

FOR RESEARCH USE ONLY!

HiFidelity™ 2X PCR MasterMix

12/20

(Catalog # M1507-800; 800 Rxns; Store at -20 °C)

I. Introduction:

Biovision's HiFidelity™ 2X PCR MasterMix sets new standards for sensitive, robust, and high-fidelity PCR performance in a uniquely-formulated buffer with a gel loading dye. This strategically-engineered polymerase has exceptional sensitivity and can amplify even the most difficult templates. In addition, HiFidelity™ has high fidelity and ultra-low error rates (over 1,000X less than Taq polymerase), making it incredibly useful for a variety of PCR applications demanding high fidelity such as Next Generation Sequencing and molecular cloning.

II. Key Features:

- Easy, convenient, ready-to-use mixture
- **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
- High reproducibility

III. Applications:

- Next Generation Sequencing and molecular cloning

IV. Contents:

Components	M1507-800 (800 Rxns)	Part Number
HiFidelity™ 2X PCR MasterMix*	10 ml	M1507-800-1

*Buffer contains 1.5 mM Mg²⁺

V. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- PCR tubes
- Nuclease free water
- Primers (forward and reverse)
- DNA Template
- Agarose
- Ethidium Bromide
- Thermal Cycler

VI. Shipping and Storage Conditions:

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

VII. Protocol:

1. Thoroughly thaw and mix the individual components before use, and **assemble the reaction** on ice. Start with high quality, purified DNA template to achieve even greater PCR success.

Component	Volume
HiFidelity™ 2X PCR MasterMix	12.5 µl
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
Template DNA	Variable (100 ng genomic DNA)
Nuclease-free water	up to 25 µl

2. Gently mix the reaction components and briefly centrifuge, then transfer tube to a thermal cycler. Use 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 SafelImage™ Basic DNA Stain (Cat No. M1193) staining.

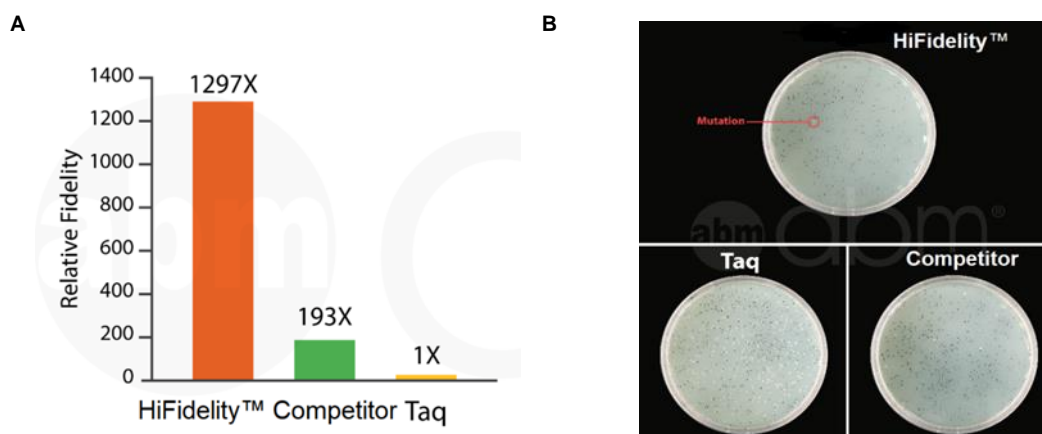


Table 1

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 (Cat. No. M1505) used in HiFidelity™ 2X PCR MasterMix shows ~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.

VIII. Related Products:

BioVision Product Name	Cat. No.	Sizes
ExpressTaq™ DNA Polymerase	M1504	400 Rxns
HiFidelity™ DNA Polymerase	M1505	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
HiFidelity™ One Step RT Kit	M1503	100 Rxns

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