

Accuris™ High Fidelity DNA Polymerase

Description

High Fidelity Polymerase represents the next level of polymerases, engineered for shorted extension times, greater sensitivity and successful PCR of crude samples. This enzyme exhibits a strong 5'-3' activity along with a 3'-5' proofreading activity. An error rate of 4.55×10^{-7} makes this enzyme the perfect partner for cloning applications. The enzyme has been modified for increased solubility and performance across a broad range of conditions. The included 5X buffer has been formulated specifically to work with the unique nature of this high fidelity polymerase.

-Greater than 50 times greater fidelity when compared to wild-type Taq polymerase

-Proprietary 5X reaction buffer includes enhancers for maximizing enzyme activity and reaction speed

-Improved yields across a variety of templates, including those that are GC and AT rich

Storage

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, this product will retain its activity for 12 months from date of receipt. The product may also be stored 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 supplied 5X reaction buffer has been formulated for maximum efficiency, sensitivity and successful PCR with long and difficult templates. Proprietary PCR enhancers, optimal levels of dNTPs (5mM) and 15mM MgCl₂ are included in the buffer. 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 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 | 50 µl reaction | Final concentration |
|--|-----------------------------|----------------|---------------------|
| Accuris 5x High Fidelity Reaction Buffer | 5 µl | 10 µl | 1X |
| Forward Primer (10µM) | 1.0 µl | 2.0 µl | 400 nM |
| Reverse Primer (10µM) | 1.0 µl | 2.0 µl | 400 nM |
| Template DNA | <100ng cDNA, <500ng genomic | | variable |
| High Fidelity Polymerase(2u/µl) | 0.25 µl - 0.5 µl | 0.5 µl - 1 µl | 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 |
| | 95°C | 15 seconds |
| 25-40 cycles | 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 |
|----------------|--|
| PR1000-HF-200 | High Fidelity DNA Polymerase, 200 units |
| PR1000-HF-1000 | High Fidelity DNA Polymerase, 1000 units |

- Two-tube formulation: High Fidelity DNA Polymerase & 5X buffer
- 50X the fidelity of wild-type Taq Polymerase
- Exhibits 3'->5' proofreading activity
- High performance with gDNA & GC- & AT-rich sequences
- Ideal for cloning (blunt end), mutagenesis, & microarray applications



PR1000-HF-200



High Fidelity DNA Polymerase

PR1000-HF-200

PR1000-HF-1000

Store at -20°C upon receipt.

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