

2xABP HiFi PCR Master Mix

Catalog Number: D019-01, D019-02

Table 1. Package information and Storage

| Cat. No. | Unit Size | Storage |
|----------|-----------|---|
| D019-01 | 1 ml | Long term: -20°C Avoid freeze/thaw cycle |
| D019-02 | 5x1 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.

2xABP HiFi PCR Master Mix contains ABP HiFi DNA Polymerase, dNTP, and an optimized buffer system. The amplification can start only with the addition of primer and template, thereby easing PCR setup and improving reproducibility.

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 μ l rxn | Final concentration |
|---------------------------------|----------------|---------------------|
| 2xABP HiFi PCR Master Mix | 25 μ l | 1x |
| PCR Forward Primer (10 μ M) | 2.5 μ l | 500 nM |
| PCR Reverse Primer (10 μ M) | 2.5 μ l | 500 nM |

| | | |
|--------------------|--------------|--------------|
| DNA template | Variable | as required* |
| ddH ₂ O | Add to 50 µl | |

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

2. PCR reaction condition:

| Step | Temperature | Time | Cycle |
|--------------------|---------------------|--------------|---------|
| Initial Denaturing | 94-96°C | 1-3 min | |
| Denaturing | 94-96°C | 10-20 sec | } 25-35 |
| Annealing | T _m ±3°C | 10-30 sec | |
| Extension | 72°C | 15-30 sec/kb | |
| Final Extension | 72°C | 5-10 min | |
| Hold | 4°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°C.

The reaction system and program are as follows:

Recommended PCR System

| Components | 50 µl rxn |
|-------------------------------|--------------|
| 2×ABP HiFi PCR Master Mix | 25 µl |
| PCR Forward Primer (10 µM) | 2.5 µl |
| PCR Reverse Primer (10 µM) | 2.5 µl |
| Human Genomic DNA (100 ng/µl) | 1 µl |
| ddH ₂ O | Add to 50 µl |

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