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FAQs for Q5[®] High-Fidelity DNA Polymerase

1. What is the fidelity of Q5[®] High-Fidelity DNA Polymerase?

Q5 High-Fidelity DNA Polymerase (and all Q5 product formulations) has a fidelity >100X *Taq* DNA Polymerase.

Due to the very low frequency of misincorporation events being measured, the error rate of high fidelity enzymes, like Q5, is difficult to measure in a statistically significant manner. Although measurements from assays done side-by-side with *Taq* yield Q5 fidelity values from 100-300X *Taq*, we report ">100X *Taq*" as a conservative measurement.

2. How should I determine an appropriate annealing temperature for my reaction?

Please use NEB's [T_m Calculator](#) to determine the appropriate annealing temperature for your primer pair and polymerase/buffer of interest. Unlike other calculators, the NEB T_m Calculator takes buffer components that affect melting temperatures into consideration when calculating the best annealing temperature. Other online calculators may underestimate the best Q5 annealing temperature.

3. What should my primer concentration be when using Q5 High-Fidelity DNA Polymerase products?

Between 200 nM and 1 μM. 500 nM is recommended.

4. How should I set up a PCR experiment using Q5 High-Fidelity DNA Polymerase?

The general guidelines for a 50 μl reaction are:

- 1 unit Q5 High-Fidelity DNA Polymerase
- 200 μM each dNTP
- 0.5 μM each primer
- 2-50 pg plasmid or 50-500 ng genomic template
- 1X Q5 Reaction Buffer
- (1X High GC Enhancer - optional)
- Denature at 98°C
- Extend 20 s/kb at 72°C

Reactions should be set up on ice

5. My template is GC rich or supercoiled. How can I optimize my product yield using Q5 High-Fidelity DNA Polymerase?

The Q5 High GC Enhancer can improve yields of high GC or difficult amplicons. The Enhancer is NOT a buffer and should be added to the Q5 reaction mix (containing Q5 Reaction Buffer) at a final 1X concentration. The Q5 High GC Enhancer typically benefits high GC amplicons but can be inhibitory to high AT amplicons.

6. Do I need to modify my annealing temperature when using the Q5 High GC Enhancer?

No. Use of the Q5 High GC Enhancer often lowers the range of temperatures at which specific amplification can be observed, however the $T_a = T_{m_{lower}} + 3^{\circ}\text{C}$ rule used for Q5 typically yields a T_a value that will support specific amplification +/- the enhancer.

7. When should I add the High GC Enhancer?

For particularly difficult or high GC amplicons ($\geq 65\%$), the Enhancer can be added to the Q5 Reaction Buffer to improve specificity and/or yield. The Enhancer is not a buffer and should not be used alone. Final concentration of the Enhancer in the amplification reaction should be 1X. In our experience, the Enhancer may inhibit amplification of high AT templates.

8. Are the DNA fragments produced by Q5 High-Fidelity DNA Polymerase blunt ended or do they have the single-base 3' overhang that *Taq* DNA Polymerase yields?

Blunt-ended products are produced by Q5 High-Fidelity DNA Polymerase (and all Q5 product formulations).

9. There is a precipitate in the bottom of the buffer tube. Is this normal?

A precipitate is occasionally seen in the Q5 Reaction Buffer. To ensure optimal performance, please make sure all components are thawed and resuspended prior to use.

10. What length of product can be made by Q5 High-Fidelity DNA Polymerase?

From simple DNA templates (*E. coli*, lambda, etc.), we have amplified fragments up to 20kb. For complex genomic DNA templates, we have amplified fragments to 10 kb.

11. I am having trouble amplifying a template that is longer than 5kb. How can I optimize my product yield using Q5 High-Fidelity DNA Polymerase?

Use more template. Sample concentration may be too low.

Template DNA may be damaged. Use carefully purified template.

Optimize enzyme concentration by testing a titration of enzyme in the reaction (0.25-2 units/50 μ l reactions)

Increase number of cycles.

Lengthen extension time to 40s/kb.

12. Does Q5 High-Fidelity DNA Polymerase exhibit a strand displacement activity?

No. Q5 High-Fidelity DNA Polymerase (and all Q5 product formulations) has an extremely weak tendency to displace downstream non-templated sequences.

13. Will Q5 High-Fidelity DNA Polymerase incorporate dUTPs?

No. Q5 High-Fidelity DNA Polymerase will not incorporate dUTPs and is not compatible with USER[™] cloning methods or bisulfite-treated DNA. For protocols employing bisulfite-treated DNA that require a hot start enzyme, we recommend EpiMark[™] Hot Start *Taq* DNA Polymerase (NEB #M0490).

14. I'd like to clone a fragment amplified with Q5 High-Fidelity DNA Polymerase. Do I have to blunt-end clone?

Blunt-end cloning is recommended. However, if TA cloning is required, 3' A-overhangs can be added with a different polymerase. It is very important to remove all the Q5 High-Fidelity DNA Polymerase first by purifying the PCR product. The proofreading activity of Q5 is very strong, so any residual polymerase will degrade the A-overhangs as they are added. *Taq* DNA Polymerase (NEB #M0273) or Klenow (exo⁻) DNA Polymerase (NEB #M0212) are excellent options for A-overhang addition. We recommend ligating immediately so the 3' A-overhangs will not be lost during storage.

15. Do other polymerases work in Q5 Reaction Buffer?

The Q5 Reaction Buffer has been formulated specifically for use with Q5 and Q5 Hot Start High-Fidelity DNA Polymerases. Phusion and *Taq* DNA Polymerases (and related products) show more consistent results in their respective buffers.