

# Accuris™ High Fidelity Hot Start

## Master Mix

### Description

Accuris High Fidelity Hot Start Master Mix is a robust, high-fidelity DNA polymerase mix ideally suited to amplify templates including complex DNA targets and inhibitor-rich samples. The Master Mix is a hot-start 2X formulation which provides excellent sensitivity in low-copy number assays and 10X higher fidelity than Taq polymerase. The 2X master-mix contains proprietary enhancers, hot-start antibodies and a proof-reading component for trouble-free PCR reaction assembly and performance.

-Optimized 2X PCR blend provides robust hot-start PCR in a wide range of applications with 10X higher fidelity than Taq Polymerase.

-Provides greater yields and specificity than other PCR master-mixes, even in low-copy number assays, long PCR up to 10Kb, and in the presence of common PCR inhibitors.

-High Fidelity Master Mix is designed and optimized for ease-of-use and broad compatibility with DNA templates of various lengths and complexity.

-Resulting product is sticky ended

### Storage

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, product will retain its activity for 12 months from date of receipt. May also store at 4°C for up to one month.

### Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

### Quality Control

Accuris enzymes and reagents are tested under general assay conditions for activity, reproducibility, efficiency, heat activation, sensitivity, and absence of nuclease contamination and nuclease activity. This product is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

### General Guidelines

#### 1. Reaction Buffer

The 2x Mix is comprised of a high-fidelity DNA polymerase complex, 2 mM dNTPs, 6 mM MgCl<sub>2</sub>, and PCR enhancers for maximum efficiency, sensitivity and success with difficult amplicons. Use of additional PCR enhancers may have a negative effect on the reaction.

#### 2. Template

For PCR of complex genomic DNA, 5ng - 500ng of template DNA may be added per reaction. Do not add more than 100ng of DNA for cDNA or plasmid DNA

#### 3. Primers

Primers should have a predicted melting temperature of approximately 60°C, using default Primer 3 settings (<http://frodo.wi.mit.edu/primer3>). The final primer concentration should be 0.2µM to 0.6µM.

#### 4. Annealing Temperature

An initial annealing temperature of 57°C is recommended. If nonspecific products or smearing appear, increase the temperature in 2°C increments. Alternately, a temperature gradient may be performed.

#### 5. Extension

The polymerase performs optimally at 72°C. Extension time is dependent upon amplicon complexity and length. Thirty seconds per kilobase (Kb) is recommended for amplification from eukaryotic genomic DNA or cDNA

### Technical Support

For trouble-shooting and tech support, contact us at [info@accuris-usa.com](mailto: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.

### Reaction setup

Prepare the reaction as follows:

Component	25 µl reaction	Final concentration
Accuris HFHS Master Mix	12.5 µl	1X
Forward Primer (10µM)	1.0 µl	400 nM
Reverse Primer (10µM)	1.0 µl	400 nM
Template DNA	<100ng cDNA, <500ng genomic	variable
PCR-grade water	to final reaction volume	

For other volumes, adjust the amount of each component accordingly.

Gently mix the solution. If needed, spin briefly in a microcentrifuge to bring reaction mixture to the bottom of the tube. Transfer samples to a thermal cycler begin cycling.

### Routine PCR Cycling

Step	Temperature	Time
Initial denaturation	95°C	1-2 minutes
25-40 cycles	95°C	15 seconds
	57°C to 67°C*	15 seconds
	72°C	30 seconds per Kb

\*Annealing temperature determined by user


### Package contents and reordering

Cat No.	Description
PR1001-HF-200	High Fidelity Master Mix, 200 reactions (2 x 1.25mL)
PR1001-HF-500	High Fidelity Master Mix, 500 reactions (5 x 1.25mL)
PR1001-HFHS-200	High Fidelity Hot Start Master Mix, 200 reactions (2 x 1.25mL)
PR1001-HFHS-500	High Fidelity Hot Start Master Mix, 500 reactions (5 x 1.25mL)

- Single-tube formulation: Ready to use 2X Master Mix.
- Ideal for blunt end cloning
- 100X the fidelity of wild-type Taq
- High Fidelity Hot-Start Master Mix available for TA cloning
- Exhibits 3'→5' proofreading activity



PR1001-HFHS-200



**ACCURIS™**  
LIFE SCIENCE REAGENTS

## High Fidelity Hot Start Master Mix

PR1001-HFHS-200  
 PR1001-HFHS-500

Store at -20°C upon receipt.

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