BIO BASIC
Quality-Affordable Research

Product information

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Hotstart Taq DNA Polymerase

Catalog #: 3GHST81
Concentration: 5 U/µl
Size: 500U / 5x500U
Storage: -20°C

Product Description:

HotStart DNA Polymerase contains a proprietary antibody that blocks polymerase activity at low temperatures. During the initial denaturtiaon step at 94°C, the antibody dissociates from DNA polymerase and restores enzyme activity. This feature significantly reduces non-specific product formations that would otherwise compete for reagent availability, thus offering higher specificity and improved yield of PCR products. PCR products amplified up to 6 kb in length with HotStart DNA Polymerase contain a single base (A) 3' overhang.

Source:

E.coli cells carrying a cloned pol gene from Thermus aquaticus.

Components:

Components	500U	5x500U(2,500U)
Hotstart Taq DNA Polymerase (5 U/µl)	100ul	500ul
5x Buffer	1.0ml	1.0ml

Storage Buffer Components*:

50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50 % glycerol and 1.0 % Triton®X-100.

Unit Definition:

One unit of enzyme catalyzes the incorporation of 10 nmol of of deoxyribonucleotides into a polynucleotide fraction in 30 mins at 70°C.

Shipping and Storage:

Upon arrival, HotStart DNA Polymerase should be stored at -20°C. Avoid repeated freezethaw cycles of all HotStart components to retain maximum performance.

***Intellectual property included**

Basic PCR Protocol:

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of Taq Polymerase, primers, MgSO4 and template DNA) vary and need to be optimized for each specific PCR.

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination.

A negative control reaction (omiting t emplate DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components to a sterile 0.2ml PCR tube sitting on ice:

Components	Volume	Final Concentration
Template DNA	~100ng	~2ng
Forward Primer (10uM)	1 - 2.5ul	200 - 500nM
Reverse Primer (10uM)	1 - 2.5ul	200 - 500nM
5x Buffer	5ul	1X
dNTP Mixture (10 mM)	1ul	200 uM
Hotstart Taq Polymerase (5U/ul)	0.5ul	2.5 - 5U
Nuclease-free H2O	25ul	-

2. Mix contents of tube and centrifuge briefly.

3. Incubate tube in a thermal cycler at 94°C for 10 mins to completely activate the HotStart DNA Polymerase and denature the template.

4. Perform 30-35 cycles of PCR amplification as follows:
Denature: 94°C for 30 sec
Anneal: 45-72°C for 30 sec
Extend: 72°C for 1 min/1kb template

5. Incubate for an addtional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or by using an Eco-DNA Dye (Cat #s DT81413, DT81414, DT81415, DT81417, DT81418). Use appropriate molecular weight standards.

Product Performance Verification:

Experimental Purpose

Verify the product performance of Hotstart Taq DNA Polymerase in storage stability, impurity tolerance, compatibility, detection range and degree of nuclease contamination.

Experimental Materials

Hotstart Taq DNA Polymerase, African swine fever virus DNA, human 293T cell DNA.

Experimental Process and Results

Experiment Design 1-1: After freezing and thawing the Hotstart Taq DNA Polymerase for 50 times, African swine fever virus DNA was used as template to carry out three gradients of 10-fold dilution, and the target genes were detected under the same conditions. The data are as follows:

