

ClearPeak microRNA SYBR Green qPCR Assays Troubleshooting Guide

250 rxn

Cat No: qmPP250

Use this guide for the two-stage ClearPeak microRNA SYBR Green qPCR Assays workflow: stem-loop primer cDNA synthesis followed by SYBR Green qPCR. The microRNA-specific 5x RT primer and qPCR primer set should remain matched to the same Assay ID.

Before You Start - Quick Checks

1. Store at -20°C for up to the stated 12 months and avoid freeze-thaw cycles.
2. For RT use 4 uL EcoScript 5x kit, 2 uL total RNA (30 ng), 4 uL 5x RT Primer, and 10 uL ddH₂O in 20 uL total.
3. Use the datasheet RT program: 42°C for 120 min, 85°C for 5 min, then 4°C hold.
4. For qPCR use 10 uL 2x SYBR Master Mix, 1 uL microRNA SYBR Green qPCR Assays, 2-5 uL stem-loop cDNA, and water to 20 uL.
5. Use the specified two-step or three-step SYBR qPCR program.

Problem	Most likely cause	Recommended action
No microRNA amplification is detected	The microRNA-specific RT primer and qPCR primer pair may not correspond to the same Assay ID.	Confirm that the 5x RT primer and SYBR qPCR primers belong to the same microRNA-specific Assay ID.
Ct is very late	RNA input, RT reaction, or qPCR cDNA input may not match the datasheet setup.	Use 30 ng total RNA in the stated RT reaction and 2-5 uL of the resulting cDNA in the qPCR reaction.
cDNA synthesis appears weak	The RT mixture or RT temperature program may not match the datasheet.	Use 4 uL EcoScript 5x kit + 2 uL RNA + 4 uL 5x RT Primer + 10 uL water, then run 42°C for 120 min and 85°C for 5 min.
qPCR is weak despite successful RT	The qPCR mix or cDNA input may be incorrect.	Use 10 uL 2x SYBR Master Mix, 1 uL microRNA qPCR assay, and 2-5 uL stem-loop cDNA in 20 uL total.
Replicates show large Ct variation	RNA input, cDNA volume, or pipetting may differ between wells.	Keep RNA input, cDNA volume, and total qPCR volume consistent across replicates.
Results change after repeated use	Repeated freeze-thaw may have affected the assay components.	Maintain -20°C storage and minimize freeze-thaw cycles.
RT primer and qPCR primers were interchanged	The stem-loop RT primer and qPCR primer pair are used at different stages.	Use the 5x RT primer only during cDNA synthesis and the microRNA SYBR Green qPCR assay only during qPCR.

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Problem	Most likely cause	Recommended action
Two-step qPCR is unsatisfactory	The assay may require the alternative cycling program.	Use the three-step qPCR program provided in the datasheet when the two-step program does not perform adequately.
Reaction volume is incorrect	Water or cDNA volume may not have been adjusted after the other components were added.	Bring both RT and qPCR reactions to the specified 20 uL total volume.
Run-to-run values shift	Storage, RT input, cDNA volume, or qPCR program may not be standardized.	Standardize -20°C storage, 30 ng RNA input, RT program, 2-5 uL cDNA input, and the chosen qPCR program.
Assay specificity is poorer than expected	The stem-loop RT step may not have been performed with the target-specific primer.	Use the target-specific stem-loop RT primer supplied for the assay before running the matching qPCR primer pair.

Still having trouble? Confirm that the stem-loop RT primer and qPCR primer pair match the same microRNA Assay ID, then recheck 30 ng RNA, the 42°C/120-min RT step, 2-5 uL cDNA input, and -20°C storage.