Azura ExtremeTaq 2x Mix

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-1820	200 x 50 µl reactions, 2x	ExtremeTaq 2x Mix - 5 x 1ml
AZ-1850	1000 x 50 µl reactions, 2x	ExtremeTaq 2x Mix - 25 x 1ml

Description

Azura ExtremeTaq 2x Mix is a ready-to-use single tube hot-start formulation optimized for robust amplification of complex GC and AT-rich genomic DNA templates . ExtremeTaq 2x Mix generates extremely high DNA yields and 3'- A overhangs which are ideal for direct integration into TA cloning vectors. In addition, the mix delivers exceptional PCR sensitivity in low copy assays with 4-fold higher fidelity than wild-type *Taq* polymerase. The highly advanced Azura ExtremeTaq 2x Mix chemistry also allows fast cycling conditions to be used with greater efficiency, reproducibility and through-put. The new-generation PCR formulation includes enhancers for maximum PCR efficiency, sensitivity and reaction speed.

- Supplied as a ready-to-use 2x master-mix for maximum convenience and minimal liquid handling.
- Robust PCR performance across a wide range of DNA templates including low-copy assays and problematic, long templates.
- High-yields with amplicons up to 10 Kb with standard or fast cycling.

Storage

Azura ExtremeTaq 2x Mix is shipped on blue or dry ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided. When stored as specified, Azura ExtremeTaq 2x Mix is stable for 12 months from date of receipt. The Mix may also be stored at 4°C for 1 month.

Important Guidelines

Azura ExtremeTaq 2x Mix: The 2x Mix is comprised of Azura HS Taq DNA Polymerase, a proof-reading polymerase, 2mM dNTPs, 6mM $MgCl_2$, 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 5ng - 500ng per reaction; For cDNA or plasmid DNA, please use < 100ng 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 0.2µM and 0.6µM.

Annealing: We recommend performing a temperature gradient to determine the optimal annealing temperature. Alternatively, 57°C can be used as a starting point. For optimization, increase in 2°C increments. For example, if non-specific products are present or smearing is visible, a higher annealing temperature is required.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity. 15 seconds per kilobase(kb) is recommended for amplification from eukaryotic genomic DNA or cDNA amplicons less than 5kb. For amplicons which are greater than 5kb, we suggest 40 seconds to 60 seconds per kb.

Reaction setup

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

Component	50µl Reaction	Final Concentration/Notes
Azura ExtremeTaq 2x Mix	25 µl	1X
Forward Primer (10µM)	2.0 µl	400 nM
Reverse Primer (10µM)	2.0 µl	400 nM
Template DNA	<100ng cDNA, <500ng genomic DNA	variable
PCR-grade water	Up to 50 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, 1 minute to 2 minutes	Initial Denaturation, enzyme activation
25 - 40	95°C, 15 seconds 55°C to 67°C, 15 seconds 72°C, 15 seconds per Kb	Denaturation Annealing (determined by user) Extension (see notes above)

Quality Control

Azura ExtremeTaq 2x Mix is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. Azura ExtremeTaq 2x 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.