

## Product Description

AmpHifi XL Master Mix is a 2× premixed reagent composed of hot-start high-fidelity DNA polymerase, reaction buffer, and dNTP Mixture. The high-fidelity thermostable DNA polymerase is obtained through enzyme-engineering directed evolution and has strong 3'→5' exonuclease activity, resulting in an extremely low mismatch rate during PCR. Through specific antibody modification, the enzyme has no 5'→3' polymerase activity or 3'→5' exonuclease activity at room temperature, enabling highly specific hot-start PCR.

The buffer contains extension-enhancing factors to maintain stable and efficient amplification. Using human genomic DNA as the template, long amplicons of 15 kb can be readily obtained. When used with the supplied PCR Enhancer, GC-rich regions (80%) can be amplified with high specificity and efficiency. This product is suitable for routine gene cloning, long-fragment amplification, and related applications.

## Components

Components	BR3M401-03 (24 T)	BR3M401-06 (96 T)
PCR Enhancer	120 µL	480 µL
AmpHifi XL Premix	600 µL	1,200 µL×2

## Storage

Store at -20±5°C.

## Notes

1. This product is intended for scientific research purposes only.
2. Use high-quality, high-purity templates. When amplifying fragments longer than 10 kb, template quality will significantly affect the amplification result.
3. The DNA polymerase is modified with a monoclonal antibody. The reaction system can be prepared at room temperature, but keep the premix on ice before use and return it to the freezer promptly after use.
4. Primer design should follow general principles. If the 5' end contains an added sequence that is not complementary to the template, do not include that sequence when calculating the T<sub>m</sub> value.
5. If the template GC content is higher than 65% and amplification is difficult or unsatisfactory using standard methods, add 1-5 µL PCR Enhancer to a 50 µL reaction until good amplification is obtained. Adding more than 5 µL is not recommended. In general, 2 µL PCR Enhancer is sufficient for most complex templates.

## Protocol

### 1. PCR Reaction Setup

1.1 Thaw each component at room temperature or on ice, mix thoroughly, and briefly centrifuge. Prepare the reaction mix according to Table 1.

Table 1. Reaction System

Component	Volume (µL)	Remarks
Template	X	0.1 ng-500 ng
Primer 1 (10 µM)	2	0.2-0.6 µM
Primer 2 (10 µM)	2	0.2-0.6 µM
AmpHifi XL Premix	25	1×
PCR Enhancer	1-5	Optional
ddH <sub>2</sub> O	Up to 50	

1.2 Gently pipette to mix and briefly centrifuge to collect the reaction solution at the bottom of the tube.

### 2. PCR Cycling Program

2.1 Set the PCR cycling program on the thermal cycler according to Table 2.

Table 2. PCR Cycling Program

Step	Temperature	Time	Cycles
Heated lid	105°C	/	/
Initial denaturation	95°C	3 min	1 cycle
Denaturation	95°C	15 s	25-35 cycles
Annealing	60°C <sup>a</sup>	15 s	
Extension	72°C	1 min/kb <sup>b</sup>	
Final extension	72°C	5 min	1 cycle
Hold	4°C	∞	1 cycle

Note a: Adjust the annealing temperature according to primer  $T_m \pm 2^\circ\text{C}$ ; 60°C is recommended. When the amplicon is longer than 10 kb, a two-step PCR program can improve product specificity.

Note b: For fragments shorter than 10 kb, set extension to 1 min/kb. For fragments of 10-20 kb, set the total extension time to 10 min.