

## ClearPeak Random Hexamer Troubleshooting Guide

50 rxn, 100 rxn

Cat No: RH50, RH100

Use this guide for common random-primed cDNA synthesis issues with ClearPeak Random Hexamer. The product is a mixture of random d(N)6 oligonucleotides that anneal to complementary sites on DNA or RNA and serve as primers for DNA polymerase or reverse transcriptase.

### Before You Start - Quick Checks

1. Store at -20°C and avoid freeze-thaw cycles.
2. Use 1 uL Random Hexamer per 20 uL cDNA synthesis reaction as recommended.
3. The primer is supplied in distilled water.
4. The datasheet recommends use with EcoScript 5x First Strand cDNA Synthesis Kit.

| Problem                                                | Most likely cause                                                                                    | Recommended action                                                                                                                                          |
|--------------------------------------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>cDNA yield is low</b>                               | The recommended Random Hexamer amount may not have been used or the RT workflow may be inconsistent. | Use 1 uL Random Hexamer per 20 uL cDNA synthesis reaction and keep the RT workflow consistent.                                                              |
| <b>Priming varies between reactions</b>                | Primer volume or storage history may vary.                                                           | Maintain -20°C storage, avoid freeze-thaw cycles, and use the same 1 uL per 20 uL reaction.                                                                 |
| <b>Primer was added at the wrong volume</b>            | The reaction may not match the datasheet recommendation.                                             | Use 1 uL ClearPeak Random Hexamer in each 20 uL cDNA synthesis reaction.                                                                                    |
| <b>Performance changed after storage</b>               | Repeated freeze-thaw may have affected the primer preparation.                                       | Use material stored at -20°C with minimal freeze-thaw history.                                                                                              |
| <b>Primer was used outside a validated RT workflow</b> | The datasheet specifically recommends the EcoScript 5x First Strand cDNA Synthesis Kit.              | Use Random Hexamer within a validated DNA synthesis or reverse-transcription workflow, following the EcoScript instructions when using the recommended kit. |
| <b>Results differ from Oligo(dT)-primed cDNA</b>       | Random Hexamer anneals at random complementary sites rather than specifically to poly(A) tails.      | Treat Random Hexamer and Oligo(dT)-primed workflows as different priming strategies and keep the chosen strategy consistent when comparing samples.         |
| <b>Run-to-run cDNA results shift</b>                   | RNA input, primer volume, or handling may not be standardized.                                       | Standardize RNA input, 1 uL primer volume, storage, and cDNA synthesis conditions across runs.                                                              |

Still having trouble? Recheck -20°C storage, minimal freeze-thaw, the recommended 1 uL Random Hexamer per 20 uL cDNA reaction, and consistency of the reverse-transcription workflow.