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HOT START PROOF 2X MASTER MIX FS-T-71702

Long Range PCR, High-Fidelity PCR, Fast PCR

Description

Host Start Proof DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with unique structure.

Hot Start Proof 2X Master Mix 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 Proof is one of the thermostable DNA polymerases with strong 3'-5'exonuclease activity (**proofreading** activity), which results in its extreme **high fidelity.** The **Hot Start Proof** 2X Master Mix is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

Kit Contents

Contents	FS-T-71702-5
Hot Start Proof 2X Master Mix	5 ml

1 ml= 100 Reactions (20 μl volume)
1 ml= 80 Reactions (25 μl volume)
1 ml= 40 Reactions (50 μl volume)

Applications:

- Long range PCR
- High-fidelity PCR and primer-extension reactions
- Genotyping
- Library construction
- High GC amplification
- Next-generation DNA sequencing
- TA cloning

Storage Buffer:

20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA,200μg/mL BSA, 50% Glycerol, 1X Stabilizers, pH 7.4 @ 25°C

Thermal Inactivation: No
5´-3´exonuclease activity: No
3´-5´exonuclease activity: Yes
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 Hot Start Proof DNA polymerase at the end to prevent primer degradation by its strong 3´-5´exonuclease activity.

-Note: The Hot Start Proof DNA polymerase requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields

Note

Do not contaminate the Hot Start Proof 2X Master Mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Components	20μL	25μL	50μL	Total Conc.
Hot Start Proof 2X Master Mix 2X Master Mix	10μL	12.5μL	25μL	1X
Forward Primer (10 μM)	0.4μL	0.5μL	1 μL	0.2 μM
Reverse Primer (10 μM)	0.4μL	0.5μL	1 μL	0.2 μM
DNA Template*	Variable	Variable	Variable	<300 ng
Nuclease-free Water	to 20μL	to 25μL	to 50μL	N/A

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

Recommended PCR Program

Step	Temp.	Time	Cycles
Initial Denaturation	98°C	45s	1
Denaturation	98°C	10 sec.	25-35
Annealing	55 ~65°C	20-30 sec.	
Extension	72°C	10-30 sec.	
Final Extension	72°C	1-5 min.	1
Hold	4-12°C	∞	1

PCR Principle

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 50μL reaction).

***DNA TEMPLATE**

DNA	20 μL reaction	50μL reaction
Plants, animals and human gDNA	4 ng - 40 ng	10 ng~100 ng
E.coli, lambda gDNA	200 pg-75 ng	500 pg-200 ng
Plasmid DNA	0,4 pg-4 ng	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 oncentration of each primer in the PCR reaction system should be in the range of 0.1-1 μM.

3. Denaturation:

98°C pre-denaturation for 45 seconds 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

4. Annealing:

The annealing temperature of **Hot Start Proof TAQ 2x Master Mix** is usually higher than other PCR polymerases.

Generally, primers longer than 20 nt are annealed at (lower primer Tm+3)°C for 10-30 seconds;

When the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm.

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.

5. Extention:

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 conditions is 10 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 20-30 s/kb. In some cases, the extension time for cDNA templates should be less than 1 min/kb

6. Cycles:

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

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7. PCR Product

Hot Start Proof 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 Hot Start DNA polymerase before dA tailing reaction, because the proofread activity of Hot Start Proof DNA polymerase will remove the dA-overhangs

8.Complex templates :

For complex templates that cannot be amplified by conventional PCR (such as long fragments, uneven TM distribution, templates with special structures), you can try the two-step method or the touchdown method.

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

***In general, 68°C is recommended, but it can be changed according to the TM value.**

Recommended Touchdown PCR Program:

Step	Temp	Time	Cycles
Pre-denaturation	98℃	45 s	1
Denaturation	98℃	10 s	5
Extension	74℃	10-30s/kb	
Pre-denaturation	98℃	10 s	5
Extension	72℃	10-30s/kb	
Denaturation	98℃	10 s	5
Extension	70℃	10-30 s/kb	
Denaturation	98℃	10 s	25
Extension	68℃	10-30 s/kb	
Post-extension	72℃	1-5min	1
Hold	4-12℃	∞	1

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Store at:: -20°C – avoid freezing and thawing cycles.

