variables at once.
Reaction volume is incorrect	Water or cDNA volume may not have been adjusted to the specified total.	Bring the reaction to exactly 20 uL with ddH ₂ O after adding master mix, assay, and cDNA.
Results changed after storage	Repeated freeze-thaw may have reduced performance.	Use assay material with a documented -20°C storage history and minimal freeze-thaw exposure.
Different assays show unexpected Ct shifts	Different gene-specific assays may have been compared with inconsistent cDNA input.	Confirm each Assay ID and keep cDNA input and qPCR setup consistent when comparing assays.
Amplification is weak despite correct setup	The selected cycling format may not be optimal for the assay/template combination.	Compare the datasheet two-step and three-step programs while keeping reaction composition unchanged.

Still having trouble? Recheck the gene-specific Assay ID, -20°C storage, the 10 uL master mix + 1 uL assay + 2-5 uL cDNA setup, and the specified two-step or three-step qPCR program.