Product Description

AmpHifi EXL Master Mix contains a genetically engineered hot-start high-fidelity DNA polymerase with extremely high DNA affinity and processivity. It shows excellent compatibility with complex templates and partially degraded templates. Its fidelity is further improved compared with previous versions and is approximately 95-fold higher than that of Taq DNA polymerase. The buffer contains extension-enhancing factors and co-stabilizing components to maintain stable and efficient amplification.

Using human genomic DNA as the template, long amplicons of 20 kb can be obtained. This product is suitable for applications such as gene-cluster cloning and ultra-long-fragment assembly.

Components

Component	BR3M402-03 (24 T)	BR3M402-06 (96 T)
2×EXL Buffer	600 µL	1,200 µL×2
EXL DNA Polymerase	24 µL	96 µL

Storage

Store at -20±5℃.

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 it is recommended to keep the reagent on ice before use and return it to the freezer promptly after use.
4. When amplifying fragments >10 kb, primer lengths >26 nt are recommended. 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 Tm value.
5. It is recommended to add 1 U enzyme in a 50 µL reaction system. Increasing the enzyme amount can improve amplification yield, but more than 2 U is not recommended.

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. PCR Reaction Setup

Component	Volume (µL)	Remarks
Template	X	0.1 ng-500 ng
Primer 1 (10 µM)	2	0.1-0.6 µM [a]
Primer 2 (10 µM)	2	0.1-0.6 µM
2×EXL Buffer	25	1×
EXL DNA Polymerase	1	1 U
ddH_2O	Up to 50	

Note a: If non-specific amplification occurs, reducing the final primer concentration to 0.1 µM can improve specificity.

2.2 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℃	/	/
Initial denaturation	98℃	30 s [a]	1 cycle
Denaturation	98℃	10 s	25-35 cycles
Annealing	60℃ [b]	15 s	
Extension	72℃	20 s/kb [c]	
Final extension	72℃	5-10 min	1 cycle
Hold	4℃	∞	1 cycle

Note a: For most templates, set initial denaturation to 30 s. If the template has high GC content, extend the initial denaturation to 1 min.

Note : Adjust the annealing temperature according to primer Tm ±2℃; 60℃ is recommended.

Note: The extension rate can be adjusted within 15-30 s/kb. An extension time of 20 s/kb can efficiently amplify most fragments.