ExtremeTaq™ HiFi Red Mix

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-1910	200 x 25 µl reactions, 2x	ExtremeTaq™ HiFi Red Mix - 2 x 1.25 mL
AZ-1911	1000 x 25 µl reactions, 2x	ExtremeTaq™ HiFi Red Mix - 10 x 1.25 mL
AZ-1912	2000 x 25 µl reactions, 2x	ExtremeTaq™ HiFi Red Mix - 20 x 1.25 mL

Description

ExtremeTaq™ HiFi Red Mix is an optimized 2x master-mix comprised of an enhanced Taq DNA Polymerase, optimized reaction buffer, $MgCl_2$, 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. This ready-to-use mix contains an inert red dye which allows samples to be loaded directly onto agarose gels without additional gel loading buffers. The red dye migrates at the same rate as 600 bp DNA fragments and 350 bp DNA fragments in 1% and 2% agarose gels, respectively. ExtremeTaq™ HiFi Red Mix is sufficiently dense to sink to the bottom of the agarose gel wells.

- **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 $MgCl_2$ optimization.
- **Versatility** High-Performance amplification of DNA extracted from human, animal, plant, bacteria, C. elegans, soil and water.

Storage

ExtremeTaq™ HiFi Red 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 Red Mix: The 2x mix is comprised of a high-fidelity DNA polymerase complex, 2 mM dNTPs, 6 mM $MgCl_2$, inert red gel-loading dye 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 Tm 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 Red 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 Red 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 60°C, 15 seconds 72°C, 30 seconds per kb	Denaturation Annealing* (determined by user) Extension*

* See Important Guidelines.

Quality Control

ExtremeTaq™ HiFi Red Mix is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. ExtremeTaq™ HiFi Red 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 and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.