

FOR RESEARCH USE ONLY!

HiFidelity™ DNA Polymerase

11/20

(Catalog # M1505-400; 400 Rxns; Store at -20°C)

I. Introduction:

Biovision's HiFidelity™ DNA Polymerase™ is a strategically-engineered Polymerase which has exceptional sensitivity and can amplify even the most difficult templates. It sets new standards for sensitive, robust and high-fidelity PCR performance. HiFidelity™ DNA Polymerase has ultra-low error rates (over 1,000X less than Taq polymerase) making it incredibly useful for a variety of PCR applications demanding high fidelity, including Next Generation Sequencing or molecular cloning.

II. Contents:

Components	M1505-400	Part Number
HiFidelity™ DNA Polymerase	200 µl (400 rxn)	M1505-400-1
5X HiFidelity™ Buffer*	2.5 ml (2 x 1.25 ml)	M1505-400-2

Buffer contains * 1.5 mM Mg²⁺

III. Key Features:

- Fast, versatile high-fidelity PCR
- Suitable for long range PCR up to 18 kb from less difficult targets or up to 15 kb from genomic DNA
- Superior Performance
- High Sensitivity

IV. Shipping and Storage Conditions:

The kit is shipped in gel pack. All the components of the kit should be stored at -20 °C.

V. Protocol:

1. Thoroughly thaw and mix individual components before use and assemble reaction on ice.

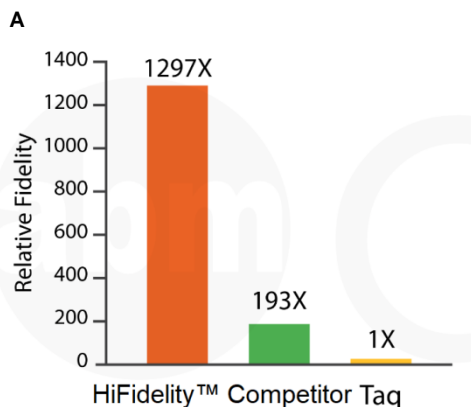
Component	Volume
5X HiFidelity™ Buffer	5 µl
dNTP Mix (10 mM)	0.5 µl
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
Template DNA	Variable (100 ng genomic DNA)
HiFidelity™ DNA Polymerase	0.5 µl
Nuclease-free H ₂ O	up to 25 µl

2. Gently mix the reaction components and briefly centrifuge. Run thermocycling conditions for standard PCR (1 kb template):

Step	Temperature	Duration
Initial Denaturation	98 °C	30 sec
25-35 cycles	98 °C	5-10 sec
	50-72 °C	10-30 sec
	72 °C	20-30 sec/kb*
Final Extension	72 °C	2 min

*20-30 sec/kb, increase as needed.

3. After PCR, maintain the reaction at 4°C or store at -20°C until use.
4. Analyze the amplification products by agarose gel electrophoresis.
5. Visualize by ethidium bromide or Safe Image™ Basic DNA Stain (Cat. No. M1193) staining.



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Table 1

Blue/White Screening	Total Colonies	White Colonies	Error rate/base	Fold over Taq
Taq Polymerase	30,192	17,589	$2.7 \times 10^{-4} \pm 0.8 \times 10^{-4}$ (1 per 3, 700 bases)	-
Competitor Polymerase	22,296	119	$1.4 \times 10^{-6} \pm 0.6 \times 10^{-6}$ (1 per 710, 000 bases)	193 ± 101
HiFidelity™ DNA Polymerase	33,880	62	$2.1 \times 10^{-7} \pm 0.6 \times 10^{-7}$ (1 per 4, 800, 000 bases)	1297 ± 371

Fig A. HiFidelity™ DNA Polymerase has ~1300X better proofreading than other enzymes. It shows the highest accuracy rate compared to other DNA Polymerases as tested using blue/white colony screening. **Fig B.** HiFidelity™ DNA Polymerase has the highest fidelity. An 866 bp sequence containing the lacZa gene (used for blue white screening) was amplified using various DNA polymerases and subcloned into the pUC19 vector. Colonies without mutations within the PCR amplified lacZa would appear blue when plated on X-gal-containing growth media, while those that have mutations introduced by PCR causing insertions, deletions, frameshift mutations or substitution in amino acid sequence would appear as white colonies. The higher the fidelity of the DNA polymerase, the greater the ratio of blue colonies compared to white colonies. **Table 1.** Relative fidelity of HiFidelity™ DNA Polymerase to Taq DNA Polymerase (Taq = 1X). PCR cycle number and mutations resulting in non-phenotypic changes are accounted for.

VI. Related Products:

BioVision Product Name	Cat. No.	Sizes
ExpressTaq™ DNA Polymerase	M1504	400 Rxns
FireTaq™ DNA Polymerase	M1506	400 Rxns
Taq DNA Polymerase	9001	500, 2500 units
PFU DNA Polymerase	9003	500, 2500 units
Laq™ DNA Polymerase	9004	500, 2500 units

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