



ABP HiFi DNA Polymerase

Catalog Number: D018-01, D018-02

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
ABP HiFi DNA Polymerase (Cat. No. D018-01, 100U)			The product is stable for at least one year when stored as directed.
ABP HiFi DNA Polymerase (2.5 U/ μ l)	40 μ L	-20 °C, avoid repeated free-thaw	
5 x HiFi Buffer (with 7.5 mM MgCl ₂)	1.25 mL		
50 mM MgCl ₂	1 mL		
ABP HiFi DNA Polymerase (Cat. No. D018-02, 500U)			
ABP HiFi DNA Polymerase (2.5 U/ μ l)	200 μ L	-20 °C, avoid repeated free-thaw	
5 x HiFi Buffer (with 7.5 mM MgCl ₂)	1.25 mL x 5		
50 mM MgCl ₂	1 mL		

Product Description

ABP HiFi DNA Polymerase is a new generation of ultra-fidelity DNA polymerase based on Pfu DNA Polymerase. It has high amplification efficiency and wide template adaptability, and is suitable for almost all PCR reactions. ABP HiFi DNA Polymerase is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of ABP HiFi DNA Polymerase is 100-fold lower than that of conventional Taq and 10-fold lower than that of Pfu. In addition, ABP HiFi DNA Polymerase has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. ABP HiFi DNA Polymerase has 5'→3' polymerase activity and 3'→5' exonuclease activity, and the amplified product is blunt-ended, suitable for fragment amplification of the seamless cloning kit and amplification of the second-generation sequencing library.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of whole dNTPs into acid-insoluble products in 30 minutes at 74 °C with activated salmon sperm DNA as the template/primer.

Quality Control

Residual *E.Coli* gDNA Test: Detecting the residual nuclear acid in 10 U of this enzyme with *E.Coli* 16S rDNA-specific TaqMan qPCR, the genome DNA of *E.Coli* is less than 10 copies.

Residual Endonuclease Test: Incubate 10 U of this enzyme and 200 ng of Lambda DNA at 37 °C for 4h, the DNA electrophoresis bands remain unchanged.

Function Assay: Load 1 U of this enzyme into a 50 μ l PCR system and use 10 ng of λ DNA as template. After 30 cycles, use 1/10 of the PCR products to perform 1% agarose gel electrophoresis and EB staining, then there shall be a specifically single band responding to expect.

Experimental Process

1. Keep all components on ice during the experiment. All components need to be mixed up thoroughly after thawing and put back to -20°C immediately for storage after using.

Components	50 μl rxn	Final concentration
5 x HiFi Buffer (with 7.5 mM MgCl_2)	10 μl	1x
dNTP Mix (10 mM each)	1 μl	200 μM
PCR Forward Primer (10 μM)	2.5 μl	500 nM
PCR Reverse Primer (10 μM)	2.5 μl	500 nM
50 mM MgCl_2	optional	as required ^a
DMSO	optional	as required ^b
DNA template	Variable	as required ^c
ABP HiFi DNA Polymerase	0.5 μl	
ddH ₂ O	Add to 50 μl	

Note:

a. For most reactions, the optimal final concentration of Mg^{2+} is 1.5-2 mM. The system already contains a final concentration of 1.5 mM Mg^{2+} . If necessary, use 50 mM MgCl_2 provided in the kit to explore the optimal concentration of Mg^{2+} at intervals of 0.2-0.5 mM.

b. The amount of DMSO can be increased by 1% and the adjustment range is 0-8%.

c. Suggested amount of DNA template in 50 μl rxn system: Genomic DNA: 5 ng - 200 ng; E. coli genomic DNA: 100 pg - 100 ng; λ DNA: 10 pg - 10 ng; Plasmid or viral DNA: 10 pg - 10 ng.

2. PCR reaction condition:

Step	Temperature	Time	Cycle
Initial Denaturing	94-96 $^{\circ}\text{C}$	1-3 min	
Denaturing	94-96 $^{\circ}\text{C}$	10-20 sec	} 25-35
Annealing	$T_m \pm 3^{\circ}\text{C}$	10-30 sec	
Extension	72 $^{\circ}\text{C}$	15-30 sec/kb	
Final Extension	72 $^{\circ}\text{C}$	5-10 min	
Hold	4 $^{\circ}\text{C}$		

Application example

Taking human genomic DNA as templates, the target fragments of 0.6 kb, 1.0 kb, 2.6 kb, 3.0 kb, 4.0 kb, 5.1 kb, 6.2 kb, 7.1 kb, 8.5 kb, 10.6 kb, 17.8 kb, 20.3 kb, and 21.4 kb were amplified, respectively. The T_m of all primers are approximately 60 $^{\circ}\text{C}$.

The reaction system and program are as follows:

Recommended PCR System

Components	50 μ l rxn
5 x HiFi Buffer (with 7.5 mM MgCl ₂)	10 μ l
dNTP Mix (10 mM each)	1 μ l
PCR Forward Primer (10 μ M)	2.5 μ l
PCR Reverse Primer (10 μ M)	2.5 μ l
Human Genomic DNA (100 ng/ μ l)	1 μ l
ABP HiFi DNA Polymerase	0.5 μ l
ddH ₂ O	Add to 50 μ l

Recommended PCR Program

Step	Temperature	Time	Cycle
Initial Denaturing	95°C	3 min	
Denaturing	95°C	15 sec	} 35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	
Hold	4°C		

Electrophoresis Results of the PCR Products



M: DL15,000 DNA Marker

- 1: 0.6 kb
- 2: 1.0 kb
- 3: 2.6 kb
- 4: 3.0 kb
- 5: 4.0 kb
- 6: 5.1 kb
- 7: 6.2 kb
- 8: 7.1 kb
- 9: 8.5 kb
- 10: 10.6 kb
- 11: 17.8 kb
- 12: 20.3 kb
- 13: 21.4 kb