

EcoScript 5x First Strand cDNA Synthesis Kit

Troubleshooting Guide

50 rxn, 100 rxn, 200 rxn

Cat No: ERT50, ERT100, ERT200

Use this guide for common first-strand cDNA synthesis problems with the EcoScript 5x First Strand cDNA Synthesis Kit. The 5x solution already contains thermostable M-MuLV Reverse Transcriptase, RNase inhibitor, random primers, Oligo(dT) primer, dNTPs, and reaction buffer; only RNA template and water are added.

Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles.
2. For 20 uL use 4 uL EcoScript 5x kit, RNA template, and ddH₂O to 20 uL.
3. Do not exceed 2 ug total RNA or 200 ng mRNA per 20 uL reaction.
4. Use 42°C for 120 min, 85°C for 5 min, then 4°C hold as the standard datasheet program.
5. For urgent results, the datasheet notes that 42°C incubation as short as 15 min may work especially for highly expressed genes.
6. Use cDNA immediately for qPCR or store at -20°C for up to six months; for long-term storage, -70°C is recommended. Avoid repeated cDNA freeze-thaw.

Problem	Most likely cause	Recommended action
Little or no cDNA is obtained	RNA input, reaction composition, or RT incubation may not match the datasheet setup.	Use 4 uL EcoScript 5x kit in 20 uL total, keep RNA within the stated limits, and use the standard 42°C/120-min RT program.
Downstream qPCR Ct is very late	cDNA synthesis may be incomplete or RNA input may be too low for the target.	Use the standard 42°C for 120 min program before relying on the shortened urgent protocol, particularly for lower-expression targets.
Reaction contains too much RNA	RNA input may exceed the kit limits.	Keep total RNA at no more than 2 ug or mRNA at no more than 200 ng per 20 uL reaction.
Reaction volume is incorrect	Water volume may not have been adjusted after RNA addition.	Use 4 uL of the 5x kit and bring the total volume to 20 uL with RNA plus ddH ₂ O.
Results vary between RT reactions	RNA amount, freeze-thaw history, or incubation conditions may vary.	Standardize RNA input, use material stored at -20°C with minimal freeze-thaw, and keep the RT program consistent.
Short 15-minute RT gives weak results	The shortened incubation may not be sufficient for the target.	Use the standard 42°C for 120 min incubation when the shortened urgent protocol does not provide adequate cDNA.
Performance declines after repeated kit use	Repeated freeze-thaw may reduce performance.	Store at -20°C and minimize freeze-thaw cycles.

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EcoScript 5x First Strand cDNA Synthesis Kit Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
cDNA performance declines during storage	The cDNA may have been stored too long or repeatedly freeze-thawed.	Use cDNA immediately when possible; otherwise store at -20°C for up to six months or at -70°C for long-term storage and avoid repeated freeze-thaw.
Extra primers or dNTPs were added unnecessarily	The 5x kit already includes random primers, Oligo(dT) primer, dNTP mixture, RNase inhibitor, reverse transcriptase, and buffer.	Prepare the reaction as specified by adding only RNA template and water to the 5x kit unless a separate validated protocol intentionally changes the system.
RT reaction was not heat-terminated	The 85°C step may have been omitted.	Follow the datasheet program including 85°C for 5 min after the 42°C incubation.
Different targets show different cDNA performance	The shortened RT option is described mainly for urgent work and especially highly expressed genes.	Use the full 120-minute RT incubation when comparing targets of different expression levels.
Run-to-run cDNA quality changes	Kit storage, RNA input, RT time, or cDNA storage may not be standardized.	Standardize -20°C kit storage, RNA amount, 20 uL setup, RT program, and downstream cDNA storage conditions.

Still having trouble? Recheck the 4 uL kit volume in a 20 uL reaction, RNA input limits, the standard 42°C/120-min program, -20°C kit storage, and cDNA storage/freeze-thaw history before changing the workflow.