HiFi Seq Hotstart DNA Polymerase

FP1509106

Storage -20℃. Avoid freeze/thaw cycle.
Shipping Ultra-low temperature transportation.

Introduction

HiFi Seq Hotstart DNA polymerase is an engineered enzyme modified via genetic engineering. It enhances the affinity for DNA templates without the need for auxiliary proteins or additional DNA-binding domains. Compared to other wild-type B-family DNA polymerases, HiFi Seq Hotstart DNA polymerase features robust processivity, which can significantly increase the yield, amplification rate, and sensitivity of amplified products. Meanwhile, its ability to amplify long fragments and complex templates (such as those with high AT or high GC content) is also notably improved.

HiFi Seq Hotstart DNA polymerase possesses 5'→3' polymerase activity and 3'→5' exonuclease activity (proofreading activity), but lacks 5'→3' exonuclease activity. The powerful 3'→5' exonuclease activity ensures the accuracy during DNA amplification. The fidelity of HiFi Seq Hotstart DNA polymerase is 100 times that of wild-type Taq DNA polymerase and 10 times that of other B-family DNA polymerases.

Component List

FP1509106	Components	100U	500U	Storage
FP1509106A	HiFi Seq Hotstart DNA polymerase (1U/μL)	100μL	500 μL	-20℃
FP1509106B	5×HiFi seq buffer	1.0 mL	5×1.0 mL	-20℃
FP1509106C	10×Enhancer	500μL	2×1.25 mL	-20℃

Application Scope

Enrichment of NGS DNA libraries, gene expression cloning, site-directed mutagenesis, and analysis of intracellular gene point mutations.

Usage Method

1. Take out 5×HiFi seq buffer and 10×Enhancer in advance and thaw them at room temperature. If precipitation occurs, mix thoroughly until dissolved before use. Keep HiFi Seq Hotstart DNA polymerase on ice for later use.
2. Prepare the PCR system according to the table below. Note that this step should be performed on an ice bath.

Component	Volume (μL)
5×HiFi seq buffer	10
10×Enhancer	5
10mM dNTP	1
HiFi Seq Hotstart DNA polymerase	1
Primer (each 5μM)	2-5
templates	—
ddH_2O	—
Total	50

2.1. The reaction volume can be set between 10–50 μl, and the reagent dosage can be adjusted proportionally. Using a volume > 50 μl is not recommended.

2.2. 1×HiFi seq buffer contains 2.5 mM Mg^{2+} (1×). Additional Mg^{2+} can be added if a higher concentration is required for the system.

2.3. For initial testing, the recommended amount of genomic template in a 50 μl reaction system is 10–100 ng, and 0.1–1 ng for slightly complex templates.

Reaction Procedure

Step Name	Temperature	Time	Cycles
pre-denaturation	95℃	3 min	1
Denaturation	**98℃**	**20 s**	
Annealing	**50-68℃**	**15s**	**10~35**
Extension	**72℃**	**15-60s**	
Final Extension	72℃	1min	1

3. Set the PCR instrument program according to the table below, turn on the heated lid, and set the temperature to 105℃.

3.1. A pre-denaturation step of 3 min at 95℃ is sufficient for regular templates. For templates with GC content ≥ 70%, extend the pre-denaturation time to 5 min.

3.2. High salt ion concentration in the system can affect DNA dissolution. To ensure complete denaturation of complex GC-rich templates, adjust the denaturation temperature to 98℃. High-salt buffers also affect primer annealing, so the optimized annealing temperature should be slightly higher than that of conventional PCR systems. For the first reaction, use 56℃ as the annealing temperature; if the reaction result is unsatisfactory, adjust the temperature in 3℃ increments to determine the optimal annealing temperature.

3.3. For the two-step amplification program, the recommended annealing/extension temperature range is 65–68℃, and the extension time is calculated as 30–60 sec/kb.

3.4. Under conventional conditions, if the target fragment ≤ 1 kb, calculate the extension time as 15 sec/kb; if the target fragment > 1 kb or higher product yield is required, calculate the extension time as 30 sec/kb.

3.5. To achieve high fidelity, the number of amplification cycles is recommended to be ≤ 25. The higher the number of amplification cycles, the higher the mismatch rate.