

## ExtremeTaq™ HiFi Mix

| Catalog No. | Pack Size and Concentration | Components and Volume               |
|-------------|-----------------------------|-------------------------------------|
| AZ-1900     | 200 x 25 µl reactions, 2x   | ExtremeTaq™ HiFi Mix - 2 x 1.25 mL  |
| AZ-1901     | 1000 x 25 µl reactions, 2x  | ExtremeTaq™ HiFi Mix - 10 x 1.25 mL |
| AZ-1902     | 2000 x 25 µl reactions, 2x  | ExtremeTaq™ HiFi Mix - 20 x 1.25 mL |

### Description

ExtremeTaq™ HiFi Mix is an optimized 2x master-mix comprised of an enhanced Taq DNA Polymerase, optimized reaction buffer, MgCl<sub>2</sub>, and ultra-pure dNTPs. This versatile master-mix is ideally suited to all routine end-point PCR applications and challenging DNA targets such as complex GC-rich DNA and low-copy number samples. The formulation contains Taq Polymerase, proprietary enhancers, hot-start antibodies, and a proof-reading component for trouble-free PCR reaction assembly and consistent performance.

- **Assay Flexibility and Accuracy:** With up to 10x the fidelity of Taq Polymerase, our ready-to-use 2x PCR mix provides robust hot-start PCR in a wide range of applications.
- **Robust Amplification:** Provides greater yields and specificity than other Taq master-mixes, even in low-copy number assays, long PCR up to 10 kb, and in the presence of common PCR inhibitors.
- **Convenience of Minimal Optimization:** ExtremeTaq™ HiFi Mix is designed and optimized for ease-of-use and broad compatibility with DNA templates of various lengths and complexity, without the need for MgCl<sub>2</sub> optimization.
- **Versatility** High-Performance amplification of DNA extracted from human, animal, plant, bacteria, *C. elegans*, soil and water.

### Storage

ExtremeTaq™ HiFi Mix is shipped on blue ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided.

### Important Guidelines

**ExtremeTaq™ HiFi Mix:** The 2x mix is comprised of a high-fidelity DNA polymerase complex, 2 mM dNTPs, 6 mM MgCl<sub>2</sub>, and PCR enhancers for maximum efficiency, sensitivity and success with difficult amplicons. We do not suggest the use of additional PCR enhancers.

**Template:** For complex genomic DNA, we suggest the use of 5 ng – 500 ng per reaction; For cDNA or plasmid DNA, please use < 100 ng per reaction.

**Primers:** Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (<http://frodo.wi.mit.edu/primer3/>). The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

**Annealing:** We strongly recommend performing a temperature gradient to determine the optimal annealing temperature. Alternatively, 58°C can be used as a starting point. The optimal annealing temperature is usually 2-5 °C below the lower T<sub>m</sub> of the pair. Depending on the reaction, the annealing time can also be reduced to 5 seconds.

**Extension:** Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity. For low complexity template such as plasmid DNA, an extension time of 15 seconds is sufficient for amplicons under 1 kb. For amplification from eukaryotic genomic DNA or cDNA, 30 seconds per kb is recommended.

**Reaction setup: Allow ExtremeTaq™ HiFi Mix to thaw and mix well by inversion. Centrifuge prior to use.**

1. Prepare a PCR master mix based on following table:

| Component              | 25 µl Reaction                    | Final Concentration/Notes |
|------------------------|-----------------------------------|---------------------------|
| ExtremeTaq™ HiFi Mix   | 12.5 µl                           | 1x                        |
| Forward Primer (10 µM) | 1.0 µl                            | 400 nM                    |
| Reverse Primer (10 µM) | 1.0 µl                            | 400 nM                    |
| Template DNA           | <100 ng cDNA, <500 ng genomic DNA | variable                  |
| PCR-grade water        | Up to 25 µl final volume          |                           |

\* For alternative total reaction volumes (eg. 20 µl), scale all components proportionally to maintain final concentrations.

2. PCR cycling:

| Cycles  | Temperature & Time  | Notes   |
|---------|---|---|
| 1       | 95°C, 2 minutes   | Initial Denaturation, enzyme activation                       |
| 25 - 40 | 95°C, 15 seconds<br>60°C, 15 seconds<br>72°C, 30 seconds per kb | Denaturation<br>Annealing* (determined by user)<br>Extension* |

\* See Important Guidelines.

### Quality Control

ExtremeTaq™ HiFi Mix is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. ExtremeTaq™ HiFi Mix is manufactured under a comprehensive quality management system, following ISO 9001:2015 standards.

### Limitations of Use

This product is intended for research purposes only and is not intended for any animal or human therapeutic use.

### Technical Support

For Trouble-shooting and Technical Guidance, please contact us at [tech@azuragenomics.com](mailto:tech@azuragenomics.com) and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.

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