2X UltraFidelity™ Master Mix with dye

Introduction

2X UltraFidelity™ Master Mix with dye is an efficient and convenient premix specifically designed for high-fidelity DNA amplification. It employs a thermostable DNA polymerase with 3'→5' exonuclease activity and incorporates the Sso7d DNA domain to enhance processivity, ensuring the stability of the DNA amplification process. The error rate of this enzyme is as low as 1/280 that of Taq DNA polymerase, making it suitable for experiments requiring high fidelity, processivity, yield and robust amplification, such as long amplicon or high GC-content fragments amplification, and cloning.

2×UltraFidelity™ Master Mix with dye contains polymerase, dNTPs, buffer, and dye. When diluted to a 1× concentration, the final concentration of Mg++ is 2 mM. Compared to conventional PCR enzymes, our product provides higher fidelity.

After PCR amplification, the product can be directly subjected to electrophoresis without adding loading buffer. If the subsequent experiment involves cloning, it is recommended to choose the dye-free version (**Cat. No. K1501**).

Components and Storage

Components \ Size	1 mL	5×1 mL	20×1 mL	100×1 mL	Storage
2X UltraFidelity™ Master Mix with dye	1 mL	5×1 mL	20×1 mL	100×1 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 with dye	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 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.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com