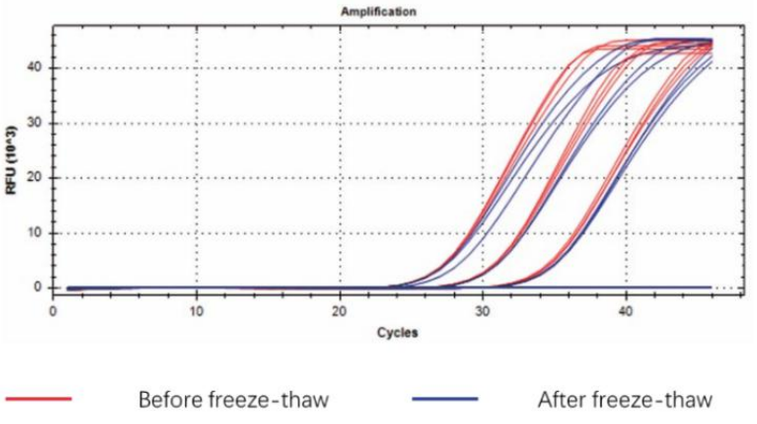

Fig. 1

Experiment Design 1-2: The Hotstart Taq DNA Polymerase was placed at 4°C, 25°C and 37°C for 7 days respectively. Using African swine fever virus DNA as template, fluorescence quantitative detections were conducted to compare the storage conditions at -20°C. The data are as follows:

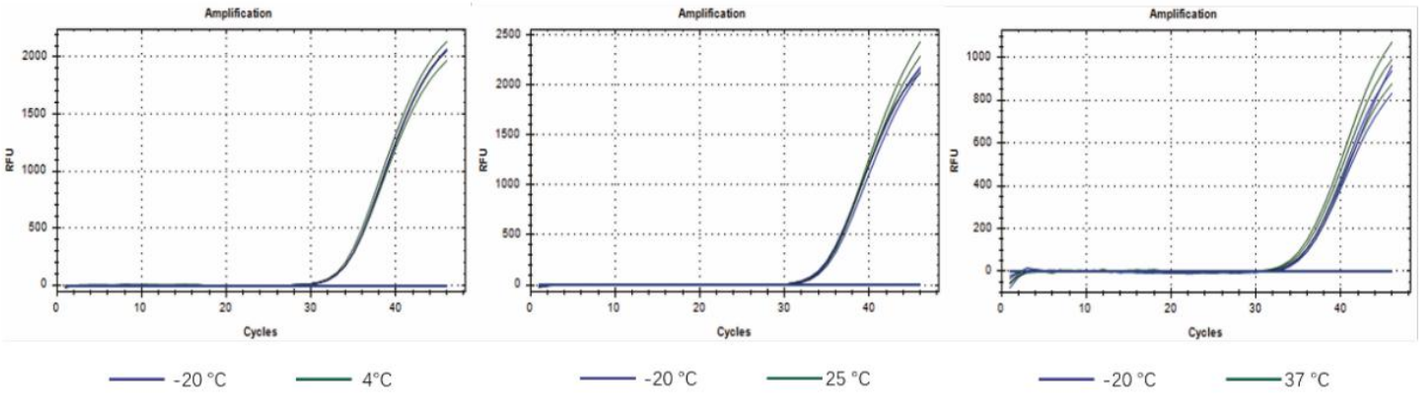

Fig. 2

The results show that Bio Basic's Hotstart Taq DNA Polymerase shows stable storage performance at 4°C, 25°C and 37°C for 7 days, and repeated freeze-thaw cycles for 50 times.

Experiment Design 2: Hotstart Taq DNA Polymerase was used to prepare premix, African swine fever virus DNA was used as the template, whole blood and blood preservation related substances (EDTA, sodium citrate) were selected as the impurity background, and similar products of Company A were compared. The data are as follows:

