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	5x1.0ml

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.