

FOR RESEARCH USE ONLY!

HiFidelity™ DNA Polymerase

Rev 06/21

(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 |

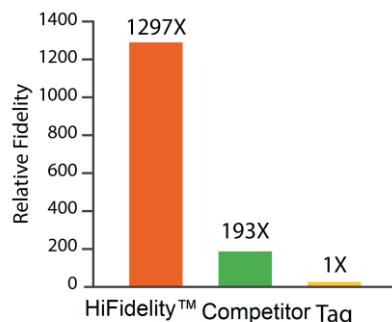
2. Gently mix the reaction components and briefly centrifuge. Run thermocycling conditions for standard PCR (1 kb template):

| 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 |

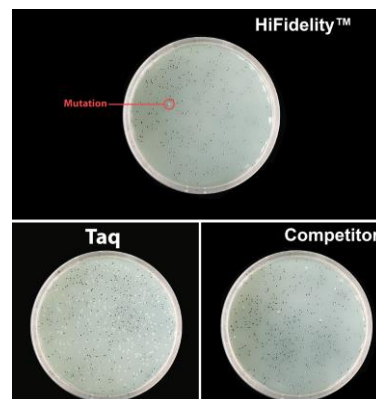
*20-30 sec/kb, increase as needed.

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.

A



B



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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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