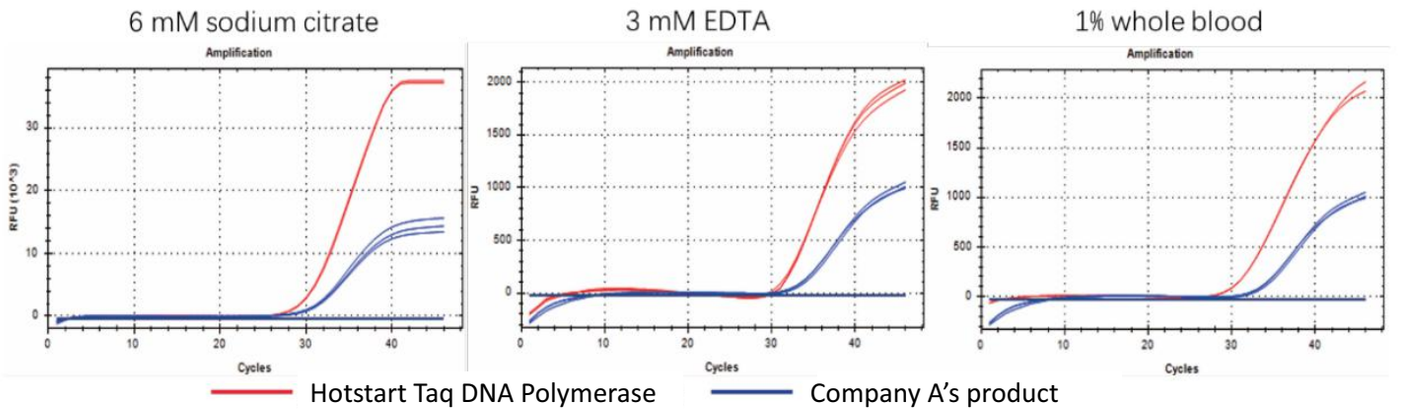

Fig. 3

The results show that Bio Basic's Hotstart Taq DNA Polymerase has good impurity tolerance and higher amplification efficiency.

Experiment Design 3: African swine fever virus DNA was used as template, and Hotstart Taq DNA Polymerase was used to perform fluorescence quantitative detections with standard and rapid program separately under the same conditions. The data are as follows:

Standard Program				
Stage 1	Predenaturation	Rep: 1	95 ℃	30 sec
Stage 2	Cycles	Rep: 45	95 ℃	10 sec
			60 ℃	30 sec
Amplification time: 30 min (total amplification time may vary depending on the instrument)				

Rapid Program				
Stage 1	Predenaturation	Rep: 1	95 ℃	20 sec
Stage 2	Cycles	Rep: 45	95 ℃	1 sec
			60 ℃	20 sec
Amplification time: 16 min (total amplification time may vary depending on the instrument)				

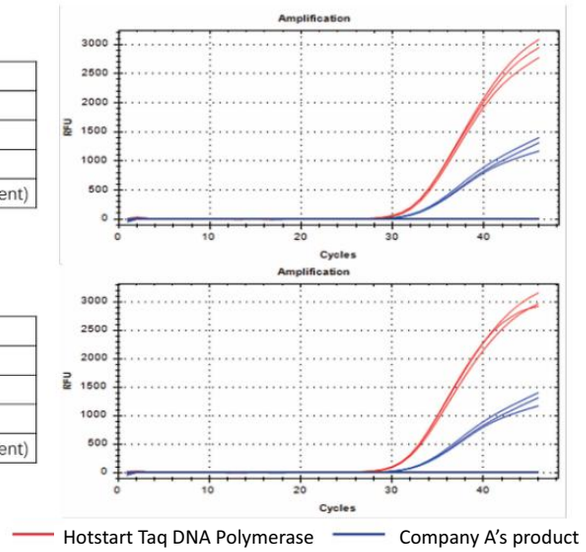

Fig. 4

The results show that Hotstart Taq DNA Polymerase can be compatible with rapid programs to meet the requirements.

Experiment Design 4-1: African swine fever virus DNA was used as template, and Hotstart Taq DNA Polymerase was used to perform 3-plex fluorescence quantitative detections. The data are as follows:

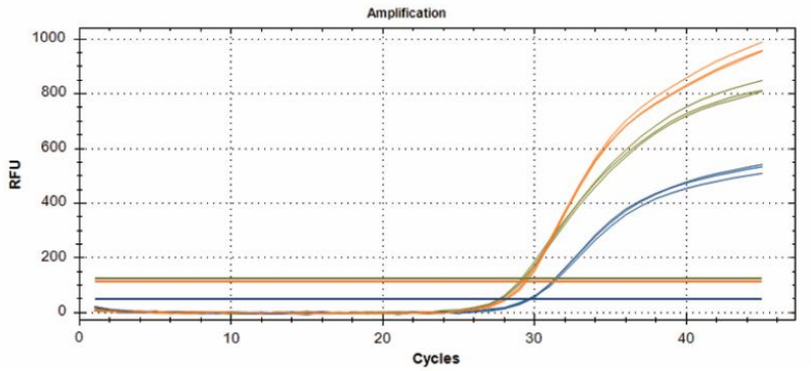

Fig. 5

Experiment Design 4-2: Human 293T cell DNA was used to carry out five gradients of 10-fold dilution, and Hotstart Taq DNA Polymerase was used to detect target gene. The data are as follows:

