FISHER MOLECULAR BIOLOGY

HOT - START TAQ DNA POLYMERASE (with GC-Buffer) FS-T-2131-1

Description

HOT START DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with comparable performance to Pyrococcusiosus DNA polymerase. With unique structure HS 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. HOT START is one of the thermostable DNA polymerases with strong 3 ' -5 ' exonuclease 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 HS 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.

Contents	FS-T-2131-200
Hot Start DNA Polymerase (2000U/mL)	200 RNXS
5X HF PCR Buffer	2x 1 mL
2.5X GC PCR Buffer	4X 1 mL
dNTPs (10 mM each)	200 µL

Thermal Inactivation: No
Product End: Blunt end

Standard Protocol

1. It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98℃.It is recommended to prepare all reaction.

2. All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add Hot Start 2X Master Mix with Dye at the end to prevent primer degradation by its strong 3´-5´ exonuclease activity.

Note: The Hot Start 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 Protocol

On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

5X HF(High Fidelity) Buffer ReactionSystem

Component	25 µl reaction	50 µl reaction	Final Conc.
Hot Start DNA Polymerase (2000 U/mL)	0.5 ul	1 ul	2U/50ul
5X HF PCR Buffer	5 ul	10 ul	1 X
dNTP mix (10 mM each)	0.5 ul	1 ul	0.2mM
DNA Template	variable	variable	<300ng
Forward Primer (10µM)	0.5 ul	1 ul	0.2uM
Reverse Primer(10µM)	0.5 ul	1 ul	0.2uM
Distilled water	to 25 ul	to 50ul	N/A

2.5 X GC BUFFER Reaction System

Component	25 µl reaction	50 µl reaction	Final Conc.
Hot Start DNA Polymerase (2000 U/mL)	0.5 ul	1 ul	2U/50ul
2,5X GC PCR Buffer	10 ul	20 ul	1x
dNTP mix (10 mM each)	0.5ul	1ul	0.2mM
DNA Template	Variable	variable	< 300 ng
Forward Primer (10µM)	0.5 ul	1 ul	0.2uM
Reverse Primer (10µM)	0.5 ul	1 ul	0.2uM
Distilled water	to 25 ul	to 50ul	N/A

General Cycling Conditions:

Step	Temp (°C)	Time	Cycle
Initial Denaturation	98	45 sec.	1
Denature	98	10 sec.	25 ~35
Anneal	55~65	20 ~ 30 sec.	
Extend	72	10 ~ 30 sec. **s/kb***	
Final Extension	72	5 min.	1
Hold	4-12		1

***Note:** Properly extending the extension time can improve the amplification yield. For complex amplification templates, such as genomic DNA, it is recommended to extend at a speed of 60 s/kb, and more recommended conditions please refer to the basic principles of PCR below.

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 (For a 50 µL reaction):

DNA	IMPUT Amount
Plants, animals and human gDNA	**10 ng-300 ng**
E.coli, lambda gDNA	**10 ng-100 ng**
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 0.1-1 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 HS 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℃ 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℃, 5-10 s.

6. Annealing
The annealing temperature of HS DNA polymerase is usually higher than other PCR polymerases. Generally,primers longer than 20 nt are annealed at (lower primer Tm+3)℃ 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. Extention
The recommended extension temperature is 72℃ . 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
HS DNA polymerase produces blunt-end PCR products, which might be directly used in the sequential blunt-end cloning.

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

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