TruFi™ 2x PCR Mix

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-1720	200 x 25 µL reactions, 2x	TruFi™ 2x PCR Mix - 2 x 1.25 mL
AZ-1725	1000 x 25 µL reactions, 2x	TruFi™ 2x PCR Mix - 10 x 1.25 mL
AZ-1750	2000 x 25 µL reactions, 2x	TruFi™ 2x PCR Mix - 20 x 1.25 mL

Description

TruFi™ 2x PCR Mix is a new generation ultra high-fidelity master mix 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.

- **Assay Flexibility and Accuracy**: With 50x the fidelity of Taq Polymerase formulations, our ready-to-use 2x PCR mix provides robust PCR in a wide range of applications.
- **Robust Amplification**: Provides greater yields and higher fidelity than other 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**: TruFi™ 2x PCR 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 GC-rich and AT-rich genomic DNA.

Storage

TruFi™ 2x PCR Mix is shipped on blue ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided.

Important Guidelines

TruFi™ 2x PCR Mix: The 2x mix is comprised of TruFi™ DNA polymerase, 2 mM dNTPs, 6 mM $MgCl_2$, 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 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, 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. For routine amplification of targets < 3 kb, 30 seconds per cycle is sufficient. For amplification of complex and longer targets > 3 kb, 30 seconds per kilobase (kb) is recommended.

Reaction setup: Allow TruFi™ 2x PCR 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
TruFi™ 2x PCR 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 to 65°C, 15 seconds 72°C, 30 seconds per kb	Denaturation Annealing* (determined by user) Extension*
1	72°C, 5 minutes	Optional final hold

* See Important Guidelines

Quality Control

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

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