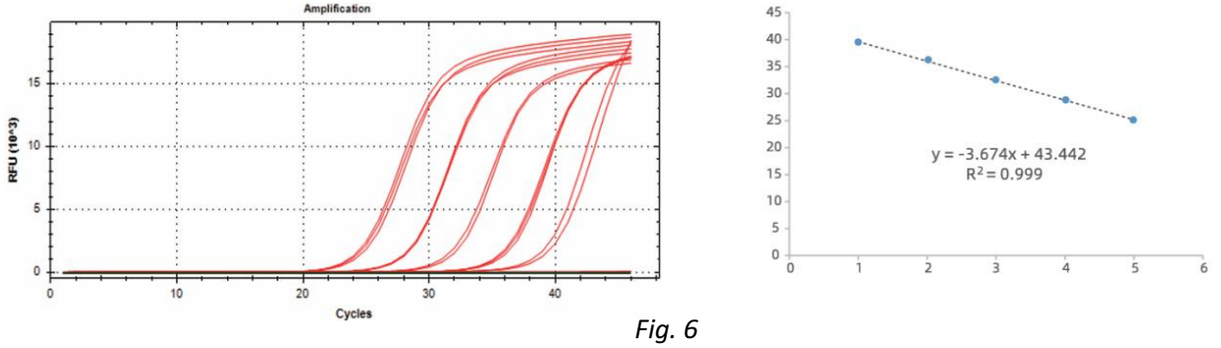

Fig. 6

The results show that Hotstart Taq DNA Polymerase's multiplex assay has high amplification efficiency and can obtain good linear relationship over a wide quantitative region.

Experiment Design 5-1: Detection of endonuclease activity: 100 ng 2000 bp linear DNA fragments were taken, mixed with 400 U Hotstart Taq DNA Polymerase and incubated at 37°C for 4 hours. The DNA band changes were detected by gel electrophoresis.

With Hotstart Taq DNA Polymerase

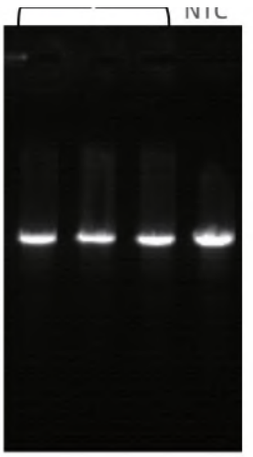

Fig. 7

Experiment Design 5-2: Detection of endonuclease activity: 500 ng Human Genomic DNA was taken, mixed with 400 U Hotstart Taq DNA Polymerase and incubated at 37°C for 16 hours. The DNA band changes were detected by gel electrophoresis.

With Hotstart Taq DNA Polymerase

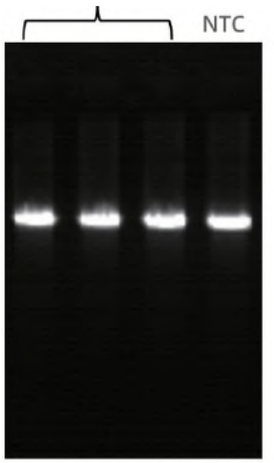

Fig. 8

The results show that Bio Basic's Hotstart Taq DNA Polymerase has no endonuclease and exonuclease activity.

Conclusion

Bio Basic's Hotstart Taq DNA Polymerase has excellent performance in storage stability, impurity tolerance, compatibility, detection range, and is free of nuclease contamination.