HotStart PCR Kit

Catalog: NP100053
Size: 50 RXN (50 µL/RXN)
Concentration: 2X

HotStart DNA Polymerase (2, 000U/mL)
5X HF Buffer
2.5X GC Buffer
dNTPs (10mM each)

Product Description

The HotStart DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with comparable performance to *Pyrococcus furiosus* DNA polymerase. With unique structure, the HotStart DNA polymerase contains a recombinant synthesis enhancement domain to increase fidelity and extension speed. The antibody-mediated hot-start feature significantly inhibits non-specific amplifications at room temperature.
The HotStart DNA polymerase has a strong 3'-5' exonuclease activity (proofreading activity), which results in its extreme high fidelity, 10-15 times higher than *Taq* DNA polymerase and 6 times higher than *Pyrococcus furiosus* DNA polymerase.
The HotStart PCR Kit is supplied with a 5X HF Buffer and a 2.5X GC Buffer. The 5X HF Buffer is an optimized buffer for general high fidelity amplifications while the 2.5X GC Buffer is used in the amplifications of problematic or GC-rich templates.

Storage

Upon receipt, store all components at -20°C.

Thermal Inactivation

None

Product End

Blunt end

Standard Protocol

- It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98 ° C.
- All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add the HotStart DNA polymerase at the end to prevent primer degradation by its strong 3´-5´ exonuclease activity.
- Note: HotStart DNA polymerase requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields.

Recommended Reaction

5X HF Buffer Reaction System:

Components	25 µL	50 µL	Total Concentration
5X HF Buffer	5µL	10 µL	1X
Forward Primer (10 µM)	0.5 µL	1 µL	0.2 µM
Reverse Primer (10 µM)	0.5 µL	1 µL	0.2 µM
DNA Template*	Variable	Variable	<300 ng
dNTPs (10mM)	0.5 µL	1 µL	0.2 µM
HS DNA Polymerase	0.5 µL	1 µL	2U/50uL
Nuclease-free Water	to 25 µL	to 50 µL	N/A

** Note : The optimal reaction concentration varies with different DNA templates. Please refer to the basic principles of PCR below.*

2.5X GC Buffer Reaction System:

Components	25 µL	50 µL	Total Concentration
2.5X GC Buffer	10 µL	20 µL	1X
Forward Primer (10 µM)	0.5 µL	1 µL	0.2 µM
Reverse Primer (10 µM)	0.5 µL	1 µL	0.2 µM
DNA Template*	Variable	Variable	<300 ng
dNTPs (10mM)	0.5 µL	1 µL	0.2 µM
HS DNA Polymerase	0.5 µL	1 µL	2U/50uL
Nuclease-free Water	to 25 µL	to 50 µL	N/A

Recommended PCR Program

Step	Temp	Time	Cycles
Pre-denaturation	98°C	45 s	1
Denaturation	98°C	10 s	
Annealing	55-65°C	20-30 s	25-35
Extension	72°C	10-30 s/kb	
Post-extension	72°C	1-5 min	1
Hold	4-12°C	∞	1

PCR Principles

1. Template

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below.

Recommended Input (For a 50µL reaction)

DNA	Input Amount
Plants, animals and human gDNA	10 ng-300 ng
E.coli, lambda genome	10 ng-100ng
Plasmid DNA	1 pg-10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately.

2. Primers

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.2-1 µM.

3. Enhancer

The Enhancer solution is an optional component to increase the amplification efficiency for problematic templates, such as GC-rich sequence or genes with strong secondary structure. Note: Since the enhancer is included in the 2.5X GC Buffer, additional enhancer is not recommended with the use of 2.5X GC Buffer. Excess amount of enhancer may be inhibitory.

4. Buffer

The HotStart PCR Kit contains a 5X HF Buffer and a 2.5X GC Buffer. The 5X HF Buffer is designed for general high fidelity PCR amplification , and the 2.5X GC Buffer is optimized for the amplifications of GC-rich templates.

5. Denaturation

98 ° C pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation. Generally, the recommended denaturation condition for low-complexity DNA templates is 98 ° C, 5-10 s.

6. Annealing

The annealing temperature of HotStart DNA polymerase is usually higher than other PCR polymerases. Generally, primers longer than 20 nt are annealed at (lower primer Tm+3) ° C for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm should be used. When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

7. Extension

The recommended extension temperature is 72 ° C. The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is

10-30 s / kb. For high-complexity amplicons, such as genomic DNA, it is recommended to increase the extension time to 1 min / kb. In some cases, the extension time for cDNA templates should be less than 1 min / kb.

8. Cycles

To obtain enough yield of PCR products, 25-35 cycles are recommended.

9. PCR Products

The HotStart DNA polymerase produces blunt-end PCR products, which might be directly used in the sequential blunt-end cloning. For T/A cloning, the PCR products should be further purified to remove HotStart DNA polymerase before dA tailing reaction, because the proofread activity of HotStart DNA polymerase will remove the dA-overhangs.