TruFi™ II DNA Polymerase

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-1720	200 units, 2 U/µL	TruFi™ II Polymerase - 1 x 0.1 mL	5x TruFi™ II Buffer – 3 x 1.5 mL
AZ-1750	1000 units, 2 U/µL	TruFi™ II Polymerase - 5 x 0.1 mL	5x TruFi™ II Buffer – 15 x 1.5 mL

Description

TruFi™ II DNA Polymerase is an ultra-high fidelity proofreading DNA polymerase exhibiting both 5'→ 3' DNA polymerase activity and 3'→ 5' proofreading exonuclease activity, enabling the correction of mismatches during the amplification process. The DNA binding domain of this polymerase ensures robust amplification and more reliable results with a broad range of amplicons (from high AT to high GC). In addition, TruFi™ II DNA Polymerase possesses a rapid elongation rate of 10 seconds per kb, and is highly efficient in long PCR with amplicons greater than 15 kb. With an error rate which is greater than 65x lower than Taq Polymerase, TruFi™ II DNA Polymerase is recommended for applications which require extremely high fidelity such as cloning, NGS applications, and mutagenesis.

- >65-fold higher fidelity than *Taq* DNA Polymerase.
- Ideal for fast PCR with a rapid elongation rate of 10 sec/kb, which is 4x faster than *Taq*.
- Robust PCR performance across a wide range of DNA templates including GC-rich targets, DNA of sub-optimal purity, and long templates.
- Supplied with a 5x reaction buffer containing optimal levels of $MgCl_2$ for convenience.

Storage

TruFi™ II 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 TruFi™ II Buffer: The 5x reaction buffer contains 7.5 mM $MgCl_2$. The buffer composition has been developed to deliver maximum efficiency, sensitivity and success with most amplicons. Completely thaw 5x buffer and thoroughly mix by inversion for proper resuspension of precipitate. In some cases, a higher concentration of $MgCl_2$ or the addition of 1M -2M betaine can improve performance.

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, 58°C can be used as a starting point. For optimization, increase in 2°C increments. Due to the high salt concentration in the 5x reaction buffer, the optimal annealing temperature may be higher than with traditional PCR buffers.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity. 10 to 30 seconds per kilobase (kb) is recommended for amplification from eukaryotic genomic DNA or cDNA. For simple targets such as plasmid DNA and short targets less than 2 kb, 10 seconds per kb is typically sufficient.

Reaction setup

1. Prepare a PCR mixture on ice based on following table, adding TruFi™ II DNA Polymerase last to prevent primer degradation:

Component	25 µL Reaction	Final Concentration/Notes
Azura 5x TruFi™ II Buffer	5 µL	1x
dNTP Mix (10 mM each)	0.5 µL	0.2 mM of each dNTP
Forward Primer (10µM)	1.0 µL	400 nM
Reverse Primer (10µM)	1.0 µL	400 nM
Template DNA	<100 ng cDNA, <500 ng genomic DNA	variable
TruFi™ II DNA Polymerase (2 U/µL)	0.125 µL – 0.5 µL	variable
PCR-grade water	Up to 25 µL final volume	

* For alternative total reaction volumes (eg. 50 µl), scale all components proportionally to maintain final concentrations.

2. PCR cycling:

Cycles	Temperature & Time	Notes
1	98°C, 2 minutes	Initial Denaturation
25 - 40	98°C, 15 seconds 58°C to 66°C, 15 seconds 72°C, 10 to 30 seconds per kb	Denaturation Annealing (determined by user) Extension

Quality Control

TruFi™ II DNA Polymerase is tested extensively for robust activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. TruFi™ II 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 tech@azuragenomics.com and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.