Accuris™ AccuFi™ High Fidelity Hot Start PCR Mix

Accuris AccuFi HFHS PCR Mix is optimized 2X mastermix that includes AccuFi High Fidelity polymerase, a proprietary reaction buffer system, $MgCl_2$, and ultra-pure dNTPs. The engineered polyerase exhibits rapid extension kinetics (~10 s/kb), enabling efficient fast cycling protocools while maintaining high processivity. The formulation supports robust long-range amplification, with reliable performance on templates exceeding 15 kb.

The enzyme possesses both 5'→3' DNA polymerase activity and intrinsic 3'→5' exonuclease proofreading activity, resulting in **ultra-low error rate** (below the detection limit) compared to Taq DNA polymerase. The high fidelity of the enzyme supports applications where sequence integrity is critical, such as molecular cloning, next-generation sequencing (NGS) library construction, and site-directed mutagenesis.

The optimized buffer system enhances amplification efficiency across challenging templates, including GC-rich and partially impure DNA samples, while maintaining high yield and and specificity with minimal reaction optimization.

Highlights:

- **Ultra-high fidelity**: Exhibits highly accurate DNA synthesis supported by coupled 5'→3' DNA polymerase activity and intrinsic 3'→5' exonuclease proofreading.
- **Hot-Start DNA polymerase**: minimizes nonspecific amplification, reduced background and enhances reproducibility in PCR
- **Strong amplification performance**: Supports efficient amplification across challenging templates, including GC-rich sequences, low-quality, and long-range targets, while maintaining high yield.
- **Fast extension rate**: Delivers rapid DNA synthesis at approximately 1 kb/10 seconds, supporting accelerated PCR cycling.

Storage

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cyycles When stored as direct-

PCR Master Mix Guidelines:

Component	25 µL reaction	50 µL reaction	Final concentration*
AccuFi™ PCR Mix	12.5 µL	25 µL	1X
Forward Primer (10 µM)	1.0 µL	2.0 µL	400 nM
Reverse Primer (10 µM)	1.0 µL	2.0 µL	400 nM
DNA Template	< 100 ng cDNA <500 ng genomic DNA		variable
PCR-grade H_2O	Up to 25 µL	Up to 50 µL	

***** For alternative total reaction volumes adjust the amount of each component proportional to the final concentration.

PCR Cycling Guidelines:

Stage	Temperature	Time	# Cycles
Initial Denaturation	95 ºC	1 minute	1
Denaturation	95 ºC	15 secodns	
Annealing* (varies)	60-65 ºC	15 seconds	25-40
Extension*	72 ºC	30 seconds per kb	

***** Use general guidelines to troubleshoot annealing and extensions steps.

General Guidelines

1. *AccuFi PCR Mix*

AccuFi PCR Mix is a pre-formulated 2× hot-start PCR master mix containing AccuFi DNA Polymerase, an optimized proprietary buffer system, 3.0 mM $MgCl_2$, and ultra-pure dNTPs. This formulation is engineered to enhance amplification robustness, sensitivity, and overall reaction efficiency across a broad spectrum of DNA templates. Prior to use, the mix should be fully thawed and homogenized by gentle inversion to ensure resuspension of any buffer-associated precipitate.

2. *Template input*

For complex genomic DNA, a mass range of 5–500 ng per reaction is recommended. For plasmid DNA or cDNA, input should not exceed 100 ng per reaction to maintain reaction efficiency and specificity.

3. *Primers Design*

Primers should be designed with a target melting temperature of approximately 60 °C, calculated using standard Primer3 parameters (https://primer3.ut.ee/). Final primer concentration in the reaction should be maintained between 0.2 and 0.6 µM to balance specificity and yield.

4. *Annealing Conditions*

Gradient PCR is recommended for empirical determination of the optimal annealing temperature. Alternatively, 60 °C may be used as an initial reference point, with optimization performed in 2 °C increments. Due to the elevated ionic strength of the master mix, the optimal annealing temperature may be higher relative to conventional PCR buffer systems.

5. *Extension conditions*

DNA synthesis is optimally performed at 72 °C. Extension time is dependent on amplicon length and template complexity. For targets below 3 kb, a 30-second extension per cycle is generally sufficient. For longer or structurally complex templates above 3 kb, an extension rate of approximately 30 seconds per kb is recommended.

Technical Support

For troubleshooting and technical support, contact us at **info@accuris-usa.com** or call **908 769-5555**. Accuris is not responsible for consequential or incidental damages, whether direct or indirect, resulting from use of this product. Accuris guarantees the performance of this product as described when used in accordance with these instructions.

Ordering Information

Catalog Number	Description	Components
PR1002-HFHS-100	AccuFi HFHS Master Mix, 100 reactions	1.25 mL of PCR Mix
PR1002-HFHS-200	AccuFi HFHS Master Mix, 200 reactions	2.5 mL of PCR Mix in 1.25 mL aliquots
PR1002-HFHS-500	AccuFi HFHS Master Mix, 500 reactions	6.25 mL of PCR Mix in 1.25 mL aliquots