

TruFi™ DNA Polymerase

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-1702	200 units, 2u/μl	TruFi™ Polymerase - 1 x 100 μl	5x TruFi™ Buffer – 3 x 1ml
AZ-1710	1000 units, 2u/μl	TruFi™ Polymerase - 5 x 100 μl	5x TruFi™ Buffer – 15 x 1ml

Description

TruFi™ DNA Polymerase is a new generation, ultra high-fidelity DNA polymerase developed specifically to overcome the many disadvantages of conventional proof-reading enzymes such as poor sensitivity, significant inhibition in crude PCR assays, long extension times and the tedious optimization of buffer conditions and reaction parameters. TruFi™ DNA Polymerase exhibits robust 5' -3' DNA polymerase activity and 3' – 5' proof-reading exonuclease activity with an error-rate of 4.55×10^{-7} . Several point mutations have selectively modified specific amino acid residues in order to improve protein solubility and performance across a wide range of ionic conditions. TruFi™ DNA Polymerase is provided with a companion 5x reaction buffer delivering superior performance with minimal optimization across a broad range of DNA amplicons, regardless of high AT or GC content. In addition, the hydrophilic nature of TruFi™ provides for significant improvements to reaction processivity for faster cycling, greater sensitivity and less inhibition with crude DNA samples.

- >50-fold higher fidelity than wild-type Taq DNA Polymerase.
- New-generation PCR buffer formulation including enhancers for maximum PCR efficiency and reaction speed.
- Robust PCR performance across a wide range of DNA templates including genomic DNA and GC-rich and AT-rich sequences.
- Supplied with 5x Reaction Buffer containing optimal levels of dNTPs and MgCl₂ for convenience.

Storage

TruFi™ DNA Polymerase 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, TruFi™ DNA Polymerase is stable for 12 months from date of receipt. The Kit may also be stored at 4°C for 1 month.

Important Guidelines

5x TruFi™ Buffer: The 5x reaction buffer contains proprietary PCR enhancers, optimal levels of dNTPs (5mM) and 15mM MgCl₂. The buffer has been designed to deliver maximum efficiency, sensitivity and success with difficult amplicons. We do not suggest the use of additional PCR enhancers or dNTPs.

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, 60°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. 30 seconds per kilobase(Kb) is recommended for amplification from eukaryotic genomic DNA or cDNA.

Reaction setup

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

Component	50µl Reaction	Final Concentration/Notes
Azura 5x TruFi™ Buffer	10 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
TruFi™ DNA Polymerase (2u/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, 1 minute	Initial Denaturation
25 - 40	95°C, 15 seconds 57°C to 67°C, 15 seconds 72°C, 30 seconds per Kb	Denaturation Annealing (determined by user) Extension

Quality Control

TruFi™ DNA Polymerase is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. TruFi™ DNA Polymerase 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.

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