

PR1MA™ 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

PR1MA 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 tech@midsci.com or call 800 227-9997.

MidSci is not responsible for consequential or incidental damages, whether direct or indirect, resulting from use of this product. MidSci 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
PR1MA 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

PR1MA High Fidelity Polymerase is supplied at a concentration of 2 units/µl and is available in 200 and 1000 unit packages. Supplied with 5X High Fidelity Buffer.

PR1MA High Fidelity Polymerase, Sample Pack

Catalog number PR1000-HF-S
Includes 10 µl of enzyme and 5X buffer.

PR1MA High Fidelity Polymerase, 200 units

Catalog number PR1000-HF-200
Includes 100 µl of enzyme and 3ml of 5X buffer in 1ml aliquots.

PR1MA High Fidelity Polymerase, 1000 units

Catalog number PR1000-HF-1000 Includes 500 µl of enzyme in 100 µl aliquots, and 15ml of 5X buffer in 1ml aliquots. PR1MA offers a full line of PCR enzymes and master mixes. Visit www.PR1MA-usa.com for details.

High Fidelity
Polymerase
PR1000-HF

2 units/µl, supplied with 5X Buffer
Package contains:
200 units High Fidelity Polymerase (1x100µl)
3ml 5X High Fidelity Buffer (3x1ml)
Store at -20°C upon receipt

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