Product Description

AmpHifi HS DNA Polymerase -V3 is a next-generation hot-start high-fidelity DNA polymerase. Through point mutation and domain recombination expression, its DNA affinity and processivity have been greatly enhanced, resulting in excellent compatibility with GC-rich templates and low-input templates. Its fidelity has also been improved compared to the previous version, reaching approximately 150 times that of Taq polymerase. AmpHifi HS DNA Polymerase III is suitable for both conventional PCR amplification and sequencing library amplification.

Components

Components	BR3P103-51 (100 U)	BR3P103-54 (500 U)	BR3P103-56 (1000 U)
5×HiFi Buffer III	1,000 μL	1,000 μL×5	1,000 μL×10
AmpHifi HS DNA Polymerase III	100 μL	500 μL	500 μL×2

Storage

Store at -20±5℃.

Notes

1. It is recommended to use nuclease-free consumables and to regularly clean the experimental area.
2. Other reagents required for PCR must be prepared by the user or purchased separately.
3. Use high-quality, high-purity template.
4. This product is for research use only.

Protocol

1. Preparation of PCR reaction mix

1.1 Thaw all components at room temperature or on ice, then flick to mix and briefly centrifuge. Keep on ice until use. Prepare the reaction mix according to Table 1:

Table 1. PCR reaction mix

Component	Volume (μL)	Remarks
Template DNA	X [a]	< 250 ng
Forward Primer (10 μM)	2 [b]	0.4 μM
Reverse Primer (10 μM)	2	0.4 μM
5×HiFi Buffer III	10	1×
AmpHifi HS DNA Polymerase III	1 [c]	1 U/50 μL
ddH2O	up to 50	
Total	50	

Note a: Genomic DNA: 50 ng–250 ng; plasmid or viral DNA: 1 pg–10 ng.

Note b: The final primer concentration may be appropriately reduced to 0.1 μM–0.2 μM; when amplifying sequencing libraries, a final primer concentration of 1 μM is recommended.

Note c: The amount of enzyme may be adjusted according to the specific application, but it is recommended not to exceed 5 U/50 μL.

1.2 Gently pipette up and down to mix, then briefly centrifuge to collect the reaction mixture from the tube wall to the bottom.

2. PCR amplification program

2.1 Set up the PCR amplification program on the thermal cycler according to Table 2:

Table 2. PCR amplification program

Step	Temperature	Time	Cycles
Heated lid	105℃	/	/
Initial denaturation	98℃	30 sec [a]	1
Denaturation	98℃	10 sec	25-35
Annealing	55-72℃ [b]	10-30 sec	
Extension	72℃ [c]	20 sec/Kb	
Final extension	72℃	3-5 min	1
Hold	4℃	∞	1

Note a: Initial denaturation at 98℃for 30 sec to 1 min is recommended.

Note b: The annealing temperature can be adjusted within Tm ± 2℃, depending on primer length and GC content. Appropriate annealing temperature greatly improves amplification success. Optimization is recommended before formal experiments.

Note c: If the primer Tm is ≥ 72℃, the annealing and extension steps may be combined into a single step, with extension time set to 30 sec per Kb.