

ClearPeak One Step qRT-PCR Probe Kit

Troubleshooting Guide

100 rxn

Cat No: 1SQP100

Use this guide for signal, specificity, ROX, and cycling problems with the ClearPeak One Step qRT-PCR Probe Kit. Reverse transcription and probe qPCR occur consecutively in one closed tube, using RNA template, the 2x Probe Buffer, Enzyme Mix, and a TaqMan-type assay probe.

Before You Start - Quick Checks

1. Store at -20°C protected from light; for frequent use, 2-8°C storage is permitted by the datasheet. Avoid repeated freeze-thaw cycles.
2. Select ROX according to the real-time PCR instrument: no ROX, low ROX, or high ROX as listed in the datasheet.
3. For 1 mL buffer add 20 uL 100x ROX for high-ROX instruments, 2 uL for low-ROX instruments, or no ROX when correction is not required.
4. Keep thawed RNA, primers, buffer, enzyme mix, and RNase-free water on ice until use; mix gently and avoid foaming.
5. For 20 uL use 10 uL 2x Probe Buffer, 1 uL Enzyme Mix, 1 uL TaqMan Assay Probe (20x), RNA <100 ng, and water to 20 uL.
6. Use the specified two-step program first; a three-step program may be attempted for primers with lower T_m values.

Problem	Most likely cause	Recommended action
No amplification is detected	The RNA template, probe assay, enzyme mix, or reaction setup may be incomplete.	Rebuild the 20 uL reaction with all specified components and keep RNA input below 100 ng as stated.
Ct is very late	RNA input or reverse-transcription conditions may not be suitable for the target.	Verify RNA input, use the specified 50°C reverse-transcription step for 15-30 min, and keep the remainder of the reaction setup unchanged.
Fluorescence baselines vary between wells	ROX concentration may not match the instrument requirement.	Use the instrument-specific no/low/high ROX setting from the datasheet and premix ROX into the 2x Probe Buffer at the specified amount.
Unexpected signal normalization occurs	The wrong ROX amount may have been added to the buffer.	For 1 mL buffer use 20 uL 100x ROX for high-ROX systems, 2 uL for low-ROX systems, or no ROX where correction is not required.
Reaction performance declines after repeated use	Repeated freeze-thaw or repeated handling of a ROX-premixed buffer may reduce performance.	Minimize freeze-thaw cycles and avoid repeated freeze-thaw of ROX-premixed buffer.
Foaming is visible during setup	Components may have been mixed too aggressively.	Mix gently but thoroughly as stated in the datasheet and avoid foaming.
Two-step cycling gives poor results with low-T_m primers	The two-step annealing/extension condition may not suit lower-T _m primers.	Use the datasheet three-step option when lower-T _m primers do not perform well in the two-step program.

Table continues on next page.

ClearPeak One Step qRT-PCR Probe Kit Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Non-specific amplification is suspected	Template, primer structure, or cycling conditions may require adjustment.	Use the datasheet recommendation to optimize PCR setup and reaction conditions according to template, primer structure, and target fragment size.
Reaction was prepared above 20 uL or below 20 uL	Water volume may not have been adjusted after adding RNA and assay components.	Bring the final reaction to exactly 20 uL with RNase-free water.
RNA input exceeds the stated limit	More than the datasheet-specified amount of RNA may have been added.	Keep RNA template below 100 ng per 20 uL reaction.
Weak signal on a high-GC or structured target	The target may still require assay-specific optimization despite the kit being designed for difficult templates.	Keep the one-step setup intact and optimize the PCR setup/cycling only within the datasheet guidance for different templates and primer structures.
Run-to-run variation is high	Storage, ROX preparation, RNA input, or cycling may not be standardized.	Standardize light-protected storage, ROX selection, RNA input, 20 uL setup, and the chosen two-step or three-step program between runs.

Still having trouble? Recheck the instrument-specific ROX requirement, RNA input below 100 ng, the 10 uL buffer + 1 uL enzyme + 1 uL probe setup, gentle mixing, and the specified RT/qPCR program before changing other variables.