# TruFi™ II Ultra 2x PCR Red Mix

| Catalog No. | Pack Size and Concentration | Components and Volume |
|---|---|---|
| AZ-1930 | 100 x 50 µl reactions, 2x | TruFi™ II Ultra 2x PCR Red Mix - 2 x 1.25 mL |
| AZ-1931 | 500 x 50 µl reactions, 2x | TruFi™ II Ultra 2x PCR Red Mix - 10 x 1.25 mL |
| AZ-1932 | 1000 x 50 µl reactions, 2x | TruFi™ II Ultra 2x PCR Red Mix - 20 x 1.25 mL |

**Description**

TruFi™ II Ultra 2x PCR Red Mix is an optimized 2x master-mix comprised of TruFi™ II DNA Polymerase, optimized reaction buffer, $MgCl_2$, inert red dye and ultra-pure dNTPs. TruFi™ II Ultra 2x PCR Red Mix is ideal for fast cycling, as the polymerase exhibits a rapid elongation rate of 10 seconds per kb. The ready-to-use 2x mix is also 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 Ultra 2x PCR Red Mix is recommended for applications which require extremely high fidelity such as cloning, NGS applications, and mutagenesis. To enable direct-to-gel analysis following amplification, TruFi™ II Ultra 2x PCR Red Mix includes an inert red gel loading dye for direct-to-gel analysis. The red dye front migrates at the rate of 1000 bp - 2000 bp DNA fragments in 0.5% to 1.5% agarose gels.

- **Ultra High-Fidelity**: provides fidelity that is >65x higher than Taq Polymerase and exhibits both 5'→ 3' DNA polymerase activity and 3'→ 5' proofreading exonuclease activity
- **Robust Amplification**: delivers greater yields with less optimization than other 2x master-mixes, even in amplification of GC-rich targets, DNA of sub-optimal purity, and long templates. TruFi™ II Ultra 2x PCR Red Mix has a high elongation rate of 10 sec/kb, which is 4x faster than *Taq*

**Storage**

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

**Important Guidelines**

**TruFi™ II Ultra 2x PCR Red Mix:** The 2x mix is comprised of TruFi™ II DNA Polymerase, optimized red reaction buffer, 3.0 mM $MgCl_2$, and ultra-pure dNTPs. The composition has been developed to deliver maximum efficiency, sensitivity and success with most amplicons. Completely thaw 2x mix 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 2x mix, 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 – Allow TruFi™ II Ultra 2x PCR Red Mix to thaw and mix contents thoroughly by inversion.**

1. Prepare a PCR mixture on ice based on following table, adding template DNA to individual tubes containing PCR mixture:

| Component | 50 µl Reaction | Final Concentration/Notes |
|---|---|---|
| TruFi™ II Ultra 2x PCR Red Mix | 25 µl | 1x |
| 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 |
| PCR-grade water | Up to 50 µl final volume | |

* For alternative total reaction volumes (eg. 25 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<br>58°C to 66°C, 15 seconds<br>72°C, 10 to 30 seconds per kb | Denaturation<br>Annealing* (determined by user)<br>Extension |

* We suggest performing a temperature gradient to determine the optimal annealing temperature. See Important Guidelines.

**Quality Control**

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