

ClearPeak Gene Expression Assays (FAM)

Troubleshooting Guide

250 rxn, 750 rxn

Cat No: GEA250, GEA750

Use this guide for common setup and signal problems with ClearPeak Gene Expression Assays (FAM). The assay is a pre-designed, pre-validated primer/probe set intended for use with ClearPeak 2x Probe Master Mix in a 20 uL probe qPCR reaction.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles.
2. Confirm the correct gene-specific Assay ID before preparing reactions.
3. For a 20 uL reaction use 10 uL ClearPeak 2x Probe Master Mix, 1 uL Gene Expression Assay, 2-5 uL template cDNA, and ddH₂O to 20 uL.
4. Use the datasheet cycling program: 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 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 rebuild the 20 uL reaction using 10 uL 2x Probe Master Mix, 1 uL assay, and 2-5 uL cDNA.
Ct is later than expected	The amount of template cDNA may be at the low end of the specified 2-5 uL range or the reaction setup may vary between wells.	Keep the reaction composition consistent and evaluate the cDNA input within the datasheet-specified 2-5 uL range.
Replicate Ct values are inconsistent	Pipetting differences or repeated freeze-thaw may be affecting assay consistency.	Avoid freeze-thaw cycles, thaw and mix consistently, and use the same reaction composition for all replicates.
No FAM signal is recorded	The FAM assay may not be monitored with the correct fluorescence channel or the probe reaction may be incomplete.	Confirm that the instrument run is configured to collect FAM fluorescence and that the assay and Probe Master Mix were both added.
Amplification is weak despite adequate cDNA	Cycling conditions may not match the datasheet conditions.	Run the specified 95°C/60°C cycling program before changing other variables.
Reaction volume is incorrect	The components may not have been adjusted to the specified 20 uL total volume.	Bring each reaction to exactly 20 uL with ddH ₂ O after adding master mix, assay, and cDNA.
Results changed after storage	The assay may have undergone repeated freeze-thaw or storage outside -20°C.	Use material with a documented -20°C storage history and minimize freeze-thaw cycles.
Different assays show unexpected differences	Different gene-specific assays may have been compared without confirming assay identity and template consistency.	Verify each Assay ID and keep template cDNA amount and qPCR setup identical when comparing assays.

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 95°C/60°C cycling program before changing assay conditions.