

ExtremeTaq DNA Polymerase

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-1905	500 units, 5 U/μL	ExtremeTaq - 1 x 100 µL	5x ExtremeTaq Buffer – 2 x 1.25 mL
AZ-1915	1000 units, 5 U/μL	ExtremeTaq - 2 x 100 µL	5x ExtremeTaq Buffer – 4 x 1.25 mL
AZ-1950	5000 units, 5 U/μL	ExtremeTaq - 10 x 100 µL	5x ExtremeTaq Buffer – 5 x 5 mL

Description

ExtremeTaq[™] DNA Polymerase is an enhanced formulation containing Taq Polymerase, proprietary enhancers, hot-start antibodies, and a proof-reading component for trouble-free PCR reaction assembly and consistent performance. ExtremeTaq Polymerase delivers a unique balance of PCR sensitivity, high fidelity, and value.

- New generation 5x PCR buffer formulation including optimal levels of MgCl₂ and PCR enhancers for maximum PCR efficiency and speed.
- Robust PCR performance across a wide range of DNA templates including multiplex assays and problematic templates.
- High-yields with amplicons up to 5 kb with standard or fast cycling.

Storage

ExtremeTaq DNA Polymerase is shipped on blue ice and should be stored at -20°C upon receipt. Excessive freeze/thawing should be avoided.

Important Guidelines

5x ExtremeTaq Buffer: The 5x reaction buffer contains proprietary PCR enhancers and 15 mM MgCl₂. The buffer has been designed to deliver maximum efficiency, sensitivity and success with difficult amplicons in end-point PCR. We do not suggest the use of additional PCR enhancers. Please note: The 5x ExtremeTag reaction buffer is not recommended for qPCR applications.

Template: For complex genomic DNA, we suggest 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 reagents to thaw and mix well by inversion. Centrifuge prior to use.

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

Component	50 μL Reaction	Final Concentration/Notes
5x ExtremeTaq Buffer	10 μL	1x
100 mM dNTPs (25 mM each)	0.5 μL	1mM
Forward Primer (10 µM)	2.0 μL	400 nM
Reverse Primer (10 µM)	2.0 µL	400 nM
Template DNA	<100 ng cDNA, <500 ng genomic DNA	variable
ExtremeTaq DNA Polymerase (5 U/μL)	0.50 μL - 1 μL	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, 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 DNA Polymerase is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. ExtremeTaq DNA Polymerase 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 <u>tech@azuragenomics.com</u> and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.

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