

ClearPeak One Step qRT-PCR SYBR Kit Troubleshooting Guide

100 rxn

Cat No: 1SQ100

Use this guide for amplification, specificity, ROX, and handling problems with the ClearPeak One Step qRT-PCR SYBR Kit. Reverse transcription and SYBR Green I qPCR occur sequentially in one tube, with primer concentration and cycling program available for optimization within the datasheet ranges.

Before You Start - Quick Checks

1. Store at -20°C protected from light; for frequent use, 2-8°C storage is permitted. Avoid repeated freeze-thaw cycles.
2. Choose no, low, or high ROX according to the instrument; for 1 mL buffer use 0, 2, or 20 uL of 100x ROX as specified.
3. Keep thawed reagents on ice, mix gently, avoid foaming, and protect the SYBR-containing buffer from strong light.
4. For 20 uL use 10 uL 2x SYBR Buffer, 1 uL Enzyme Mix, 0.4 uL each of 10 uM forward/reverse primer, RNA <100 ng, and water to 20 uL.
5. A typical final primer concentration is 0.2 uM; the datasheet gives 0.1-1.0 uM as an optimization range.
6. Use the two-step program first; a three-step program is available when primer T_m requires it.

Problem	Most likely cause	Recommended action
No amplification is detected	One or more one-step components may be missing, RNA input may be unsuitable, or primer concentration may be too low.	Rebuild the 20 uL reaction using the specified buffer, enzyme mix, primers, RNA <100 ng, and water; if efficiency remains low, primer concentration may be increased within the 0.1-1.0 uM range.
Amplification efficiency is low	Primer concentration or cycling conditions may not suit the target.	Use 0.2 uM as the typical primer concentration and optimize within 0.1-1.0 uM; use the three-step program when lower-T _m primers require it.
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 datasheet optimization range and adjust cycling according to template, primer structure, and target size.
Fluorescence baselines differ across wells	ROX concentration may not match the instrument.	Use the listed no/low/high ROX requirement and add the specified ROX amount to the SYBR Buffer.
SYBR signal is weak after storage or setup	The fluorescent dye may have been exposed to strong light or the product may have undergone repeated freeze-thaw.	Protect the kit from light and minimize freeze-thaw cycles during storage and preparation.
Foaming occurs during reagent preparation	The buffer may have been mixed too vigorously.	Mix gently but thoroughly and avoid foaming, as stated in the datasheet.
Ct values vary between replicates	RNA amount, primer amount, ROX setting, or total reaction volume may differ between wells.	Standardize RNA input, primer concentration, ROX condition, and the 20 uL final reaction volume across replicates.

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Problem	Most likely cause	Recommended action
Two-step program performs poorly	Primer T _m may not be suitable for the two-step annealing/extension condition.	Use the three-step program described in the datasheet, with annealing in the 55-65°C range.
RNA input is excessive	More than the stated RNA amount may have been added.	Keep RNA template below 100 ng per 20 uL reaction.
Reaction volume is incorrect	Water volume may not have been adjusted after all components were added.	Bring each reaction to 20 uL with RNase-free water after adding buffer, enzyme, primers, and RNA.
Run-to-run performance changes	Storage, light exposure, ROX preparation, primer concentration, or cycling may not be standardized.	Use consistent light-protected storage and setup, the same ROX condition, primer concentration, RNA input, and cycling program across runs.
Target-specific performance remains poor	The datasheet notes that templates, primer structures, and target fragment sizes may require optimization.	Optimize PCR setup and reaction conditions for the specific template and primer structure while keeping the one-step kit composition within the stated limits.

Still having trouble? Recheck light-protected storage, instrument-specific ROX, RNA below 100 ng, the 20 uL setup, primer concentration within 0.1-1.0 uM, and the two-step versus three-step program before making further changes.