

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 – 5 x 5 mL

Description

ExtremeTaq HiFi Mix is a robust, high-fidelity DNA polymerase complex ideally suited to a wide range of DNA templates including the most challenging and complex DNA targets and inhibitor-rich samples. ExtremeTaq HiFi Mix is a hot-start 2x formulation which provides excellent sensitivity in low-copy number assays with 10x higher fidelity than *Taq* polymerase. The 2x master-mix contains proprietary enhancers, hot-start antibodies and a proof-reading component for trouble-free PCR reaction assembly and performance.

- **Assay Flexibility and Accuracy:** Optimized 2x PCR blend provides robust hot-start PCR in a wide range of applications with 10x higher fidelity than *Taq* Polymerase.
- **Robust Amplification:** Provides greater yields and specificity than other PCR master-mixes, even in low-copy number assays, long PCR up to 10Kb, 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.

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 MgCl₂, 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 recommend performing a temperature gradient to determine the optimal annealing temperature. Alternatively, 55°C can be used as a starting point. For optimization, increase in 2°C increments. ExtremeTaq HiFi Mix contains a proprietary enhancer which may alter the optimal annealing temperature in comparison with traditional PCR buffers and master-mixes.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity. 45 seconds per kilobase (Kb) is recommended for amplification from eukaryotic genomic DNA or cDNA. For Multiplex PCR, we suggest an initial annealing temperature gradient from 55°C to 65°C in order to determine the highest level of specificity. In addition, we recommend an initial extension time of 90 seconds to maximize yield and specificity.

Reaction setup – Allow ExtremeTaq HiFi Mix to thaw and mix contents thoroughly by pipetting up and down gently.

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 55°C to 60°C, 15 seconds 72°C, 45 seconds per Kb	Denaturation Annealing* (determined by user) Extension (see notes for multiplex)

* We suggest performing a temperature gradient to determine the optimal annealing temperature. 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 9001:2008 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 and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.

Azura Genomics guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Azura Genomics shall not be liable for any direct, indirect, consequential or incidental damages arising from the use, the result of use or the liability to use this product.