UltraFidelity™ DNA polymerase

Introduction

UltraFidelity™ DNA Polymerase is a high-fidelity, thermostable DNA polymerase that possess 3'→5' exonuclease activity and incorporates the Sso7d domain, which enhances its processivity to achieve stable and efficient DNA amplification. Its error rate is only 1/280 of that of Taq DNA polymerase, it is highly suitable for experiments requiring high fidelity, processivity, yield and robust amplification, such as long amplicon amplification, high GC-content fragments, and cloning. The 5×Reaction Buffer contains 2 mM Mg^{2+} at 1×concentration. Our product is an ideal choice for various applications including seamless cloning, long fragment PCR, high-fidelity PCR, and multiplex PCR.

Components and Storage

Components \ Size	K3108-100 U	K3108-500 U	K3108-1000 U	存储条件
UltraFidelity™ DNA polymerase (2 U/μL)	50 μL	250 μL	500 μL	-20°C
5×Reaction buffer	1 mL	5×1 mL	10×1 mL	-20°C
Shipping: Dry Ice	**Shelf life:** 12 months			

Protocol

1. Set up the PCR Reaction

Set your reaction as the following table. Or use your own system and conditions. Thoroughly thaw the Reaction buffer 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
5×Reaction buffer	5 μL	10 μL	1X
10 mM dNTPs	0.5 μL	1 μL	200 μM
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	< 1,000 ng
UltraFidelity™ DNA polymerase	0.25 μL	0.5 μL	0.02 U/μL
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.

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