

ClearPeak microRNA Assays (FAM)

Troubleshooting Guide

150 rxn, 750 rxn

Cat No: MCA150, MCA750

Use this guide for issues that can arise in the two-stage ClearPeak microRNA Assays (FAM) workflow: microRNA-specific cDNA synthesis followed by probe qPCR. Keep the microRNA-specific RT primer and matching assay probe paired throughout the workflow.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles.
2. For cDNA synthesis use 4 uL EcoScript 5x kit, 2 uL total RNA (30 ng), 4 uL ClearPeak microRNA 5x RT primer, and 10 uL ddH₂O in 20 uL total.
3. Use the RT program specified in the datasheet: 16°C, 42°C, 85°C, then 4°C hold.
4. For probe qPCR use 10 uL 2x Probe Master Mix, 1 uL microRNA Assay Probe, 2-5 uL cDNA, and ddH₂O to 20 uL.
5. Use 95°C for 2 min, then 40 cycles of 95°C for 10 sec and 60°C for 30 sec.

Problem	Most likely cause	Recommended action
No microRNA signal is detected	The microRNA-specific RT primer and assay probe may not correspond to the same assay ID.	Confirm the microRNA-specific Assay ID and use the matching RT primer and assay probe as one assay pair.
Ct is very late	The cDNA synthesis input or qPCR cDNA amount may not match the datasheet setup.	Use 30 ng total RNA in the stated 20 uL RT reaction, then use 2-5 uL of the resulting cDNA in the 20 uL qPCR reaction.
cDNA synthesis appears unsuccessful	The RT reaction mixture or temperature program may be incomplete.	Rebuild the RT reaction using 4 uL EcoScript 5x kit, 2 uL RNA, 4 uL microRNA 5x RT primer, and 10 uL water, then follow the stated RT temperature sequence.
qPCR gives no amplification after RT	The probe qPCR setup may be incomplete or the wrong cDNA may have been used.	Use cDNA generated with the matching microRNA RT primer and prepare qPCR with 10 uL Probe Master Mix plus 1 uL assay probe.
Replicates show large Ct variation	RNA input, cDNA volume, or pipetting may differ between wells.	Keep RNA input and qPCR cDNA volume consistent across replicates and prepare all reactions to the same 20 uL final volume.
FAM fluorescence is not detected	The run may not be collecting the FAM channel or the probe assay may be missing.	Confirm FAM fluorescence acquisition and verify that the microRNA Assay Probe was added to every qPCR reaction.
Results decline after repeated use	Repeated freeze-thaw may have reduced assay performance.	Aliquot when practical and keep the assay at -20°C while avoiding repeated freeze-thaw cycles.

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Problem	Most likely cause	Recommended action
RT and qPCR components were interchanged	The RT primer and qPCR probe have different roles in the workflow.	Use the 5x RT primer only during cDNA synthesis and the microRNA Assay Probe only in the subsequent probe qPCR.
Reaction volume is not 20 uL	Water or template volume may have been added without accounting for the other components.	Adjust ddH ₂ O so both the RT and qPCR reactions finish at the datasheet-specified 20 uL total volume.
Signal differs strongly between runs	Storage history or reaction setup may not be consistent.	Standardize -20°C storage, RNA input, RT setup, cDNA volume, qPCR composition, and cycling conditions between runs.
Probe qPCR is weak despite apparently successful RT	The qPCR cycling program may not match the datasheet.	Use 95°C for 2 min followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec before changing other variables.

Still having trouble? Verify that the microRNA-specific RT primer and FAM assay probe belong to the same Assay ID, then recheck the 30 ng RNA RT setup, the 2-5 uL cDNA qPCR input, -20°C storage, and the specified RT and qPCR programs.