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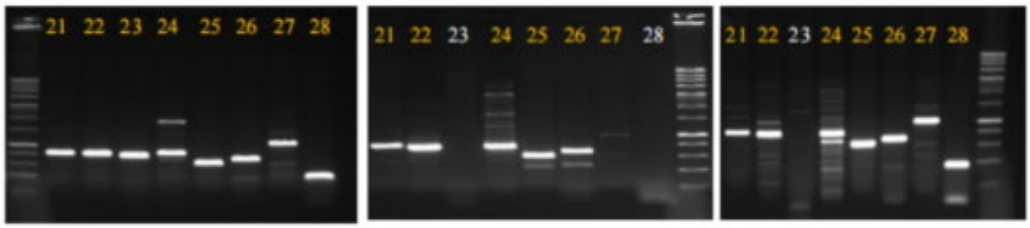
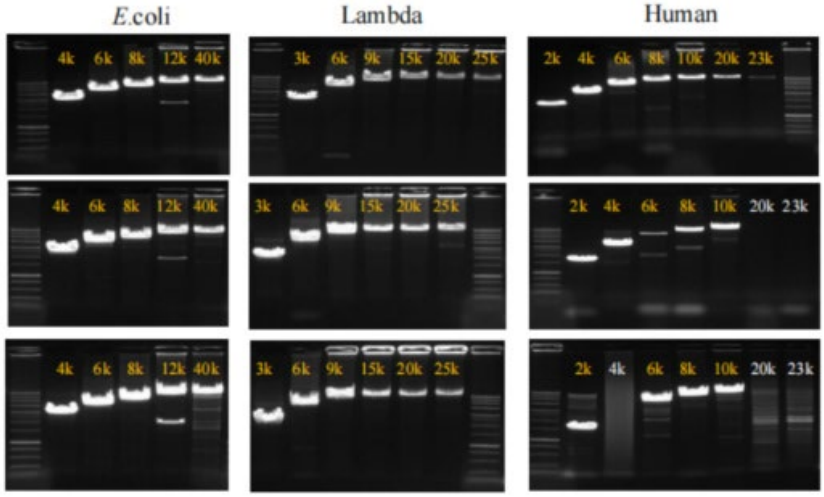
1. GC-rich, AT-rich Template

GC rich: 21: GC-38 (62,7%), 22: GC-7 (69,5%), 23: GC-46 (71,1%), 24: GC-18(78,1%)
AT rich: 25: AT-13 (41%), 26: AT-12 (34%), 27: AT-4 (27%), 28: AT-1(23%)

1. HOT START PROOF 8/8 **2.** COMPETITOR 1 6/8 **3.** COMPETITOR 7/8



2. Long fragment Amplification



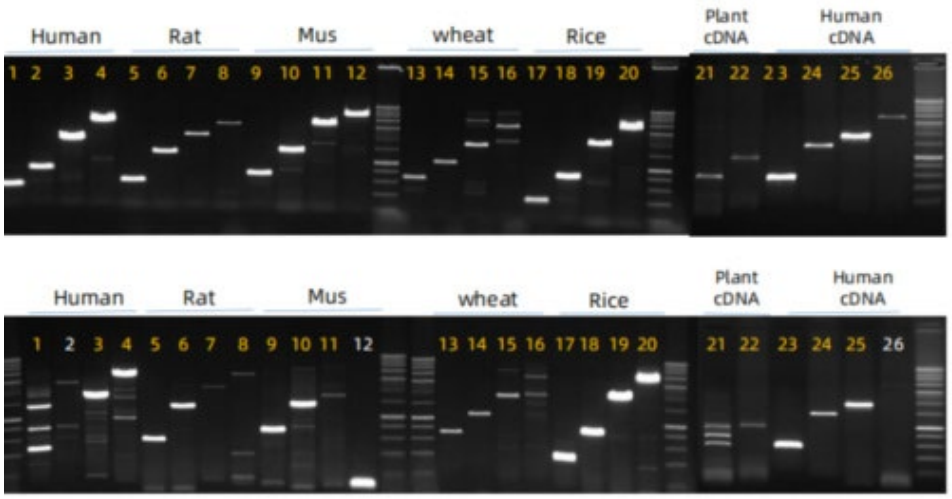
Hot Start Proof 18/18

Competitor 1. 16/18

Competitor 2. 15/18

For long fragment amplification, the amplification success rate of Hot Start Proof is comparable to or better than that of competitors.

3. Universal (Human, Rat, Mouse, Wheat, Rice, Plant, Human cDNA)



Hot Start Proof 26/26

Competitor 1. 23/26

Amplification results of 26 pairs of primers were analyzed, and Hot Start Proof showed high amplification success rate for different types of templates, comparable to international brands.

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