2X UltraFidelity™ Master Mix

Introduction

2X UltraFidelity™ Master Mix is a highly efficient and convenient premix, specifically designed for high-fidelity DNA amplification. This product contains a thermostable DNA polymerase with 3'→5' exonuclease activity. The error rate of this enzyme is approximately 1/280 of Taq DNA polymerase, ensuring the stability of the DNA amplification process. It is suitable for experiments involving long amplicons or difficult sequences. The premix has a concentration of 2X and includes dNTPs, Mg++, and a proprietary buffer. Efficient amplification can be achieved by simply adding primers and DNA template, regardless of the GC content of the template. Compared to conventional low-fidelity polymerase, it offers significantly higher fidelity.

Components and Storage

Size / Components	1mL	5x1mL	20x1mL	100x1mL	Storage
2X UltraFidelity™ Master Mix	1 mL	5x1 mL	20x1 mL	100x1 mL	-20°C
Shipping: Dry Ice		**Shelf life:** 12 months			

Protocol

1. Set up the PCR Reaction

Set your PCR reaction system as the following table. Or use your own system and conditions. Thoroughly thaw the Master Mix and all components, mix well, and briefly centrifuge before use. It is recommended to set up the reaction on ice.

Component	25 μL Reaction	50 μL Reaction	Final Concentration
2X UltraFidelity™ Master Mix	12.5 μL	25 μL	1X
10 μM forward primer	1.25 μL	2.5 μL	0.5 μM
10 μM reverse primer	1.25 μL	2.5 μL	0.5 μM
Template DNA	variable	variable	< 1000 ng
ddH_2O	To 25 μL	To 50 μL	/

2. Mix the reaction thoroughly and centrifuge.

3. Transfer the PCR tubes to the PCR machine and start the thermal cycling. The PCR cycling process are as follows:

Procedure	Temperature	Time	Cycles
Initial denaturation	98°C	30 s	1 Cycle
Denaturation	98°C	5-10 s	25-35 Cycles
Annealing	50-72°C	10-30 s	
Extension	72°C	20-30 s/kb	
Final extension	72°C	2 min	1 Cycle
Hold	4-10°C	+∞	1 Cycle

Note

1. It's recommended to set the PCR reactions in the clean bench and use filtered pipette tips. Always keep the positive/negative control DNA or other templates separate from other components. The PCR tubes used should be nuclease-free.
2. The primers used are typically 20-40 nucleotides in length and preferably have a GC content of about 40-60%. Professional software is recommended to design or analyze primers. Optimal results are usually generated when the final concentration of each primer is 0.5 μM.
3. The recommended extension temperature is 72°C. For complex genomic samples, the extension time is typically 20-30 s/kb. However, for simple templates (plasmids, E. coli, etc.) or complex templates < 1 kb, it can be reduced to 10 s/kb. For cDNA or complex templates, the extension time can be increased to 40 s/kb.
4. Usually, 25-35 cycles are ideal to acquire appropriate yield. Detection of low-copy templates may require up to 45 cycles. Note that too many cycles may lead to an increase in non-specific amplifications.
5. The optimal annealing temperature for UltraFidelity™ DNA polymerase is typically higher than that of Taq DNA polymerase. When using it for the first time, it is recommended to start with a temperature at least 3°C higher than the lower Tm value of the primers. Alternatively, you can use a temperature gradient to optimize the annealing temperature.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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