

Technical Bulletin - March 2022

Choosing the Right High-Fidelity Reagent For Your PCR Reaction

<u>When is a High-Fidelity Enzyme the</u> <u>Right Choice?</u>

PCR applications that require highly accurate amplification (amplified DNA with minimal sequence errors) will require high fidelity enzymes. High fidelity enzymes are commonly used in cloning, sequencing and mutagenesis work where inaccurate copies would lead to experimental failure. Depending on a variety of factors, a specific type of high-fidelity enzyme or master mix will be better suited for certain amplifications.

New Enzyme Technology to the Rescue

The first high-fidelity enzymes were introduced in 2005 to address some of the shortcomings of native Taq polymerase. It is well known that native Taq is unstable at high temperatures, error prone, and has difficulty amplifying DNA with high GC content or strong secondary structures. High fidelity DNA polymerases solve these issues by having strong proofreading abilities, increased affinity for double-stranded DNA, and impressive stability at high temperatures.

Accuris Life Science Reagents has introduced a variety of high-fidelity enzymes for specific applications. These enzymes have up to 100x the fidelity of Taq polymerase. Accuris' high-fidelity enzymes can accept crude DNA samples without inhibiting amplification. These enzymes have been modified to create blunt-end or A-overhang products for cloning applications as well. The optimized buffers include PCR enhancers, which result in better sensitivity and higher activity while ensuring more reproducible performance with a wide range of targets.

Accuris High Fidelity DNA Polymerase

The original *High-Fidelity Polymerase* from *Accuris* has been genetically modified for better solubility and higher activity across a broad range of ionic conditions. This *two-tube* kit includes the enzyme in one tube, and buffer in separate tubes. The 5X buffer contains an optimized concentration of dNTPs and enhancers. A 3'- 5' proofreading exonuclease activity and an error rate of 4.55×10^{-7} makes this *High-Fidelity Polymerase* the perfect enzyme for accurate amplification of a wide range of targets , including those that are GC/AT rich. Even "crude" samples that have not been purified can be successfully amplified. This product leaves blunt ends in cloning applications and is recommended for

amplicons up to 10kb in length. Testing has confirmed 50x the fidelity of native Taq polymerase.



Accuris High Fidelity Master Mix

The Accuris High Fidelity Master Mix has been modified to deliver a unique balance of PCR sensitivity, high fidelity, versatility, and tolerance to inhibitors. The single-tube 2X formulation contains proprietary enhancers and a proofreading component to ensure trouble-free PCR reaction assembly and performance. The formulation provides extreme sensitivity in low copy number assays, 100x the fidelity of native Taq polymerase, and leaves a blunt end for blunt end cloning applications.

> High Fidelity Master Mix PR1001-HF-200



<u>Accuris High Fidelity Hot Start Master</u> Mix

The Accuris High Fidelity Hot Start Master Mix incorporates an antibody-mediated hot-start polymerase mixed with proprietary enhancers. The enzyme's proof-reading component ensures trouble-free PCR reaction assembly and performance. The single-tube 2X master mix reduces the number of pipetting step while improving throughput and reproducibility. The enhanced buffer formulation and hot-start blend provide the ideal conditions for high-performance PCR. The enzyme is inactive at room temperature, so non-specific amplification will not occur. This formulation is ideal for difficult high-GC content sequences up to 10kb , has 10x the fidelity of native Taq polymerase, and generates an Aoverhang for TA cloning application.



Choosing the right high-fidelity enzyme allows you to optimize your PCR reaction. Each unique high-fidelity enzyme has a different performance characterization, level of accuracy, parameter for amplicon size, and end result for cloning (bluntend & A-overhang) applications. Accuris offers technical support to help you optimize your PCR reaction; Please reach out to us for advice and additional details on our full line of End-Point and qPCR reagents.